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Extraction of *Gnetum gnemon* L. (Melinjo) Leaves Using Deep Eutectic and Conventional Solvents: Phytochemical Contents, Antioxidant Capacity, and Toxicity Assessment

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Abstract

Gnetum gnemon L. (Melinjo) is widely recognised for its rich bioactive compounds with potential health benefits. This study evaluates the effectiveness of deep eutectic solvents (DESs) and conventional solvents using ultrasound-assisted extraction on the extraction efficiency, antioxidant activity, and toxicity of *G. gnemon* leaf extracts (GGLE). Four DESs, namely choline chloride-lactic acid (ChCl-LA), choline chloride-glycerol (ChCl-Gly), betaine-lactic acid (Bet-LA), and L-proline-citric acid (LP-CA), were compared with conventional solvents, namely water, methanol, and ethanol. Extraction performance was evaluated based on crude extract yield (CEY), phytochemical content through chlorogenic acid content (CAC), total phenolic (TP), and total flavonoid (TF), as well as antioxidant activity assessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and ferric reducing antioxidant power (FRAP) assays. Toxicity was assessed using the brine shrimp lethality assay (BSLA). Among all solvents, ChCl-LA yielded the highest CEY (20.23 ± 0.68%), CAC (13.96 ± 0.31 µg/g), TP (92.54 ± 0.74 mg GAE/g), DPPH (84.95 ± 1.49%), ABTS (91.25 ± 0.34%), and FRAP (481.14 ± 2.31 mg TE/g) ($p < 0.05$), while exhibiting no toxicity with a high LC_{50} value (6,339.43 µg/mL). Nonetheless, ethanol yielded the highest TF (2.98 ± 0.47 mg QE/g), followed by ChCl-Gly (2.45 ± 0.27 mg QE/g), although non-significant ($p > 0.05$), with ChCl-LA (2.14 ± 0.27 mg QE/g) placed as the third highest. Overall, DESs, particularly ChCl-LA, demonstrated promising extraction efficiency and non-toxicity compared to conventional solvents, supporting their potential as greener alternatives for extracting bioactive compounds from *G. gnemon* leaves.

Keywords: *Gnetum gnemon*, Deep eutectic solvents, Conventional solvents, Ultrasound-assisted extraction, Chlorogenic acid, Phenolics, Flavonoids, Antioxidant, Toxicity

Introduction

Extracting bioactive compounds from plants is essential for developing functional foods and nutraceutical products. However, conventional extraction methods often rely on organic solvents that

are toxic, flammable, and environmentally harmful due to their non-biodegradable nature [1]. To address these concerns, green extraction techniques have been explored to reduce environmental impact without compromising efficiency [2]. Among these, deep eutectic solvents (DESs) have emerged as promising green alternatives owing to their biodegradability, low toxicity, cost-effectiveness, and capacity to enhance the release of phenolics, flavonoids, and other phytochemicals through strong hydrogen bonding and van der Waals interactions [3-5]. Composed of inexpensive and renewable materials such as glycerol and choline chloride, DESs are increasingly studied for natural product extraction as sustainable substitutes for conventional solvents [6].

Complementing the use of green solvents, ultrasound-assisted extraction (UAE) has also gained attention for enhancing the yield and extraction efficiency of bioactive compounds from plant materials. This technique uses ultrasonic waves to improve mass transfer and generate cavitation bubbles that disrupt cell walls, thereby reducing processing time and increasing extraction rates [7]. When combined with green solvents, UAE can further reduce solvent consumption and energy usage [8,9]. The combination of DESs and UAE has shown promising results in extracting valuable bioactive compounds from various plants such as *Moringa oleifera*, *Rosa canina*, and *Baphicacanthus cusia* [10-12]. However, there is limited research applying this technique to extract bioactive compounds from *Gnetum gnemon* L. (melinjo). Its leaves are rich in beneficial compounds such as flavonoids, tannins, polyphenols, and resveratrol, which are known for their strong antioxidant properties [13,14]. Extracting these compounds using a combination of DESs and UAE offers a more sustainable strategy for harnessing their health benefits.

This study aims to compare the extraction efficiency, which includes crude extract yield (CEY), chlorogenic acid content (CAC), total phenolic (TP), and total flavonoid (TF), the bioactivity measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and ferric reducing antioxidant power (FRAP), and the safety assessed using the brine shrimp lethality assay (BSLA), of *G. gnemon* leaf extracts (GGLE) obtained using various deep eutectic solvents (DESs) and conventional solvents, both assisted by ultrasonication. Through this comparison, the study seeks to identify the most effective and environmentally responsible method for maximising the extraction potential of *G. gnemon* leaves, with potential applications in sustainable functional foods and nutraceutical products.

