Food Chemistry Advances 11 (2026) 101328

Contents lists available at ScienceDirect

Food Chemistry Advances

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

Comparative extraction strategies for maximizing phytochemicals and antioxidant potential in red and yellow cashew (*Anacardium occidentale* L.) leaf varieties

Mithul Aravind [a], Chakkaravarthi Saravanan [b], Lijing Ke [c], Dania Cheaha [d], Teerapat Teetharatkul [e], Santad Wichienchot [a,f,*]

[a] Functional Food and Nutrition Program, Faculty of Agro-Industry, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand
[b] Department of Interdisciplinary Sciences, National Institute of Food Technology Entrepreneurship and Management, Kundi, District-Sonepat, Haryana 131028, India
[c] Faculty of Environment, School of Food Science and Nutrition, University of Leeds, Leeds LS2 9JT UK
[d] Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand
[e] Faculty of Medicine, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand
[f] Center of Excellence in Functional Foods and Gastronomy, Faculty of Agro-Industry, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand

ARTICLE INFO

Keywords:
Cashew leaf
Polyphenol
Flavonoid
Extraction
Anti-depression
Antioxidant

ABSTRACT

Cashew (*Anacardium occidentale* L.) leaves (CL) are a rich yet underutilized source of bioactives, particularly phenolics, which exhibit potent antioxidant and diverse biological properties. Despite growing interest in exploiting plant-based antioxidants, comparative data on how extraction methods influence the recovery of bioactives from different CL (yellow and red varieties) remain limited. The present study aimed to evaluate the effect of different extraction techniques on the bioactive composition and antioxidant potential of two CL varieties. Various sample pre-treatments were applied, followed by extraction using diverse solvent profiles through conventional (CE) and ultrasound-assisted (UAE) methods. The resultant extracts were analyzed for free and bound phenolic and flavonoid contents as well as antioxidant activity, with yellow leaves from CE showing free and bound flavonoid contents of 251.46 and 134.02 mg QE/g, respectively, compared to 189.8 and 97.95 mg QE/g for the 5- min UAE treatment. Our findings demonstrated that extraction efficiency varied significantly ($p < 0.05$) with solvent and leaf variety. Between the two varieties, UAE of yellow variety showed higher antioxidant activity (343 μM Trolox equivalent/g) than red variety (337 μM Trolox equivalent/g) indicating a stronger correlation between phenolic concentration and radical scavenging ability. The CE and 10-min UAE (1 mg/mL) increased the RAW 264.7 cell survival rate upto 120% and exerted anti-inflammatory activity (around 99.02%) by inhibiting nitric oxide. Thus, our study underscores the importance of extraction parameters in recovering bioactives from yellow and red CL providing a methodological basis for optimizing extraction protocols. Further studies will focus on formulating functional ingredients or nutraceuticals from processed CL with potential anti-depressive and antioxidant activities.

1. Introduction

Polyphenols are among the most studied classes of bioactive plant secondary metabolites, recognized for their redox-active properties and their role in mitigating oxidative stress–induced pathologies (Muscolo et al., 2024). Most plant parts biosynthesize polyphenols that impart characteristic color, aroma, and flavor to plant-derived materials. Cashew (*Anacardium occidentale* L.) is one of the most widely cultivated tropical crops among diverse botanicals. Compared to nuts and cashew apples, cashew leaf consumption is geographically limited (Oliveira et al., 2020). Flavonols, phenolic acids, flavan-3-ols, and hydrolyzable tannins are among the many phenolic compounds in cashew leaves. These compounds are bioactive molecules with strong radical scavenging, metal chelating, and antimicrobial effects (Galletta et al., 2026). Accordingly, cashew leaf polyphenols could be used in natural preservatives, nutraceuticals, and functional foods. The applicability of

* Corresponding author at: Functional Food and Nutrition Program, Center of Excellence in Functional Foods and Gastronomy, Faculty of Agro-Industry, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand.
E-mail address: santad.w@psu.ac.th (S. Wichienchot).

https://doi.org/10.1016/j.focha.2026.101328

Available online 11 May 2026

such compounds in food and functional food items constitutes new opportunities for food science and plant biochemistry. However, the stability and biological activities of polyphenols depend not exclusively on their intrinsic composition but rather on the processing and extraction processes applied for their recovery and application.

Processing conditions, such as solvent extraction, fermentation, drying, and blanching, can have a profound influence on polyphenol structural stability, solubility, and bioaccessibility. Thermal processing, for example, may hydrolyze cell wall matrices and liberate bound phenolics, but can also lead to polymerization or oxidative deterioration from prolonged heat exposure (Sławińska & Olas, 2024). Similarly, recovery efficiency and selectivity towards specific phenolic sub-classes are controlled by manipulation of solvent polarity, extraction temperature, pH, and time (Bolat et al., 2024). These changes influence both the quantitative yield of the phenolics and qualitative factors, such as antioxidant activity, storage potential, and interactions with other macromolecules (Shi et al., 2022). Studies on other leafy biomaterials have indicated that the integrity of phenolic compounds and the reproducibility of functional outcomes are strongly influenced by the optimization of processing parameters (Huo et al., 2025).

The impact of processing conditions on polyphenol composition directly affects biological quality in scientific and commercial applications; hence, it is important to understand the influence of processing conditions on cashew leaf polyphenols. A systematic investigation of the modulation of the phenolic profile using various treatments is required to enhance bioactivity, improve extraction yield, and design stable products. Moreover, these studies contribute to increasing the economic value of cashew byproducts, which aligns with recent trends in circular bioeconomy and sustainable bioprocessing. This study aims to clarify the interactions between processing parameters and phenolic dynamics, thereby establishing a foundation for the optimal utilization of cashew leaves as a source of natural bioactive compounds for health promotion, including potential antidepressant applications.

2. Materials and methods

2.1. Chemicals and reagents

The standards, including Trolox, gallic acid, and quercetin, were ≥ 98% purity. Distilled water was used for all analyses. All other chemicals, solvents, and reagents used in this study were of analytical grade. The Folin–Ciocalteu reagent and sodium hydroxide (NaOH) were procured from Loba Chemie Pvt. Ltd., Mumbai, India. Aluminum chloride ($AlCl_3$) and sodium nitrite ($NaNO_2$), as well as other chemicals, were obtained from Kemaus (New South Wales, Australia). Cell culture media and related accessories were procured from Gibco™, including Dulbecco's Modified Eagle Medium (DMEM; Gibco, Thermo Fisher Scientific, Grand Island, NY, USA). The Griess reagent was obtained from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany). Other cell culture reagents, including fetal bovine serum and trypsin, were obtained from HyClone™ (Cytiva, Logan, UT, USA).

2.2. Plant materials

The yellow and red variety cashew leaves were collected from a local plantation in Khiri Rat Nikhom (9°1′48″N 98°57′12″E), Surat Thani Province, Thailand. Botanically, the leaves from positions I (apical) to III (sub-apical) were collected for this study. The samples were procured in October 2024 and no pesticide or insecticide was used. The freshly harvested leaves were washed with running tap water and drained. The leaves were handled carefully to avoid any physical damage. The yellow and red cashew leaves were designed as YCL and RCL, respectively.

2.3. Sample preparation

2.3.1. Wet grinding

The leaves (RCL and YCL) were ground into a fine paste with a hand-held blender (Bosch – ErgoMixx 800 W). The blender blade (food-grade stainless steel) and sample jar were thoroughly cleaned with potable water before use. The samples were homogenized with distilled water at a ratio of 1:0.5 (w/v) using turbo mode until a fine paste was obtained. The resulting paste was designed as WG and stored in an airtight container at −20 °C until further analysis.

2.3.2. Freeze-drying

The washed leaves (RCL and YCL) were gently patted dry and frozen at −20 °C overnight. The frozen leaves were successively freeze-dried (CHRIST Delta 2–24 LSCplus freeze drier) under the pressure of 5 mbar at −50 °C for 3–4 days. The freeze-dried samples were designed as FD, ground into a fine powder, sieved through an 80-mesh sieve, and stored at −20 °C in airtight pouches until further analysis.

2.3.3. Microwave-assisted vacuum drying

The leaves (RCL and YCL) were dried in a microwave-assisted vacuum drier (MAVD) (Marchcool Industry, Bangkok), using a frustum basket characterized by the following dimensions: 26.2 cm height, 21.1 cm inner base radius, and 26.3 cm outer bases. The irradiation time and microwave power were controlled through the central digital control system with a working frequency of 2450 MHz. Around 100 - 120 g of both YCL and RCL were dried at 300 W and 450 W. The dried samples were ground to a fine powder, allowed to pass through an 80-mesh sieve, and stored at −20 °C until further analysis in air-tight pouches.

2.4. Moisture content

For the obtained samples, moisture content was examined using the hot air oven method using the modified procedure from Aravind et al. (2025). Briefly, 3 g of the sample was weighed in a pre-dried aluminum moisture dish and dried in an oven for 1.5 h at 130 °C. After concurrent drying, the final weight was recorded. The difference in the weight was multiplied by 100 to calculate the percentage moisture content in the sample.