Materials and methods

Chemicals, reagents, and standards

All solvents and reagents used were of analytical grade. Chlorogenic acid standard (≥ 95%), gallic acid (≥ 98%), quercetin (≥ 95%), aluminium chloride hexahydrate (≥ 99%), sodium carbonate (≥ 99.5%), DPPH reagent (≥ 97%), ABTS reagent (≥ 98%), potassium persulfate (≥ 99%), ferric chloride (≥ 98%), 2,4,6-tripyridyl-s-triazine (TPTZ), sodium acetate (≥ 99%), and hydrochloric acid (HCl, 37%) were used, together with the Folin-Ciocalteu reagent, ethanol, methanol, glycerol, lactic acid, betaine, L-proline, citric acid, and choline chloride (all ≥ 99% purity for solvents). All chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA) or Merck (Darmstadt, Germany) unless otherwise specified.

Sample collection and preparation

Fresh *G. gnemon* leaves were collected from the Faculty of Food Science and Nutrition, Universiti Malaysia Sabah. The leaves were thoroughly rinsed with tap water to remove dirt and surface impurities, then gently blotted dry using tissue paper. To ensure uniformity, the leaves were sorted by size and cut into pieces measuring 6.5 cm in width and 4.5 cm in length, with a thickness of 0.1 mm. The leaves were subsequently dried in a microwave oven (Samsung, Kuala Lumpur, Malaysia) at 180 W for 20 min. The dried leaves (DGGL) were then ground to a fine powder using an electric blender (Elba, Borso del Grappa, Italy) and stored in plastic containers at room temperature for subsequent analysis.

Preparation of deep eutectic solvents

DESs were prepared by mixing hydrogen bond acceptors (HBAs) with hydrogen bond donors (HBDs). Four different DES formulations were developed using choline chloride, betaine, and L-proline as HBAs, and lactic acid, glycerol, and citric acid as HBDs, in specific molar ratios as listed in **Table 1**. The mixtures were

prepared through a modified heating and stirring method, based on the protocol described by Wang *et al*. [15]. A small amount of deionised water was added to adjust the viscosity of the DESs. Each mixture was heated to 70 °C in a beaker with continuous stirring until a clear solution was obtained. The resulting DESs were kept at room temperature for further analysis.

Table 1 Formulations and molar ratios of DESs**.**

DES formulation	DES code	Molar ratio (HBA:HBD)	Reference
Choline chloride-lactic acid	ChCl-LA	1:2	[16]
Choline chloride-glycerol	ChCl-Gly	1:1	[17]
Betaine-lactic acid	Bet-LA	1:1	[18]
L-proline-citric acid	LP-CA	1:1	[19]

Crude extract yield

The prepared DESs and conventional solvents, namely ethanol, methanol, and water, were used for the extraction process. The protocol described by Zulkifli *et al*. [20] was followed with minor modifications to obtain the CEY from GGLE. A sample-to-liquid ratio of 1:30 g/mL was used, consisting of 5 g of DGGL and 150 mL of solvent. The mixture underwent UAE using a sonicator (QSonica, Newtown, CT, USA) operating at 60% amplitude for 10 min. After extraction, the mixture was filtered through filter paper to remove solid residues. Excess solvent was evaporated using a rotary evaporator (Heidolph, Schwabach, Germany) at 50 °C to concentrate the extract, followed by drying in a convection oven (Binder, Neckarsulm, Germany) for 24 h at 50 °C. The CEY was calculated using Eq. (1).

$$\text{CEY (\%)} = \frac{\text{Dried mass of crude extract (g)}}{\text{Mass of raw material (g)}} \times 100 \quad (1)$$

Phytochemical content

Using high-performance liquid chromatography (HPLC), the quantification of CAC was performed based on the method described by Saini *et al*. [21] with slight modifications. A chlorogenic acid standard (1 mg/mL) diluted in methanol was used as the standard reference. The analysis was conducted using an Agilent 1100 system (Agilent Technologies, Santa Clara, CA, USA) equipped with an InertSustain C18 column (5 µm, 150 × 4.6 mm^2) and an ultraviolet-visible (UV-Vis) detector. The mobile phases consisted of (A) 0.1% acetic acid in ultrapure water and (B) acetonitrile, operated under isocratic elution. The total run time was 20 min, with the column temperature set at 30 °C and the flow rate maintained at 0.8 mL/min. A 20 µL sample was injected into the system, and detection was carried out at a wavelength of 325 nm. Before injection, each sample was filtered through a 0.22 µm syringe filter. The CAC of GGLE was determined using Eq. (2).