2.5. Color analysis

The color measurement of the processed cashew leaves powder was measured using a HunterLab ColorFlex EZ colorimeter with the CIE Lab* color scale (Aravind et al., 2025). The instrument was equipped with standard illuminant C and a 45°/0° geometry. Before analysis, the instrument was calibrated using standard white and black tiles. The reference values were recorded using the standard white tile ($L^* = 94.9$, $a^* = -1.17$, $b^* = 0.88$). Subsequently, a 32 mm sample port (ring) was fixed, and a 64 mm optically clear glass sample cup was used for analysis. The samples were carefully packed and compacted in the sample holder. External light interference was excluded by covering the sample with an opaque black lid. The CIE color coordinates (L*, a*, and b*) were used to calculate hue angle (°H) and chroma (C). The mathematical expressions for these parameters were given in the following equations.

$$^{\circ}\mathrm{H} = \tan^{-1}\left(\frac{b^*}{a^*}\right)$$

$$\mathrm{C} = \sqrt{a^{*2} + b^{*2}}$$

2.6. Extraction

The extraction of bioactive compounds from the prepared samples (solid-liquid extraction) was performed using three different solvents, namely water (Aq), 80% ethanol (EtOH), and acidified ethanol (AE)

with pH ~ 2.2. The acidification was done using 0.1% (v/v) concentrated HCl in 80% ethanol. For comparison, two different extraction techniques were used, namely conventional extraction (CE) and ultrasonic-assisted extraction (UAE). The resultant extract was termed the free polyphenol extract (FPE).

2.6.1. Conventional extraction

Around 1.0 g of the prepared samples was extracted with continuous stirring using the above-mentioned solvents for 3 h at room temperature. The solute to solvent ratio (1:30 w/v) was kept constant for all samples. The mixtures were then centrifuged (Eppendorf Centrifuge 5430 R at 2800 x *g*) at 4 °C for 20 min to remove solid particles. The collected supernatant was stored under refrigerated conditions for further analysis. The residue was used for bound phenolic extraction.

2.6.2. Ultrasound-assisted extraction

Around 1.0 g of the prepared samples was mixed with 30 mL of solvent and extracted using a lab-scale UAE unit (Hielscher UIP1000 hdT generator). The amplitude was set at 60%. The extraction was carried out for two different times, including 5 and 10 min. An ice bath was used to avoid excessive heat generation during sonication. Subsequently, the extracts were centrifuged at 2800 x *g* for 20 min at 4 °C. The collected supernatant was stored under refrigerated conditions for further analysis. The residue was used for bound phenolic extraction.

2.6.3. Bound phenolic extraction

The residue left after FPE extraction was further subjected to bound phenolic extraction according to Sassi et al. (2022). Briefly, one gram of residue was mixed with 10 mL of sodium hydroxide (4 M) solution and extracted for 1 h. The pH was then adjusted to 2.0 using 1 M hydrochloric acid (HCl) and 1 M NaOH. The mixtures were centrifuged at 4 °C for 15 min at 4000 x *g*. Subsequently, hexane was added in a 1:1 (v/v) ratio and mixed vigorously to remove fats and lipids. The hexane layer was discarded. Then, a mixture of ethyl acetate and diethyl ether (1:1, v/v) was added to the aqueous phase and extracted three times, and the organic phases were pooled. The combined extract was evaporated under vacuum using rotary evaporator (Buchi, Vacuum Controller V-700 with Rotavapor R-215). The residue was redissolved in ethanol, stored at −20 °C, and used for phenolic and flavonoid analyses.

2.7. Phenolic content assay

The prepared extracts were analyzed for the phenolic content using the Folin-Ciocalteu (FC) reagent assay (Chakkaravarthi et al., 2025). Briefly, 20 µL of the sample was mixed with 40 µL FC reagent and incubated in the dark for 6 min. Further, 40 µL sodium carbonate and 100 µL distilled water were added. The mixture was incubated for an hour in the dark, and the absorbance was recorded at 765 nm in a microplate reader (Thermo Fisher Scientific, Varioskan LUX 3020–81, 501). Skanlt software 6.1.1 was used for the absorbance computation, and gallic acid was used as the standard reference compound (R^2 = 0.975) in this assay. The results were expressed as mg gallic acid equivalent (GAE)/g.

2.8. Flavonoid content assay

The total flavonoid content was determined using the aluminum chloride assay (Chakkaravarthi et al., 2025). Briefly, a 20 µL sample extract was treated with 40 µL of 5% sodium nitrite and 10% aluminum chloride, with an incubation of 5 and 6 min, respectively. Besides, 1 M sodium hydroxide (100 µL) was added and again incubated for 30 min. All the incubation in this assay was done in the dark. Ultimately, the absorbance was measured at 510 nm. The standard calibration plot was made using quercetin as a standard (R^2 = 0.991), and hence the results were interpreted in terms of mg quercetin equivalent (QE)/g.

2.9. Antioxidant assay

The freshly prepared extracts were tested for their antioxidant activity through the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, according to the modified procedure from Feng et al. (2021). Briefly, a 20 µL sample was mixed with 180 µL methanolic DPPH and incubated for 30 min in the dark. The optical density was measured at 517 nm. Trolox was used as the internal standard. The total antioxidant activity of the samples was expressed in terms of mM Trolox equivalent (TE)/*g* sample (R^2 = 0.966). The inhibitory concentration (IC_{50}) was calculated by extrapolating the regression line from the inhibition plot. The percentage inhibition and the Trolox equivalent antioxidant capacity (TEAC) were calculated according to the following mathematical formulae.

$$\text{Inhibition ratio } (\%) = \frac{\text{Control Abs.} - \text{Sample Abs.}}{\text{Control Abs}} \times 100$$

$$\text{TEAC} = \frac{\text{IC50 of Trolox (ug/mL)}}{\text{IC50 of sample (ug/mL)}}$$

2.10. Cell culture assay

2.10.1. Cell culture

RAW264.7 immortalized mouse macrophage cell lines (cat. no. TIB-71; American Type Culture Collection) was cultured using RPMI 1640 medium supplemented with 10% fetal bovine serum (100 U/mL) and 1% penicillin-streptomycin (Invitrogen, USA). The cells were incubated at 37 °C in a humidified chamber with 5% CO_2.

2.10.2. Cytotoxicity

RAW264.7 cells were seeded (5×10^5 cells) in a 96-well plate and incubated for 24 h with FD-processed samples, extracted with aqueous CE and UAE, at different concentrations (Pansai et al., 2021). After incubation, 10 µL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (5 mg/mL) was added to each well and incubated again for 2 h, at 37 °C. Successively, the supernatant was discarded and 200 µL of dimethyl sulfoxide solution was added to dissolve the formed formazan crystals. The absorbance was measured at 570 nm using a microplate reader.

2.10.3. Nitric oxide (NO) production

RAW264.7 cells were seeded (5×10^5 cells) in a 96-well plate and incubated for 24 h with the test samples at different concentrations (with and without 2 µg/mL lipopolysaccharide treatment) for 24 h (Pansai et al., 2021). After incubation, the nitric oxide production was estimated by collecting 100 µL supernatant and reacting with 100 µL Greiss reagent (Merck, Germany) for 10 min at room temperature. The reaction mixture was measured at 570 nm through a microplate reader.

2.11. Statistical analysis

All analyses were performed in triplicate, and the data were presented as mean ± standard deviation. Statistical analysis was performed using SPSS software (version 27), with a confidence level of 95% ($p < 0.05$). One-way analysis of variance (ANOVA), followed by Duncan's multiple range test, was used to assess statistically significant differences among multiple groups. A Student's *t*-test was employed to compare differences between two independent means.

3. Results and discussion

3.1. Moisture content

Moisture plays a crucial role in determining the quality of products and is influenced by various processing and drying treatments. The current work has compared the wet-ground (WG), freeze-dried (FD), and

microwave-assisted vacuum-dried (MAVD; 300 W and 450 W) cashew leaf samples. The observed moisture content of the freshly produced samples is presented in Supplementary Table 1. The moisture content of fresh cashew leaves, without processing, was reported to be around 80–85%. Particle size reduction through wet grinding significantly increased the moisture content by 6–8% ($p < 0.05$). Additionally, the fresh cashew leaves were freeze-dried to moisture contents of 4.81% for the yellow variety and 5.57% for the red variety. A recent study on green tea leaves reported that the moisture content after freeze-drying was reduced to 2.5% (Li et al., 2024). Moreover, González et al. (2024) reported that functional food powders with a moisture content below 10% exhibit improved storage stability. Accordingly, the cashew leaf powder obtained through freeze-drying showed favorable moisture content (5–6%).

The moisture content from MAVD samples was observed to be around 11–12%. Notably, an increase in the microwave power from 300 W to 450 W lowered the moisture content in a shorter time. Consistent findings were reported by Koç (2020), who demonstrated that increasing microwave power from 180 W to 540 W resulted in a reduction in moisture content from 7.81% to 5.38%. In our current study, the cashew leaves attained a moisture content of 11.69% (YCL) and 11.81% (RCL) in 25 min. Beyond this duration, the leaves developed a slightly burnt surface and the quality was compromised. Meanwhile, drying at 450 W further alleviated the moisture content to 11.09% and 11.15%, respectively, for the yellow and red varieties in 20 min. Nevertheless, the difference in moisture content remained insignificant ($p > 0.05$). Hence, the statistical difference between the sample treatments was significant, and within subsets, it remained insignificant. Overall, the quality and visual appearance of the dried cashew leaves were better in the FD samples compared to the MAVD treatment. Similar findings were reported by Koç (2020), who compared the drying characteristics of red pepper and concluded that freeze-drying had a greater impact on visual appearance than microwave-assisted vacuum drying (MAVD). Likewise, Li et al. (2024) reported that in green tea leaves, freeze-drying better preserved volatile compounds compared to MAVD treatment. Technically, freeze-drying is based on the principle of sublimation, in which water is removed directly from the frozen state under vacuum conditions (typically < 500 Pa) without significantly affecting other biochemical components or altering the microstructure. In contrast, microwave drying relies on electromagnetic waves-induced molecular oscillation and dipole rotation. The resulting heat generation may adversely affect bioactive compounds, particularly under prolonged drying conditions.