$$\text{CAC (µg/g)} = \frac{\text{Mass of chlorogenic acid (µg)}}{\text{Dried mass of CEY (g)}} \quad (2)$$

The TP and TF of GGLE were determined using the Folin–Ciocalteu method described by Jinin *et al*. [22] and the aluminium chloride colorimetric method described by Stephenus *et al*. [23], respectively, with minor alterations. For TP, absorbance was measured at 765 nm using a UV-Vis spectrophotometer (PerkinElmer, Waltham, MA, USA) with gallic acid as the standard reference. For TF, absorbance was measured at 430 nm with quercetin as the standard reference. Results were expressed as mg gallic acid equivalents per gram (mg GAE/g) of extract for TP and mg quercetin equivalents per gram (mg QE/g) of extract for TF, as shown in Eq. (3).

$$\text{Standard equivalent concentration (mg/g extract)} = \frac{c \times V}{m} \quad (3)$$

where c is the concentration of crude extract (mg/mL) obtained from the standard curve, m is the mass of sample (mg), and V is the volume of sample (mL).

Antioxidant activity

The antioxidant activity of GGLE was evaluated using DPPH and ABTS radical scavenging assays, based on the methods of Rushdy *et al*. [24] and Hussen and Endalew [25], respectively, with minor

modifications. For the DPPH assay, absorbance was measured at 517 nm using a UV-Vis spectrophotometer with ascorbic acid and a blank DPPH solution as the positive and negative controls, respectively. For the ABTS assay, absorbance was recorded at 734 nm with Trolox and a blank ABTS solution as the positive and negative controls, respectively. The radical scavenging activities of GGLE in both assays were calculated using Eq. (4).

$$\text{Inhibition activity (\%)} = \frac{\text{Abs}_c - \text{Abs}_s}{\text{Abs}_c} \times 100 \quad (4)$$

where Abs_c is the control of the absorbance and Abs_s is the sample of the absorbance.

The FRAP assay was conducted based on the procedure reported by Hengpratom *et al*. [26] with slight alterations. The FRAP solution was prepared by mixing sodium acetate buffer, TPTZ in hydrochloric acid, and ferric chloride at a 10:1:1 ratio. Absorbance was measured at 593 nm, and Trolox was used as the standard reference. Results were expressed as mg Trolox equivalents per gram (mg TE/g) of extract, calculated using Eq. (3).

Brine shrimp lethality assay

The BSLA protocol was carried out based on the method described by Benjamin *et al*. [27] with slight adjustments. Eggs of brine shrimp (*Artemia salina*) were hatched in an aerated aquarium filled with seawater for 48 h. After hatching, the nauplii were collected from a well-lit area, ensuring they were free from eggshells, and subsequently used for the assay. Using a micropipette and glass capillary, a total of 100 nauplii were transferred into a petri dish containing 20 mL of seawater. For each petri dish, 1 mL of the diluted GGLE (1 mg/mL) was mixed with 20 mL of seawater containing brine shrimp larvae and left for 24 h at room temperature under light. The remaining larvae were then counted using a magnifying glass. Potassium dichromate was used as the positive control. For GGLE, various concentrations (100, 300, 500, and 1,000 ppm) were tested. The percentage of mortality was calculated using Eq. (5).

$$\text{Mortality (\%)} = \frac{\text{Number of dead nauplii}}{\text{Total number of nauplii}} \times 100 \quad (5)$$

Statistical analysis

All experimental data were collected in triplicate and are presented as mean ± standard deviation (SD). Statistical analysis was performed using IBM SPSS Statistics (Version 28). Analysis of variance (ANOVA) was applied as the data met the required assumptions, including homogeneity of variance and normality, which were tested using Levene's test and Shapiro–Wilk test, respectively. ANOVA was followed by Tukey's HSD post hoc test to identify significant differences, indicated by different letters among the samples. The significance level was set at $p < 0.05$.

Results and discussion

Assessment of CEY and CAC

Figure 1 shows the effects of different solvent systems on the CEY and CAC extracted from DGGL. For CEY, the solvents were ranked in descending order as follows: ChCl-LA > water > LP-CA > Bet-LA > ChCl-Gly > methanol > ethanol. Among all tested solvents, ChCl-LA demonstrated the highest extraction efficiency with a CEY of 20.23 ± 0.68%, followed by water (19.60 ± 0.56%), LP-CA (18.34 ± 0.29%), Bet-LA (17.83 ± 0.34%), and ChCl-Gly (14.18 ± 0.70%). In contrast, conventional solvents such as methanol and ethanol produced lower yields of 7.28 ± 0.59% and 4.12 ± 0.50%, respectively. This superior performance of ChCl-LA highlights its excellent extraction capability. Similar trends were observed in extracts from *Mangifera pajang* and *Peperomia pellucida* [16,28]. This efficiency is largely attributed to the strong hydrogen-bonding capacity of ChCl-LA, which enhances the solubilisation and release of bioactive constituents. In addition, the intrinsic properties of DESs, including tunable polarity, low volatility, and high solvation power, further contribute to their extraction performance [29].

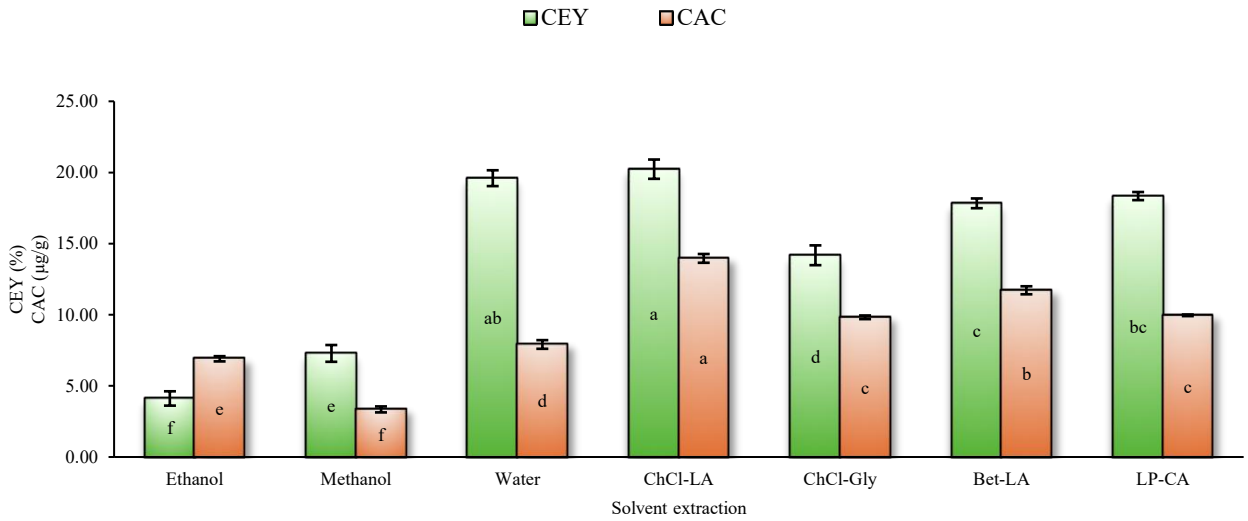

Figure 1 CEY and CAC of GGLE extracted using different types of solvents. Data are presented as mean ± SD (n = 3). Different letters within the bars indicate significant differences ($p < 0.05$).

A similar trend was observed for CAC, with solvents ranked in descending order as follows: ChCl-LA > Bet-LA > LP-CA > ChCl-Gly > water > ethanol > methanol. ChCl-LA yielded the highest CAC of 13.96 ± 0.31 µg/g, followed by Bet-LA (11.72 ± 0.28 µg/g), LP-CA (9.96 ± 0.06 µg/g), and ChCl-Gly (9.82 ± 0.13 µg/g). Conventional solvents extracted lower amounts of CAC, with water yielding 7.91 ± 0.31 µg/g, ethanol 6.90 ± 0.18 µg/g, and methanol the lowest at 3.35 ± 0.21 µg/g. The outstanding performance of ChCl-LA in extracting chlorogenic acid is consistent with previous findings from studies on *Achillea millefolium* and other phenolic-rich plants [30,31]. Its hydrophilic nature promotes strong interactions with polar phenolic acids such as chlorogenic acid, thereby facilitating their dissolution and recovery. This polarity-driven affinity plays a crucial role in enhancing extraction efficiency [32]. Therefore, ChCl-LA achieved the highest CEY and CAC among all solvents tested, reflecting its strong hydrogen-bonding capacity and polarity-driven affinity for phenolic compounds. These findings confirm its effectiveness as a sustainable solvent for optimising bioactive compound recovery.