3.2. Color

A total of eight samples were analyzed for the color, and the values were expressed in terms of L* (black and whiteness), a* (greenness to redness), b* (blueness to yellowness), C (color intensity), and °H (color shade) in Supplementary Table 2. The obtained results showed that chroma was higher in the yellow variety than in the red variety. Among the different drying conditions, freeze-drying had exerted better color intensity with the value 24.4, and the lowest was observed in the 450 W MAVD-treated samples (10.98). Moreover, increasing the power range in MAVD from 300 W to 450 W lowered the color intensity from 16.0 to 10.98 in the red variety. Coherently, the L* value was lowest (23.55) for 450 W MAVD, very near to darkness. The values clearly showed that the bright green color was well retained, with improved lightness (L* = 54.66) in freeze-drying compared to wet grinding ($p < 0.05$). Similar results were reported by Youssef and Mokhtar (2014), who observed a significant increase in lightness (L*) of purslane leaves after freeze-drying (35.9 to 57.07). This may be attributed to enzymatic activities of polyphenol oxidase and chlorophyllase, as well as structural disruption caused by freeze-drying, which increases surface area and facilitates pigment degradation. In particular, chlorophyllase converts chlorophyll to chlorophyllide by removing the phytol chain, contributing to changes in color (Li et al., 2025). These mechanisms may also explain the color variations observed in the present study. The °H values are mostly between 50° and 90°, indicating the values are comparatively brighter towards yellow. However, the hue values of 450 W MAVD-treated cashew leaves were lower (66.16°) than those of other treatments. Conversely, the freeze-dried samples exerted negative hue angles (−81.86° and −86.0° for the yellow and red varieties, respectively), indicating a shift towards a brighter green color.

The obtained a* values, with positive values indicating redness and negative values indicating greenness, are higher in the red varieties than in the yellow ones. This could mainly be due to the presence of anthocyanins and the drying treatment. The highest a* value was attained from freeze-dried red leaves (−1.59), while the lowest (1.43) was in 450 W MAVD-treated yellow leaves. The drying treatment significantly played a crucial role in influencing the redness. For instance, the intense dark color arose from the intense heat, as in MAVD, contributing to the redness. On the other hand, the b* values are relatively positive in the current study, indicating the yellowness. The yellow variety had the highest b* values, despite the drying treatment. The highest b* values were observed in the freeze-dried yellow leaves (24.16), while the lowest was 10.05 in the 450 W MAVD-treated red variety. Similar results were reported by Huo et al. (2025), who observed increased greenness in leaves, as indicated by more negative a* values. In addition, yellow cotyledons exhibited higher b* values, reflecting greater maturity. Although MAVD samples were processed for a shorter duration, freeze-drying provided better color retention due to its low temperature, oxygen-limited environment, and minimal structural damage compared to MAVD treatment. Hence, the overall outcomes clearly showed that the effect of drying significantly impacted the visual appearance of the prepared samples.

3.3. Free phenolic content

The free phenolic content showed a significant difference ($p < 0.05$) between different treatments and extracted solvents. The overall conventionally extracted phenolic profile is provided in Fig. 1a. Compared to the other dried samples, WG samples showed the least phenolic content ($p < 0.05$). The lower phenolic content in WG samples may be attributed to their higher moisture content, which dilutes free phenolics and limits cell disruption and phenolic release. Increased particle size and moisture levels may also affect pH and promote complexation of phenolics with other macromolecular components in the leaf matrix (Nemli et al., 2024). In addition, higher moisture content may enhance phenolic degradation by activating polyphenol oxidase and peroxidase enzymes (Sui et al., 2023). In the fresh cashew leaf paste, the phenolic content was found to be around 6–7 mg GAE/g when extracted with Aq. The highest free phenolics reported in the WG sample were the AE extracted samples with 16.34 mg GAE/g.

Compared to MAVD treatment, the highest phenolic content was observed in the freeze-dried samples. The yellow and red varieties of the AE extract contained 204.17 mg and 194.6 mg free phenolics, while the Aq extract contained 107.94 mg and 131.76 mg, respectively. Besides, the EtOH extract showed higher free phenolic content, with the highest in the yellow variety (182.73 mg). Moreover, increasing the power from 300 W to 450 W had a slight impact on the phenolic content. For instance, the AE extracted 300 W-treated samples contained 144.79 mg, while 450 W-treated samples contained 132.65 mg. The heat generated during processing may have affected the phenolic profile. Similar results were reported by López-Hernández et al. (2024) in basil leaves, where a reduced phenolic content was observed after 622 W MAVD treatment (38 mg/g) compared to fresh leaves (50 mg/g).

Further, solvent polarity played a crucial role in phenolic extraction, regardless of the different drying methods, with the following order in our current study: AE > EtOH > Aq. A similar trend was reported by Kiani et al. (2023), where moringa leaf extracts showed phenolic contents in the order of 17.1 mg > 16.2 mg > 4.01 mg. In the present study,

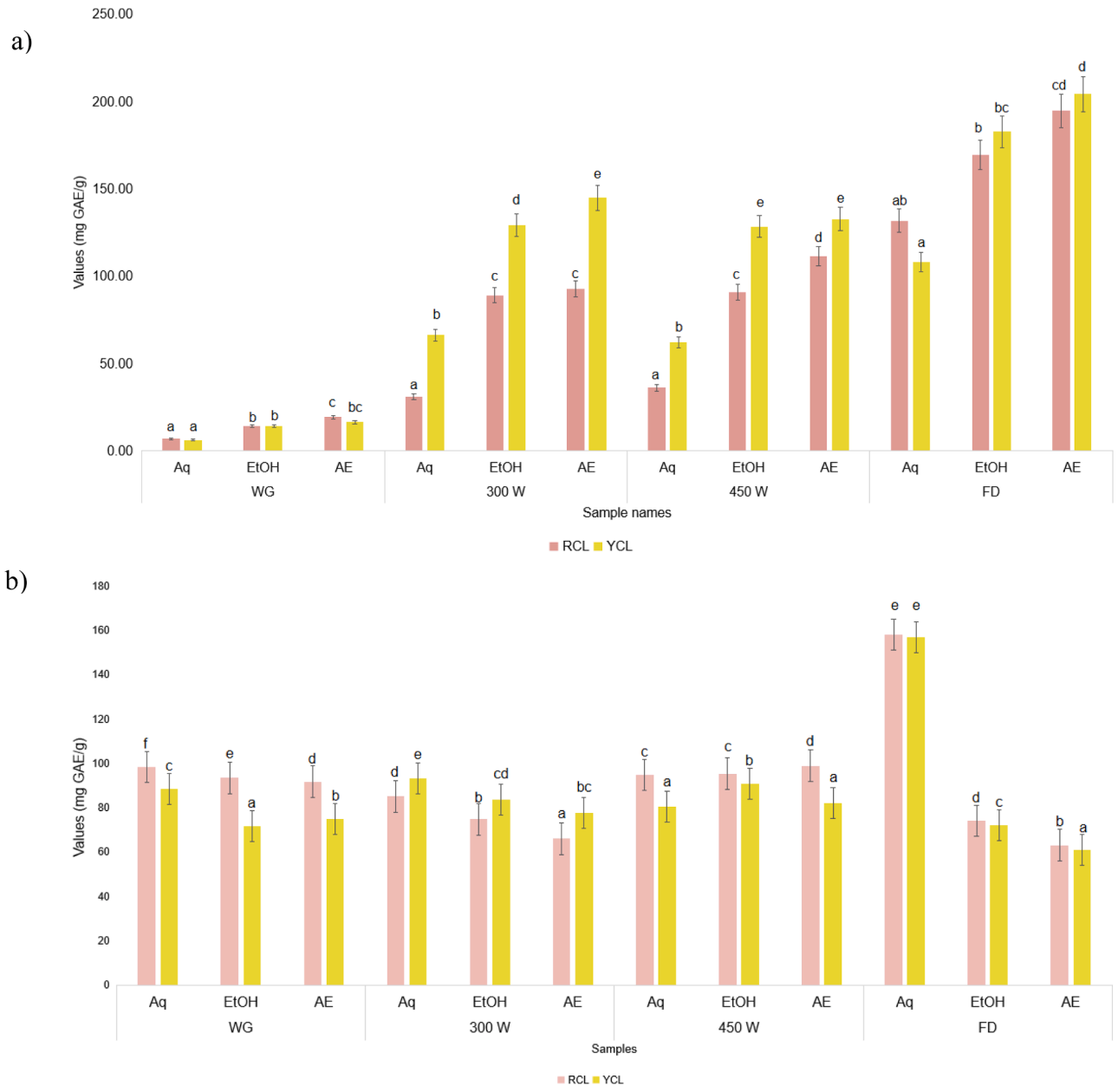


Fig. 1. (a) Free phenolic content and (b) bound phenolic content in conventional cashew leaf extracts. Error bars represent standard deviation of triplicate analyses. Different letters (a–f) indicate statistically significant differences ($p < 0.05$) within the sample groups.RCL and YCL refer to red and yellow cashew leaves, respectively. WG and FD denote wet-ground and freeze-dried samples. "300 W" and "450 W" indicate microwave-assisted vacuum drying (MAVD) power levels. Aq, EtOH, and AE represent aqueous, 80% ethanol, and acidified ethanol (pH ~2.2) solvents, respectively.

ethanol (EtOH) extraction also showed lower efficiency than acidified ethanol (AE), which is consistent with Zin et al. (2021), who reported higher phenolic content in acidified 70% ethanol (90 mg/g) compared to absolute ethanol (45 mg/g) and 70% ethanol (62 mg/g) in noni leaves. However, the free phenolic content obtained from freeze-dried methanolic extraction reported by Sassi et al. (2022) (3.45 mg/g) was lower than that observed in the present study. In contrast, Onuh et al. (2017) reported higher phenolic content in cashew leaves using methanolic extraction (103.92 mg/g), likely due to differences in extraction conditions, sample preparation, and plant variability.

Nevertheless, from Fig. 2a, it was clear that UAE significantly improved the phenolic profile compared to CE. This could be due to the release of adherent polyphenols from the complex leaf matrix, and to cell wall rupture caused by high-frequency sound waves and the consequent microbubble acoustic cavitation effect. Interestingly, the MAVD-treated red variant cashew leaf samples exhibited an increased phenolic profile after 10 min of UAE extraction. The EtOH extraction at 450 W showed 158.78 mg/g in 5 min, yet increased to 178.76 mg/g. In contrast, the freeze-dried yellow variety from AE extract using UAE showed 286.6 mg/g in 5 min, dropping to 239.22 mg/g in 10 min ($p < 0.05$). However, they are significantly higher than CE (204.17 mg/g). Therefore, increasing the time of UAE lowered the phenolic profile. This may be attributed to heat generation during the UAE process, which can affect phenolic stability. Chotphruethipong et al. (2017) reported a higher phenolic content (564.6 mg/g) following UAE treatment compared with that observed in the present study, likely due to differences in extraction conditions and sample characteristics.

a)

11 110 290
mg GAE/g

Treatment	Solvent	Time of extraction (min)	RCL	YCL
WG	Aq	5	a	ab
		10	bc	c
	AE	5	d	d
		10	e	d
	EtOH	5	d	d
		10	d	d
FD	Aq	5	a	a
		10	a	a
	AE	5	c	d
		10	c	c
	EtOH	5	b	c
		10	b	c
300 W	Aq	5	a	a
		10	a	a
	AE	5	c	de
		10	c	de
	EtOH	5	b	d
		10	c	e
450 W	Aq	5	ab	b
		10	a	b
	AE	5	c	cd
		10	de	ef
	EtOH	5	de	cd
		10	f	f

b)

20 50 80
mg GAE/g

Treatment	Solvent	Time of extraction (min)	RCL	YCL
WG	Aq	5	f	g
		10	e	f
	AE	5	b	c
		10	a	d
	EtOH	5	b	a
		10	a	a
FD	Aq	5	j	i
		10	i	h
	AE	5	c	b
		10	b	a
	EtOH	5	e	g
		10	d	f
300	Aq	5	f	f
		10	e	e
	AE	5	c	cd
		10	b	c
	EtOH	5	b	c
		10	a	a
450	Aq	5	f	g
		10	e	fg
	AE	5	d	d
		10	a	bc
	EtOH	5	d	d
		10	c	b

Fig. 2. (a) Free phenolic content and (b) bound phenolic content in ultrasound-assisted cashew leaf extracts. Error bars represent standard deviation of triplicate analyses. Different letters (a–j) indicate statistically significant differences ($p < 0.05$) within the sample groups.RCL and YCL refer to red and yellow cashew leaves, respectively. WG and FD denote wet-ground and freeze-dried samples. "300 W" and "450 W" indicate microwave-assisted vacuum drying (MAVD) power levels. Aq, EtOH, and AE represent aqueous, 80% ethanol, and acidified ethanol (pH ~2.2) solvents, respectively. The numbers 5 and 10 indicate ultrasonic treatment times (5 and 10 min).

3.4. Bound phenolic content

The residue collected from the CE was subjected to alkaline hydrolysis to release bound phenols from the leaf matrix. The bound phenolic content obtained from our present findings is shown in Fig. 1b These are components that are covalently bound to the cell wall. Following hydrolysis, ethyl acetate and diethyl ether extraction solubilize the released phenolics. In our present study, the bound phenolics were higher in CE than in the UAE ($p < 0.01$). For example, the bound phenolic content was found to be 98.35 mg/g in the WG, Aq-extracted red variety, and just 6.73 mg/g in the CE. Overall, around a 13% increase was observed. On the other hand, the 450 W treatment showed better results than the 300 W treatment ($p < 0.05$). In the case of the yellow variety, EtOH showed a higher value of 83.6 mg/g at 300 W, yet increased to 90.64 mg/g at 450 W. Interestingly, the Aq extract possessed a better profile than the other, less polar solvents used in this study. Similar outcomes were reported for *A. mongolicum* extract, in which phenolic compounds were more effectively extracted in an aqueous medium (10.2 mg/g) than in methanol (7.5 mg/g), likely due to differences in solvent polarity and compound solubility.

On the other hand, the UAE extracts showed reduced values (shown in Fig. 2b). For instance, the Aq extracted, freeze-dried yellow variety sample from CE showed the highest bound phenolic content (157.4), while after UAE, the bound phenolics were dropped to 74.6 and 70 mg/g with 5 and 10 min of extraction. A similar pattern was followed between the CE and UAE. This may be attributed to high-frequency ultrasonic waves, which disrupt cell walls and enhance the release of phenolic compounds. Bound phenolics may also be partially released into the solvent during sonication. Consistently, Dadi et al. (2019) reported that ultrasonication of moringa leaves reduced bound phenolics to 11.9 mg/g after 10 min and further to 4 mg/g with longer treatment. These results indicate that UAE conditions strongly influence the conversion of bound to free phenolics, which likely explains the trends observed in the present study.

3.5. Free flavonoid content

Cashew leaves contain abundant flavonoids, especially quercetin, kaempferol, and their conjugates. The free flavonoid content for the prepared samples (CE and UAE) is shown in Figs. 3a and 4a, respectively. In our current study, the free flavonoid content in the CE was reported to be highest in the freeze-dried cum AE extracted yellow variety (251.46 mg QE/g). The lowest was found in the Aq-extracted WG samples of the red variety (13.62 mg/g). The pattern followed similar trends to those of phenolic content. Briefly, freeze-dried samples exhibited better flavonoid content than MAVD and WG, accordingly. Further, AE > EtOH > Aq was the order for the flavonoid profile in terms of the solvent used. Moreover, the yellow variety showed higher flavonoid content than the red variety ($p < 0.05$). Zin et al. (2021) reported that less polar solvents at lower temperatures favor flavonoid extraction. In *Limnophila aromatica*, flavonoid content was higher in 75% ethanol (19.5 mg/g) than in aqueous extract (4.04 mg/g) (Do et al., 2014). However, higher flavonoid levels in aqueous extracts (230.97 mg/g) were reported in water spinach (Roy et al., 2022), indicating that extraction efficiency is strongly influenced by plant matrix and solvent interactions.

The UAE-treated samples showed a slightly lower flavonoid profile than CE. Our current study used a 60% amplitude and observed the highest content in the AE-extracted FD samples (189.8 mg/g), which was lower than that in the CE (251.46 mg/g). Lee and Lee (2023)