Assessment of TP and TF

Figure 2 presents the influence of various solvent types on the TP and TF of GGLE. For TP, the DES combination with ChCl-LA yielded the highest value of 92.54 ± 0.74 mg GAE/g, followed by ChCl-Gly (88.75 ± 1.90 mg GAE/g), Bet-LA (75.64 ± 2.11 mg GAE/g), LP-CA (72.84 ± 2.20 mg GAE/g), water (67.31 ± 2.47 mg GAE/g), methanol (63.33 ± 2.40 mg GAE/g), and ethanol (21.04 ± 1.12 mg GAE/g). These results highlight the superior extraction capacity of DESs compared with conventional solvents, particularly in recovering phenolic compounds. The strong hydrogen-bonding interactions in ChCl-LA promote the solubility, stability, and diffusion of phenolic compounds during extraction. This observation is consistent with previous studies in which ChCl-LA combined with UAE significantly improved the recovery and stability of polyhydroxy compounds [33,34].

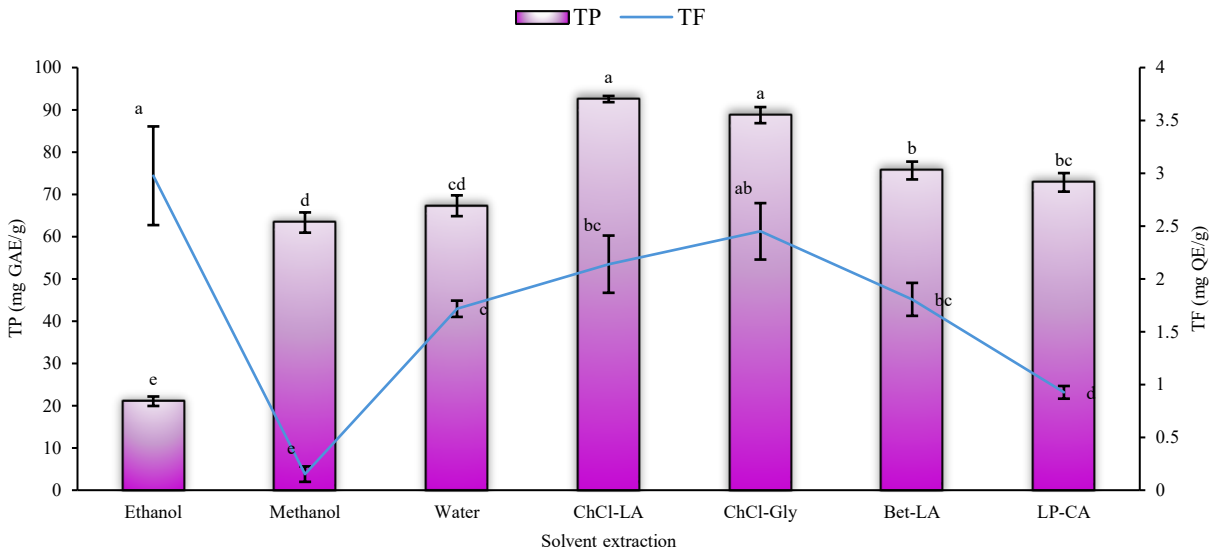

Figure 2 TP (bar) and TF (line) of GGLE extracted using different types of solvents. Data are presented as mean ± SD (n = 3). Different letters within the bar and line indicate significant differences ($p < 0.05$).

In contrast, TF followed a different pattern, with ethanol producing the highest value of 2.98 ± 0.47 mg QE/g, followed closely by ChCl-Gly (2.45 ± 0.27 mg QE/g), with no significant difference between the two solvents ($p > 0.05$). The remaining solvents were ranked as follows: ChCl-LA (2.14 ± 0.27 mg QE/g), Bet-LA (1.81 ± 0.16 mg QE/g), water (1.71 ± 0.08 mg QE/g), LP-CA (0.93 ± 0.06 mg QE/g), and methanol (0.15 ± 0.07 mg QE/g). Ethanol demonstrated high performance in flavonoid extraction, consistent with the findings of Moon *et al*. [35], who reported that ethanol extracts of *Acer tegmentosum* contained the highest flavonoid content among several solvent fractions. Additionally, Philippi *et al*. [36] found that both ethanol and ChCl-Gly produced comparable yields of polyphenols from eggplant peels, suggesting that while DESs are effective, ethanol may offer a slight advantage in some cases. Ethanol efficiently dissolves flavonoids by forming strong hydrogen bonds with solutes, whereas DESs also rely on hydrogen bonding, but their multicomponent interactions can limit flavonoid extraction [37,38]. These results indicate that although DESs such as ChCl-LA and ChCl-Gly show strong potential for phenolic extraction, ethanol remains a competitive option for flavonoid recovery. The variation in solvent effectiveness highlights the importance of selecting solvents according to the specific class of bioactive compounds targeted.