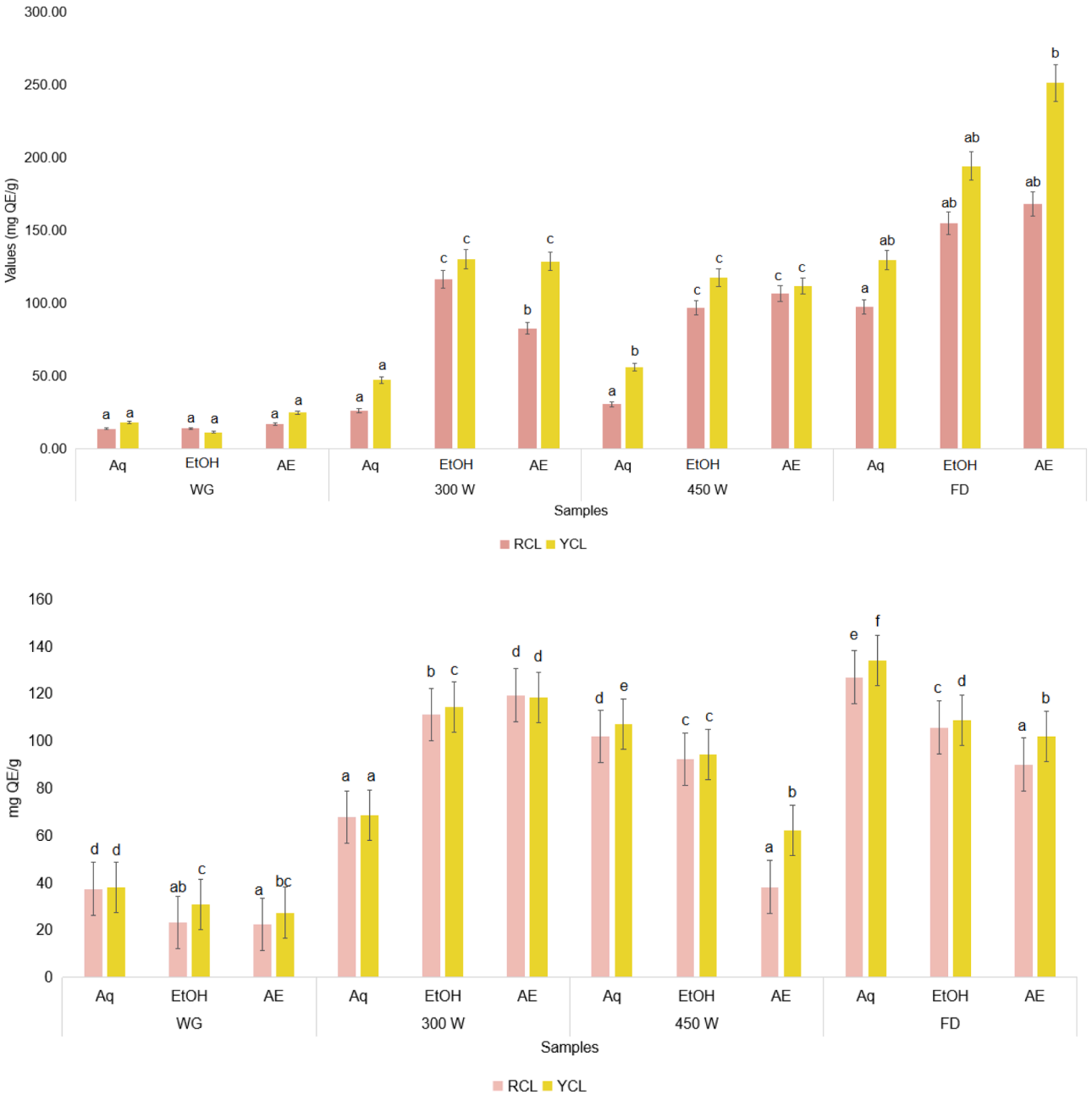
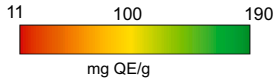


Fig. 3. (a) Free flavonoid content and (b) bound flavonoid content in conventional cashew leaf extracts. Error bars represent standard deviation of triplicate analyses. Different letters (a–f) indicate statistically significant differences ($p < 0.05$) within the sample groups.RCL and YCL refer to red and yellow cashew leaves, respectively. WG and FD denote wet-ground and freeze-dried samples. "300 W" and "450 W" indicate microwave-assisted vacuum drying (MAVD) power levels. Aq, EtOH, and AE represent aqueous, 80% ethanol, and acidified ethanol (pH ~2.2) solvents, respectively.

reported that flavonoid extraction from black soybean decreased from 19.8 to 17.8 mg/g after 7 min of ultrasonication at 60% amplitude. In contrast, Hao et al. (2023) found that UAE significantly enhanced flavonoid extraction from Indian lettuce, with optimal conditions of 60% ethanol, 30 min, and 411.4 W yielding a maximum flavonoid content of 48 mg/g. These contrasting results suggest that UAE efficiency depends strongly on extraction conditions and plant matrix characteristics. However, our current findings identified the 5-min Aq-extracted red variety to possess the lowest flavonoid profile. Although Lee and Lee (2023) reported a reduction in phenolic content after microwave-assisted vacuum drying (MAVD), the present study observed a contrasting trend. Specifically, increasing MAVD power from 300 to 450 W significantly improved flavonoid content ($p < 0.05$), suggesting that process intensity and matrix-specific responses may influence compound retention and release. For instance, 5 min of EtOH extracted red variety at 300 W yielded 67.2 mg/g. At the same time, they increased to 93.1 mg/g at 450 W. Hence, based on the treatment types, the overall flavonoid content improved significantly ($p < 0.05$).

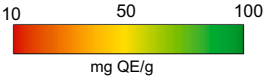
3.6. *Bound flavonoid content*

The results for bound flavonoid content showed a reduced quantity after UAE treatment. This was because the bound flavonoids are released from the cell wall that is damaged during UAE, and hence the values are affected. The values of bound flavonoid content from CE and UAE were depicted in Figs. 3b and 4b, respectively. The CE-based free flavonoid values of the freeze-dried yellow variety extracted with EtOH showed 185.3 and 140.84 mg/g. In contrast, after bound extraction from the



a)

Treatment	Solvent	Time of extraction (min)	RCL	YCL
WG	Aq	5	a	ab
		10	abcd	abc
	AE	5	abc	abcd
		10	cd	abcd
	EtOH	5	abc	abc
		10	d	bcd
FD	Aq	5	a	a
		10	a	a
	AE	5	b	b
		10	b	b
	EtOH	5	b	b
		10	b	b
300	Aq	5	a	a
		10	a	a
	AE	5	b	c
		10	b	c
	EtOH	5	b	c
		10	b	c
450	Aq	5	ab	cd
		10	ab	bc
	AE	5	e	f
		10	cd	a
	EtOH	5	de	e
		10	f	f



b)

Treatment	Solvent	Time of extraction (min)	RCL	YCL
WG	Aq	5	h	i
		10	cd	g
	AE	5	c	i
		10	b	e
	EtOH	5	b	f
		10	a	de
FD	Aq	5	h	h
		10	g	g
	AE	5	d	f
		10	b	e
	EtOH	5	d	a
		10	a	a
300	Aq	5	c	c
		10	a	b
	AE	5	e	h
		10	c	g
	EtOH	5	f	i
		10	c	d
450	Aq	5	b	c
		10	a	b
	AE	5	f	g
		10	cd	cd
	EtOH	5	e	g
		10	de	e

Fig. 4. (a) Free flavonoid content and (b) bound flavonoid content in ultrasound-assisted cashew leaf extracts. Error bars represent standard deviation of triplicate analyses. Different letters (a–i) indicate statistically significant differences ($p < 0.05$) within the sample groups.RCL and YCL refer to red and yellow cashew leaves, respectively. WG and FD denote wet-ground and freeze-dried samples. "300 W" and "450 W" indicate microwave-assisted vacuum drying (MAVD) power levels. Aq, EtOH, and AE represent aqueous, 80% ethanol, and acidified ethanol (pH ~2.2) solvents, respectively. The numbers 5 and 10 indicate ultrasonic treatment times (5 and 10 min).

UAE treatment (5 and 10 min), the values dropped ($p < 0.05$) to 85.98 and 80.52 mg/g, respectively. Overall, the current findings indicate that the Aq extract showed an improved bound flavonoid profile compared to the free flavonoids. Bound phenolics are initially retained within the matrix during aqueous extraction and are subsequently released into the ethyl acetate fraction after hydrolysis, resulting in higher flavonoid recovery in the bound form. In contrast, acidified ethanol (AE) combined with ultrasonication (pH ~2.2) disrupts the cell matrix more effectively, promoting the release of bioactive flavonoids during the free extraction step and leaving fewer compounds available for bound extraction. Additionally, flavonoids are heat-sensitive; thus, increased UAE intensity may raise temperature and promote free radical formation, leading to reduced flavonoid content (Saborirad et al., 2024).

The MAVD samples expressed lower values than FD. For instance, the red variety leaves of CE-based 400 W-treated EtOH were recorded as 92.19 mg/g, which was dropped from 111.23 mg/g in the 300 W treatment. This may be attributed to microwave irradiation, which can partially enhance the release of bound flavonoids from the leaf matrix. However, contrasting effects have been reported in the literature. Shao et al. (2011) observed that increasing microwave power from 200 to 600 W enhanced flavonoid extraction from Perilla frutescens leaves, whereas further increases to 800 W reduced flavonoid levels. Similarly, Xu et al. (2021) reported a 52.14% decrease in flavonoid extraction from Chinese bamboo when microwave power exceeded 480 W. These findings suggest that excessive microwave energy may lead to flavonoid degradation despite initial enhancement of extraction efficiency.

3.7. Total antioxidant capacity

The Trolox equivalent antioxidant capacity (TEAC) is defined as the measure of antioxidant activity. This correlates with the degree of radical scavenging potential of the phenolics present in our cashew leaf extract. Our current TEAC values closely matched those of with the bioactive constituents extracted from CE and UAE. From Table 1, the

Table 1
Trolox equivalent antioxidant capacity of cashew leaf extracts obtained using conventional extraction.

TEAC	Solvent	RCL	YCL
WG	Aq	3.41	2.02
	EtOH	2.69	2.69
	AE	4.03	2.69
300 W	Aq	20.17	17.93
	EtOH	24.65	24.65
	AE	29.13	29.13
450 W	Aq	8.52	15.68
	EtOH	11.43	20.17
	AE	24.65	24.64
FD	Aq	46.38	47.05
	EtOH	69.47	69.46
	AE	80.66	80.65

The values are expressed as μM Trolox equivalent per gram of sample. YCL and RCL indicate yellow and red cashew leaf varieties, respectively. WG = wet grinding; FD = freeze-drying; MAVD = microwave-assisted vacuum drying. Aq, EtOH, and AE refer to the solvents used for conventional extraction, namely water, 80% ethanol, and acidified ethanol, respectively.

TEAC values were recorded as the highest in the freeze-dried samples, with 80.66 and 80.65 μM Trolox equivalent per gram for FD RCL and YCL, respectively. However, no significant difference was observed between YCL and RCL ($p > 0.05$). Due to the lowest phenolic and flavonoid levels, the radical scavenging was less exhibited in WG samples. The lowest values were read in the WG RCL and YCL samples, with the values 3.41 and 2.02 μM/g, respectively. The microwave power significantly influenced the radical-scavenging potential of the prepared samples ($p < 0.05$). A similar outcome was reported by Li et al. (2017), who found that the Trolox equivalent antioxidant capacity (TEAC) of *G. axillaris* fruits significantly decreased when microwave power exceeded 400 W, suggesting possible degradation of antioxidant compounds at higher energy inputs.

The UAE samples showed a higher TEAC value due to enhanced extraction of phenolics and flavonoids, assisted by the ultra-frequency sound waves. The TEAC values of the UAE-extracted samples were tabulated in Table 2. Unlike CE-extracted samples, the UAE-treated samples showed a significant ($p < 0.05$) difference between the yellow and red varieties. In accordance with our TPC and TFC values, the TEAC values were higher in freeze-dried samples, especially in AE-extracted, 5-min treated samples, *i.e.*, 343 and 337 μM/g. The degradation of phenolics and flavonoids with increased UAE treatment also influenced their antioxidative potential.

The results indicate a positive relationship between phenolic concentration and antioxidant activity, suggesting that antioxidant properties are largely dependent on phenolic and flavonoid levels. Sinlapapanya et al. (2022) reported the presence of quercetin, kaempferol, and other flavonoids in young cashew leaves. These compounds can cross the blood–brain barrier and exhibit neuroprotective effects (Ishisaka et al., 2011), including reductions in oxidative stress and inflammatory markers such as IL-6 and IL-1β. They may also modulate neurotransmission, enhance neurogenesis, inhibit monoamine oxidase and acetylcholinesterase activities, and regulate the hypothalamic–pituitary–adrenal axis (Song et al., 2025). Owing to these bioactivities, cashew leaf compounds show potential for use in functional foods and nutraceuticals with possible antidepressant applications.

Table 2
Trolox equivalent antioxidant capacity of cashew leaf extracts obtained using ultrasound-assisted extraction.

Treatment	Solvent	Time (min)	YCL	RCL
WG	Aq	5	219	217
		10	196	199
	AE	5	320	314
		10	297	292
	EtOH	5	278	273
		10	258	252
300 W	Aq	5	199	199
		10	185	183
	AE	5	295	289
		10	272	268
	EtOH	5	256	250
		10	236	233
450 W	Aq	5	173	172
		10	157	151
	AE	5	256	250
		10	236	233
	EtOH	5	220	216
		10	207	200
FD	Aq	5	235	231
		10	218	213
	AE	5	343	337
		10	318	312
	EtOH	5	298	292
		10	276	270

The values are expressed as μM Trolox equivalent per gram of sample. YCL and RCL indicate yellow and red cashew leaf varieties, respectively. WG and FD refer to wet-ground and freeze-dried samples. "300 W" and "400 W" indicate the microwave-assisted vacuum drying (MAVD) power levels. Aq, EtOH, and AE represent the solvents used for conventional extraction, namely water, 80% ethanol, and acidified ethanol, respectively. The numbers 5 and 10 indicate ultrasonic-assisted extraction (UAE) times (5 and 10 min).

3.8. Cell viability

The CE, UAE5, and UAE10 extracts of FD-YCL and RCL were tested for viability using RAW 264.7 cells. The samples were treated at different concentrations (62.5 to 1000 ug/mL). Fig. 5D and E depict the overall viability profile of RAW 264.7 cells in response to different sample treatments. Briefly, the CE extracts supported cell growth, increasing viability above 100%, with reference to the control. The viability remained above 80% at the lowest tested concentration, *i.e.*, 62.5 μg/mL. On the other hand, the UAE5 samples exhibited cytotoxicity, lowering the viability to around 76% at the highest concentration (*i.e.*, 1000 μg/mL). At lower concentrations, the UAE5 still enhanced the cell viability. Compared to UAE5, UAE10 exerted better viability in the cells. Hence, it was clear that, the higher phenolic content in UAE5 significantly affected the cell survival. Between samples, the YCL showed slightly higher viability of the cells, though not significantly ($p < 0.05$). Similar outcomes were reported by Oliveira et al. (2026), where polyphenol-rich stem bark extracts of barbatimão exhibited cytotoxicity at higher concentrations (>125 μg/mL). Likewise, Huang et al. (2026) reported that cell viability of *H. serrata* thallus decreased below 70% at 100 μg/mL, indicating concentration-dependent cytotoxic effects of plant-derived polyphenols. Thus, the cell survival rates rely mainly on the concentration of the extracts used. The higher phenolic or flavonoid content in the extract hindered the cell survival rate significantly.

3.9. Cytotoxicity and inflammatory profile

The survival rate of the treatments was above 70%, and they were evaluated for cytotoxicity and inflammatory testing using the Greiss reagent. The cells were stimulated with the endotoxin lipopolysaccharide (LPS), which promotes nitric oxide (NO) production and activates downstream inflammatory signaling pathways. NO is a key inflammatory mediator and biomarker associated with cellular inflammatory responses. The inflammatory response of the cells with respect to the sample treatment is shown in Fig. 5A - C. Briefly, the extracts elicited a strong, dose-dependent anti-inflammatory response.

The anti-inflammatory properties were directly correlated with cell viability. Although there is no significant difference between YCL and RCL, higher dose exerted better anti-inflammatory properties. However, the higher phenolic content extracted by UAE5 exerted a cytotoxic effect, and thus the anti-inflammatory profile was coherently poor. For instance, the UAE5 from YCL (1000 μg/mL) showed a very poor inhibition (*i.e.*, 56.1%) compared to the CE (99.01%) and UAE10 (99.1%). With a reduction in concentration, the anti-inflammatory action dropped significantly ($p < 0.05$) in CE and UAE samples. The UAE10 from RCL showed the highest NO inhibition (94.9%) with 1000 μg/mL sample treatment. However, the inhibitory percentage dropped to 32.8% after treatment with 62.5 μg/mL. In contrast, the UAE5 from both YCL and RCL samples showed an altered pattern. The inflammatory inhibition response was lower (54.69%) at the highest concentration (1000 μg/mL), while the maximum effect occurred at 12.5 μg/mL, followed by a decline at higher doses. This biphasic response may be due to cytotoxicity or saturation effects at higher concentrations. Similarly, Akhtar et al. (2026) reported a decrease in anti-inflammatory response in *S. marianum* extract from 185% to 80% when the concentration increased from 50 to 1000 μg/mL.

4. Limitations and potential applications

A key limitation in botanical research is the variability of phytochemical composition across geographical regions, driven by soil