Assessment of antioxidant activity

Figure 3 presents the antioxidant activity of GGLE extracted using different solvents, as evaluated through DPPH, ABTS, and FRAP assays. In the DPPH assay, the extract obtained using the DES combination with ChCl-LA exhibited the highest radical scavenging activity of 84.95 ± 1.49%, followed by methanol (76.85 ± 1.29%), ethanol (70.58 ± 1.07%), water (67.23 ± 0.98%), LP-CA (67.10 ± 2.49%), ChCl-Gly (64.92 ± 1.87%), and Bet-LA (61.36 ± 1.63%). The strong DPPH activity of the ChCl-LA extract indicates its efficiency in extracting antioxidant compounds, largely attributed to its ability to disrupt plant cell wall structures and enhance the solubility and release of intracellular bioactive compounds. Similar findings were reported by Ghanem *et al*. [39], who showed that ChCl-LA significantly improved DPPH scavenging activity in extracts from *Salvia officinalis* leaves. In addition, the strong hydrogen-bonding capacity and modifiable polarity of ChCl-LA contribute to its superior antioxidant extraction performance [40,41].

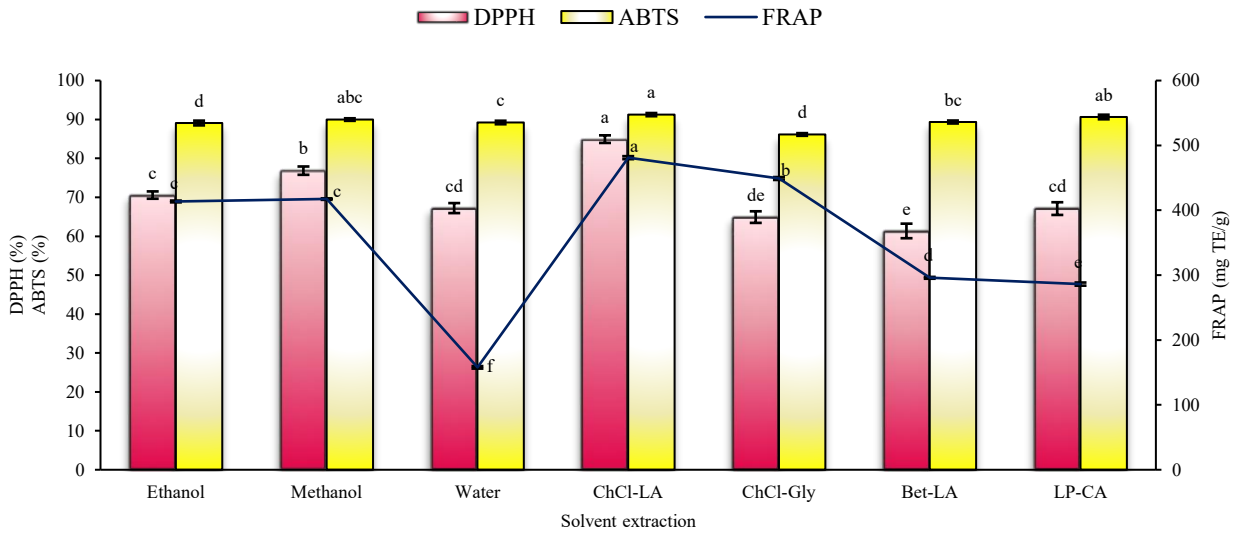

Figure 3 DPPH (bars), ABTS (bars), and FRAP (line) of GGLE extracted using different types of solvents. Data are presented as mean ± SD (n = 3). Different letters within the bars and line indicate significant differences ($p < 0.05$).

The ABTS assay showed a similar pattern, with the ChCl-LA extract again exhibiting the highest antioxidant activity of 91.25 ± 0.34%, followed by LP-CA (90.64 ± 0.52%), methanol (89.98 ± 0.45%), Bet-LA (89.35 ± 0.59%), water (89.23 ± 0.41%), ethanol (89.09 ± 0.32%), and ChCl-Gly (86.15 ± 0.38%). The strong ABTS scavenging capacity of ChCl-LA is attributed not only to its efficiency in extracting antioxidant compounds but also to the inherent antioxidant properties of its components. Lactic acid, a component of this DES, possesses known radical-scavenging potential that enhances the overall antioxidant effect [42]. Moreover, molecular dynamics simulations have demonstrated that strong hydrogen bonding between chloride ions and lactic acid contributes to a stable and reactive solvent network. This structural stability facilitates effective interactions with ABTS radicals and helps preserve active antioxidant compounds during extraction [43].