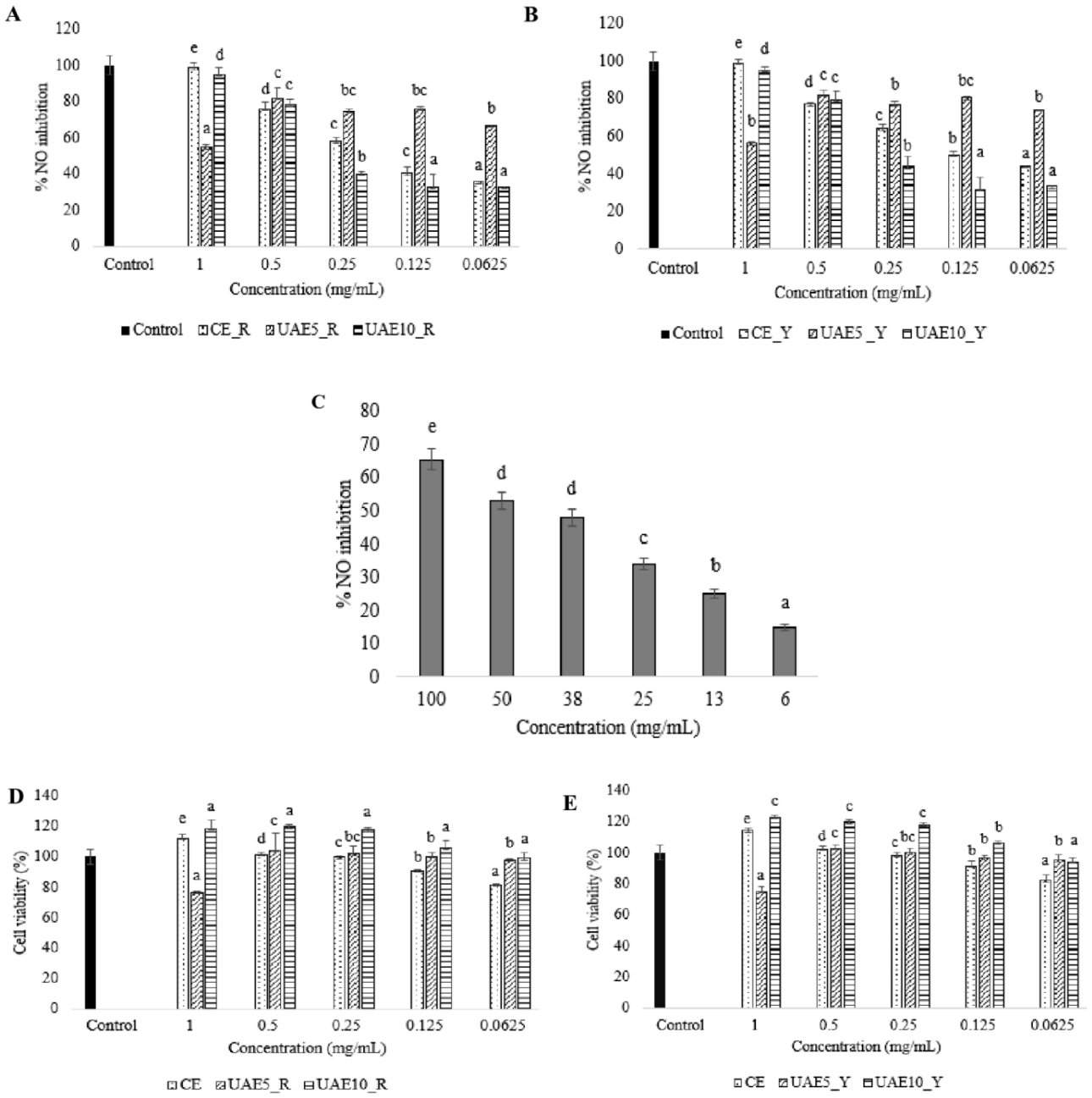


Fig. 5. Effects of freeze-dried red and yellow cashew leaf (RCL and YCL) extracts on RAW 264.7 cell viability and LPS-induced inflammation. (A) % NO inhibition of RCL obtained from different extraction methods. (B) % NO inhibition of YCL obtained from different extraction methods. (C) % NO inhibition of L-nitroarginine (positive control).

Notes: Vehicle control cells were treated with various concentrations of YCL and RCL extracts obtained from conventional extraction (CE) and ultrasound-assisted extraction (UAE) for 5 and 10 min (n = 4). Cells were co-treated with lipopolysaccharide (LPS, 2 μg/mL) and incubated for 24 h. Statistical comparisons among treatments were performed using one-way ANOVA followed by Duncan's multiple range test. Different letters indicate significant differences at $p < 0.05$.

properties, climate, seasonal variation, ecological stressors, climate change, and increasing commercial cultivation, which may introduce additional variability through agronomic and harvesting practices. These factors limit standardization, quality control, and translational applicability. The present study provides preliminary *ex vivo* evidence; thus, further *in vivo* validation is required to confirm neuronal and health-related effects.

The results support the development of flavonoid-rich products using advanced delivery systems such as microencapsulation, as previously reported for moringa leaf flavonoids (Wei et al., 2023). Alternatively, consumption of young fresh cashew leaves may better preserve native bioactive compounds compared to extraction and powder processing, whereas standardized extracts may be more suitable for targeted therapeutic applications. Importantly, cashew leaf polyphenols may exert prebiotic-like effects by modulating gut microbiota, suggesting potential relevance to mood regulation via the gut–brain axis. Future research should focus on dose optimization, behavioral and brain tissue assessments in depression-induced animal models, followed by clinical trials, particularly given their long history of safe human consumption.

5. Conclusion

The current study demonstrated that the different extraction methods (including sample treatment, solvent, and extraction method)

distinctly influenced the yield of total phenolic and flavonoid content between the YCL and RCL varieties. This highlighted the impact of geographical and biochemical factors on bioactive profiles. The ultrasonic-assisted extraction played a crucial role in maximizing the recovery of phenolics and flavonoids, thereby directly influencing the antioxidant activity.

Our current findings underscore the importance of optimizing extraction parameters, particularly for cashew leaf varieties, to fully harness their bioactive potential. Phenolic-rich cashew leaves may interact with the gut microbiota, where polyphenols are converted into bioactive metabolites that influence host health via gut–brain axis modulation, including inflammation and neurotransmission. Accordingly, they have potential applications in functional foods, supplements, and nutraceuticals for health and mood-related benefits. Besides, future *in vivo* experiments and clinical trials should focus on their bioactivity roles. These studies aim to clarify the mechanism by which polyphenols in cashew leaves affect gut-brain function and their psychiatric effects, which contribute to a healthy life.

Funding

This research was supported by Thailand's National Science, Research and Innovation Fund (NSRF) and Prince of Songkla University (Grant No. AGR7001039S-0).

CRediT authorship contribution statement

Mithul Aravind: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Chakkaravarthi Saravanan:** Writing – review & editing, Supervision, Conceptualization. **Lijing Ke:** Writing – review & editing, Supervision, Conceptualization. **Dania Cheaha:** Writing – review & editing, Supervision, Resources, Methodology, Conceptualization. **Teerapat Teetharatkul:** Writing – review & editing, Supervision, Resources, Conceptualization. **Santad Wichienchot:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare no conflict of interest for this research.

Acknowledgments

The authors would like to sincerely thank the Center of Excellence in Functional Foods and Gastronomy, Faculty of Agro-Industry, Prince of Songkla University - Hat Yai campus for the resources and facilities support. This research was funded by the National Science, Research and Innovation Fund (NSRF) and Prince of Songkla University (Grant No. AGR7001039S-0).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.focha.2026.101328.

Data availability

Data will be made available on request.

References

Akhtar, M. T., Saadia, M., & Irfan, M. I. (2026). *Silybum marianum* extract as a next-generation multifunctional therapeutic: Potent antioxidant, antidiabetic, antimicrobial, anti-inflammatory, and anti-biofilm activities validated by phytochemical profiling and molecular docking. *3 Biotech, 16*, 26. https://doi.org/10.1007/s13205-025-04636-4

Aravind, S. M., Mattshe, R. K. S., Prasanna, P., Saran, S. P., Dharanesh, D., Kumar, S. R., & Kishore, A. (2025). Valorization and characterization of red banana (*Musa acuminata*) peel powder for the development of functional cookies. *Food and Humanity, 5*, Article 100683. https://doi.org/10.1016/j.foohum.2025.100683

Bolat, E., Saritas, S., Duman, H., Eker, F., Akdaşçi, E., Karav, S., & Witkowska, A. M. (2024). Polyphenols: Secondary metabolites with a biological impression. *Nutrients, 16*(15), 2550. https://doi.org/10.3390/nu16152550

Chakkaravarthi, S., Sirinupong, T., Tampanna, N., Aravind, S. M., & Wichienchot, S. (2025). *In vitro* colonic fermentation of pigmented rice: Gut microbiome bioconversion of dietary fiber and polyphenols. *Food Research International, 64*, Article 105757. https://doi.org/10.1016/j.fbio.2024.105757

Chotphruethipong, L., Benjakul, S., & Kijroongrojana, K. (2017). Optimization of extraction of antioxidative phenolic compounds from cashew (*Anacardium occidentale* L.) leaves using response surface methodology. *Journal of Food Biochemistry, 41*(5), Article e12379. https://doi.org/10.1111/jfbc.12379

Dadi, D. W., Emire, S. A., Hagos, A. D., & Eun, J. B. (2019). Effect of ultrasound-assisted extraction of *Moringa stenopetala* leaves on bioactive compounds and their antioxidant activity. *Food Technology and Biotechnology, 57*(1), 77. https://doi.org/10.17113/ftb.57.01.19.5877

Do, Q. D., Angkawijaya, A. E., Tran-Nguyen, P. L., Huynh, L. H., Soetaredjo, F. E., Ismadji, S., & Ju, Y. (2014). Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. *Journal of Food & Drug Analysis, 22*(3), 296. https://doi.org/10.1016/j.jfda.2013.11.001

Feng, L., Tang, N., Liu, R., Nie, R., Guo, Y., Liu, R., & Chang, M. (2021). Effects of different processing methods on bioactive substances and antioxidation properties of *Lycium barbarum* (goji berry) from China. *Food Bioscience, 42*, Article 101048. https://doi.org/10.1016/j.fbio.2021.101048

Galletta, B., Mondello, F., Davi, F., Vinci, R. L., Cacciola, F., Mondello, L., Nostro, A., Miceli, N., & Taviano, M. F. (2026). *Erucastrum virgatum* subsp. *Virgatum* (Brassicaceae) endemic to Sicily (Italy): Biological properties and phenolic content of leaf extracts. *Chemistry & Biodiversity, 23*(3). https://doi.org/10.1002/cbdv.202503466

González, N., Pacheco, C., Grafia, A. L., Razuc, M. F., Acebal, C. C., & Lopex, O. V. (2024). Food functional powders with redox capacity and antioxidant properties obtained from food losses and waste of olive oil industry. *Processes, 12*(12), 2666. https://doi.org/10.3390/pr12122666

Hao, J., Wang, Z., Jia, Y., Sun, L., Fu, Z., Zhao, M., Li, Y., Yuan, N., Cong, B., Zhao, L., & Ge, G. (2023). Optimization of ultrasonic-assisted extraction of flavonoids from *Lactuca indica* L. cv. Mengzao and their antioxidant properties. *Frontiers in Nutrition, 10*, Article 1065662. https://doi.org/10.3389/fnut.2023.1065662

Huang, Y., Li, X., Dai, L., Cheng, M., Zhao, L., Shen, Y., Xie, J., & Luo, X. (2026). Antioxidant, anti-inflammatory, and chemical composition analysis of *in vitro Huperzia serrata* thallus and wild *Huperzia serrata*. *Molecules, 31*(1), 195. https://doi.org/10.3390/molecules31010195