In the FRAP assay, ChCl-LA again exhibited the highest antioxidant potential, yielding 481.14 ± 2.31 mg TE/g, followed by ChCl-Gly (449.00 ± 2.20 mg TE/g), methanol (417.31 ± 1.17 mg TE/g), ethanol (413.59 ± 1.35 mg TE/g), Bet-LA (295.91 ± 1.41 mg TE/g), LP-CA (286.23 ± 2.42 mg TE/g), and water (157.89 ± 1.93 mg TE/g). The excellent ferric-reducing ability of ChCl-LA is attributed to the presence of functional groups in the solvent system, particularly the choline cation and acidic moieties, which enhance its electron-donating capacity. These groups facilitate the reduction of ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions, which is the core mechanism assessed by the FRAP assay [44]. Collectively, the DPPH, ABTS, and FRAP assay results demonstrate the effectiveness of ChCl-LA as a robust and multifunctional green solvent for extracting antioxidant compounds.

Assessment of BSLA

Figure 4 shows the toxicity level of GGLE extracted using different solvents, as assessed through BSLA. GGLE extracted with water and all four DES formulations exhibited LC_{50} values greater than 1,000 µg/mL, indicating no toxicity and suggesting that these solvents are safe for bioactive compound extraction at the tested concentrations. In contrast, ethanol and methanol extracts showed markedly lower LC_{50} values of 383.12 and 237.70 µg/mL, respectively, indicating a higher level of toxicity. These findings confirm that solvent selection influences the cytotoxic potential of plant extracts.

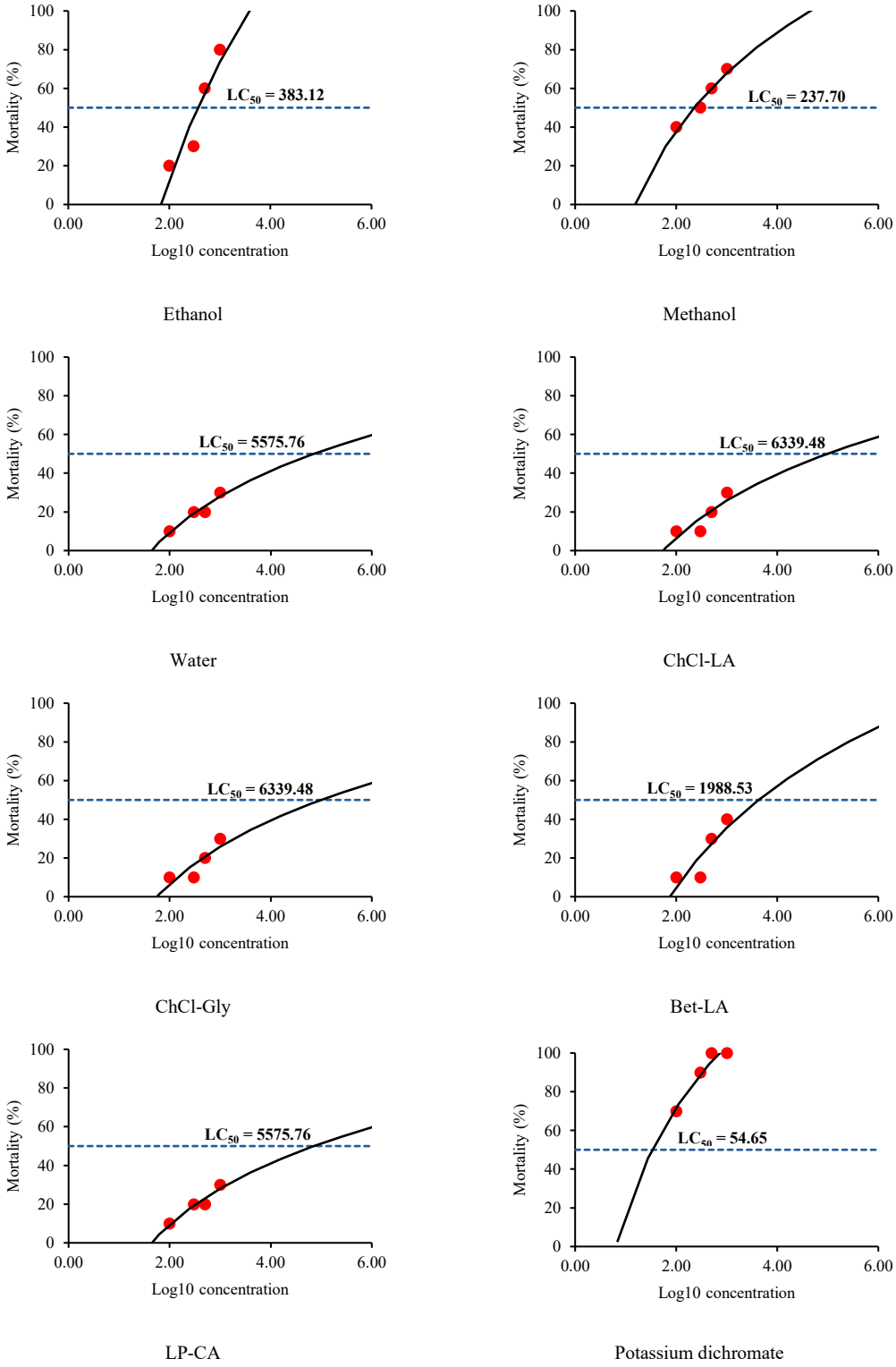

Figure 4 LC_{50} values from BSLA of GGLE extracted using different types of solvents.

BSLA is a widely used method for the preliminary evaluation of cytotoxicity in natural products and plant-based extracts [27]. The non-toxic nature of DESs has been supported by previous studies. For instance, Saini *et al*. [28] reported that the DES composed of ChCl-LA produced an LC_{50} value of 1,597.62 µg/mL when used to extract *Peperomia pellucida* leaves, indicating no observable toxicity. In contrast, methanol-extracted samples have consistently shown higher toxicity, as observed in studies involving *Sargassum polycystum*

and *Murraya exotica* leaves [45,46]. Overall, these results reinforce the potential of DESs as safer and non-toxic alternatives to conventional organic solvents in natural product extraction. Further studies are recommended to evaluate *in vivo* efficacy using appropriate animal models. Such investigations would provide a more comprehensive understanding of the bioavailability, metabolism, and physiological effects of the extracts, as well as their safety profile under biological conditions. These studies would also help bridge the gap between *in vitro* findings and potential applications in human health.

Practical implications

The findings demonstrate that solvent selection is critical in determining overall extraction efficiency. Among the tested solvents, ChCl-LA exhibited superior extraction performance, making it a promising option for food and nutraceutical applications where high recovery of bioactive compounds is essential. Its ability to achieve high yields, coupled with its biodegradable, low-toxicity, and renewable nature, highlights its potential as a green extraction medium. These characteristics align with sustainable product development objectives, as ChCl-LA reduces dependence on hazardous organic solvents, minimises environmental impact, and supports the production of safe, eco-friendly functional foods and nutraceutical products at an industrial scale.

Limitations of the study

This study was limited to a specific range of solvents and extraction parameters, highlighting opportunities for further research. Future studies could examine a broader spectrum of DES formulations, optimise extraction conditions, and compare their performance with other emerging green extraction technologies. Additionally, investigations into scalability, cost-effectiveness, and operational feasibility will provide valuable insights into the industrial applicability of DES-based extraction techniques, particularly for large-scale production of functional foods and nutraceutical products.

Conclusions

This study confirmed the effectiveness of DESs, particularly the ChCl-LA formulation, in enhancing the extraction of bioactive compounds from *G. gnemon* leaves. Compared with conventional solvents, ChCl-LA yielded higher CEY, CAC, TP, and antioxidant activities (DPPH, ABTS, and FRAP), while demonstrating non-toxic effects in the BSLA. Although ethanol produced the highest TF, ChCl-LA offered a more balanced profile in terms of extraction efficiency, safety, and recovery of diverse bioactive compounds. These findings highlight the potential of DESs as sustainable green solvents that reduce reliance on hazardous organic solvents and support eco-friendly production of functional foods and nutraceutical products. Future research should address the scalability, stability, and integration of DES-based extracts into industrial applications to optimise their potential in green extraction.

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Declaration of generative AI in scientific writing

During the preparation of this manuscript, the authors utilised ChatGPT (OpenAI) and Quillbot solely to enhance language clarity, grammar, and overall readability. Following the application of this tool/service, the author(s) thoroughly reviewed and edited the content as necessary and take full responsibility for the final published version.

CRediT author statement

Muhammad Naufal Qaweim Rushdy: Data curation, Formal analysis, Investigation, Methodology, Software, Writing - original draft preparation; **Mohd Azrie Awang:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Writing - review and editing; **Mohammad Amil Zulhilmi Benjamin:** Validation, Writing - review and editing; **Muhammad Daniel Eazzat Mohd Rosdan:** Data curation, Investigation, Visualization. **Aniza Saini:** Data curation, Investigation, Visualization**. Pichayada Somboon:** Resources, Validation.

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