Huo, H., Bao, H., Li, J., & Li, H. (2025). Optimization and antioxidant evaluation of clean and efficient recovery of polyphenols from *Phellodendron amurense* waste leaves via ultrasound-assisted extraction. *Ultrasonics Sonochemistry, 120*, Article 107512. https://doi.org/10.1016/j.ultsonch.2025.107512

Ishisaka, A., Ichikawa, S., Sakakibara, H., Piskula, M. K., Nakamura, T., Kato, Y., Ito, M., Miyamoto, K. I., et al. (2011). Accumulation of orally administered quercetin in brain tissue and its antioxidative effects in rats. *Free Radical Biology & Medicine, 51*(7), 1329–1336. https://doi.org/10.1016/j.freeradbiomed.2011.06.017

Kiani, H. S., Ahmad, W., Nawaz, S., Farah, M. A., & Ali, A. (2023). Optimized extraction of polyphenols from unconventional edible plants: LC-MS/MS profiling of polyphenols, biological functions, molecular docking, and pharmacokinetics study. *Molecules, 28*(18), 6703. https://doi.org/10.3390/molecules28186703

Koç, G.Ç. (2020). Effect of different drying techniques and microwave finish drying on the powder properties of red pepper powder (*Capsicum annuum* L.). *Journal of Food Science & Technology, 57*(12), 4576. https://doi.org/10.1007/s13197-020-04496-1

Lee, M., & Lee, K. (2023). Effect of ultrasound and microwave treatment on volatile compounds, total polyphenols, total flavonoids, and isoflavones in soymilk processed with black soybean (*Glycine* max (L.) Merr.). *Ultrasonics Sonochemistry, 99*, Article 106579. https://doi.org/10.1016/j.ultsonch.2023.106579

Li, Y., Li, S., Lin, S., Zhang, J., Zhao, C., & Li, H. (2017). Microwave-assisted extraction of natural antioxidants from the exotic *Gordonia axillaris* fruit: Optimization and identification of phenolic compounds. *Molecules, 22*(9), 1481. https://doi.org/10.3390/molecules22091481

Li, N., Yao, Z., Ning, J., Sun, L., Lin, Q., Zhu, X., Li, C., Zheng, X., & Jin, J. (2024). Comparison of different drying technologies for green tea: Changes in color, non-volatile and volatile compounds. *Food Chemistry X, 24*, Article 101935. https://doi.org/10.1016/j.fochx.2024.101935

Li, W., Liu, J., Hu, T., Hou, Y., Ma, Z., Feng, T., Guo, Z., Mao, J., & Chen, B. (2025). Genome-wide survey of chlorophyllase (CLH) gene family in seven Rosaceae and functional characterization of MdCLH1 in apple (*Malus domestica*) leaf photosynthesis. *Plant Physiology & Biochemistry, 219*, Article 109387. https://doi.org/10.1016/j.plaphy.2024.109387

López-Hernández, M., Montealegre, M.Á., Criollo, J., Váquiro, H. A., & Sandoval-Aldana, A. (2024). Quality parameters and modeling of microwave drying kinetics of basil 'Nufar' (*Ocimum basilicum* L.) leaves. *Agricultural Engineering, 6*(3), 2417. https://doi.org/10.3390/agriengineering6030141

Muscolo, A., Mariateresa, O., Guilio, T., & Mariateresa, R. (2024). Oxidative stress: Role of antioxidant phytochemicals in prevention and treatment of diseases. *International Journal of Molecular Sciences, 25*(6), 3264. https://doi.org/10.3390/ijms25063264

Nemli, E., Ozkan, G., Subasi, B. G., Cavdar, H., Lorenzo, J. M., Zhao, C., & Capanoglu, E. (2024). Interactions between proteins and phenolics: Effects of food processing on

content and digestibility of phenolic compounds. *Journal of the Science of Food & Agriculture, 104*, 2535. https://doi.org/10.1002/jsfa.13275

Oliveira, N. N., Mothe, C. G., Mothe, M. G., & Oliveira, L. G. (2020). Cashew nut and cashew apple: A scientific and technological monitoring worldwide review. *Journal of Food Science & Technology, 57*, 12–21. https://doi.org/10.1007/s13197-019-04051-7

Oliveira, C. N. P., Peixoto, T. G., Teixeira, L. G. M. L., Pessao, S. B. A., Pedrosa, N. M., Xavier, V. F., Vieira, P. M. A., Soares, C. D. V., et al. (2026). Optimization of *Stryphnodendron adstringens* (Barbatimão) extraction: Chemical evaluation, cytotoxicity, antioxidant and anti-inflammatory activities. *Molecules, 31*(2), 224. https://doi.org/10.3390/molecules31020224

Onuh, J. O., Idoko, G., Yusufu, P., & Onuh, F. (2017). Comparative studies of the phytochemical, antioxidant and antimicrobial properties of cashew leaf, bark and fruits extracts. *American Journal of Food & Nutrition, 5*(4), 115. http://pubs.sciepub.com/ajfn/5/4/1.

Pansai, N., Wichienchot, S., Raungrut, P., & Takahashi, C. Y. (2021). Inflammatory response of RAW264.7 macrophage cells treated with dragon fruit oligosaccharide on lipopolysaccharide-induced inflammation. *Food Science & Technology Research, 27*(1), 111–119. https://doi.org/10.3136/fstr.27.111

Roy, S., Zilani, N. H., Alrashada, Y. N., Islam, M. M., Akhe, F. K., Uddin, S. J., & Sarower, M. G. (2022). Profiling of bioactive compounds and antioxidant activity of aquatic weed *Ipomoea aquatica*. *Aquaculture Fish & Fisheries, 2*(5), 425. https://doi.org/10.1002/aff2.56

Saborirad, S., Baghaei, H., & Hashemi, M. H. (2024). Optimizing the ultrasonic extraction of polyphenols from mango peel and investigating the characteristics, antioxidant activity and storage stability of extract nanocapsules in maltodextrin/whey protein isolate. *Ultrasonics Sonochemistry, 103*, Article 106778. https://doi.org/10.1016/j.ultsonch.2024.106778

Sassi, A., Normah, H., Khattak, M. M. A. K., & Hanapi, M. J. (2022). Analysis of phenolic profile, total phenolic content and antioxidant activity in *A. occidentale* leaves. *Food Research, 6*(1), 20–26.

Sławińska, N., & Olas, B. (2024). Current state of knowledge about thermal processing of edible seeds: Special emphasis on their bioactive constituents and antioxidant activity. *Food Chemistry, 458*, Article 140526. https://doi.org/10.1016/j.foodchem.2024.140526

Shao, P., He, J., Sun, P., & Zhao, P. (2011). Analysis of conditions for microwave-assisted extraction of total water-soluble flavonoids from *Perilla frutescens* leaves. *Journal of Food Science & Technology, 49*(1), 66. https://doi.org/10.1007/s13197-011-0265-8

Shi, L., Zhao, W., Yang, Z., Subbiah, V., & Suleria, H. A. R. (2022). Extraction and characterization of phenolic compounds and their potential antioxidant activities. *Environmental Science & Pollution Research, 29*(54), 81112–81129. https://doi.org/10.1007/s11356-022-23337-6

Sinlapapanya, P., Sumpavapol, P., Nirmal, N., Zhang, B., Hong, H., & Benjakul, S. (2022). Ethanolic cashew leaf extract: Antimicrobial activity, mode of action, and retardation of spoilage bacteria in refrigerated Nile tilapia slices. *Foods, 11*(21), 3461. https://doi.org/10.3390/foods11213461

Song, Z., Wu, Y., Luo, L., Hu, Q., Wu, S., Wu, M., & Zhang, G. (2025). Mechanistic insights into the anti-depressant effect of quercetin: An integrated bibliometrics, bioinformatics, and animal experimentation. *Frontiers in Nutrition, 12*, Article 1612746. https://doi.org/10.3389/fnut.2025.1612746

Sui, X., Meng, Z., Dong, T., Fan, X., & Wang, Q. (2023). Enzymatic browning and polyphenol oxidase control strategies. *Current Opinion in Biotechnology, 81*, Article 102921. https://doi.org/10.1016/j.copbio.2023.102921

Wei, P., Zhang, Y., Wang, Y., Dong, J., Lin, Z., Li, W., Liu, L., et al. (2023). Efficient extraction and excellent activity of flavonoid from *Moringa oleifera* leaves and its microencapsulation. *LWT, 184*, Article 115021. https://doi.org/10.1016/j.lwt.2023.115021

Xu, J., Wu, J., Qi, J., Li, J., Liu, Y., Miao, Z., Qiu, G., & Jia, W. (2021). Microwave-assisted extraction of flavonoids from *Phyllostachys heterocycla* leaves: Optimization, mechanism, and antioxidant activity *in vitro*. *BioResources, 16*(4), 8060–8081. https://doi.org/10.15376/biores.16.4.8060-8081

Youssef, K. M., & Mokhtar, S. M. (2014). Effect of drying methods on antioxidant capacity, color and phytochemicals of *Portulaca oleracea* L. leaves. *Nutrition & Food Science, 4*(6), Article 1000322. https://doi.org/10.4172/2155-9600.1000322

Zin, Z. M., Mohamad, N., Hui, C. K., Majid, N. I., & Zainol, M. K. (2021). Effect of acidified ethanol on antioxidant properties of *Morinda citrifolia* leaf extract and its catechin derivatives. *Current Research in Nutrition & Food Science, 9*(1), 172. https://doi.org/10.12944/CRNFSJ.9.1.17