Malaysian Applied Biology
https://doi.org/10.55230/mabjournal.v54i1.3212

Research Article

Natural Deep Eutectic Solvents vs. Conventional Solvents: Effects on Crude Yield, Mangiferin Content, Antioxidant Activity, and Toxicity in *Mangifera pajang* Kosterm. Fruit Extracts

Muhammad Daniel Eazzat Mohd Rosdan[1], Mohd Azrie Awang[1,2]*, Mohammad Amil Zulhilmi Benjamin[3], Fritzgerald Anak Andrew[1], Aniza Saini[1], Siti Faridah Mohd Amin[1,2], Norliza Julmohammad[1,2]

1. Faculty of Food Science and Nutrition, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia
2. Food Security Research Laboratory, Faculty of Food Science and Nutrition, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia
3. Borneo Research on Algesia, Inflammation and Neurodegeneration (BRAIN) Group, Faculty of Medicine and Health Sciences, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia

*Corresponding author: ma.awang@ums.edu.my

ABSTRACT

Mangifera pajang Kosterm. fruits, commonly known as 'Bambangan,' are rich in natural antioxidants due to their phytochemical constituents and are categorised as an underutilised fruit. This study focused on investigating the effects of different solvents on the extraction yield (EY), mangiferin content (MC), total flavonoids (TF), total phenolics (TP), antioxidant activity, and toxicity of *M. pajang* fruit extracts (MPFE). The selected solvents included natural deep eutectic solvents (NADES), water, methanol, and ethanol. The extraction of MPFE was performed using ultrasound-assisted extraction with an ultrasonic probe. Antioxidant activities were evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and ferric-reducing antioxidant power (FRAP) assays. Toxicological assessment was conducted using the brine shrimp lethality assay (BSLA) to determine LC_{50} values. Overall, NADES proved to be the most efficient solvent for extraction, yielding the highest EY (37.09 ± 2.34%), MC (0.032 ± 0.000 mg/g), TF (0.80 ± 0.01 mg RE/g dry extract), TP (14.94 ± 1.74 mg GAE/g dry extract), and exhibits potent antioxidant activity as measured by DPPH (82.38 ± 0.24%), ABTS (86.37 ± 0.03%), and FRAP (304.57 ± 5.24 mg TE/g dry extract). Moreover, NADES demonstrated non-toxicity in the BSLA (LC_{50} = 1988.37 µg/mL) of MPFE. These findings suggest that NADES is a suitable solvent for exploring the medicinal potential of *M. pajang* fruits and their application in therapeutic development.

Key words: Antioxidant, *Mangifera pajang*, mangiferin, NADES, toxicity, ultrasound-assisted extraction

INTRODUCTION

The extraction of natural compounds from medicinal plants has gained significant attention due to their rich content of bioactive molecules, such as flavonoids, phenolics, and antioxidants. The choice of solvent for the extraction process is critical, as it directly influences the type and quantity of compounds extracted (Kumar *et al*., 2023). Solvent selection is typically based on their polarity characteristics (Rodrigues *et al*., 2023). Common solvents for extracting polar compounds, such as flavonoids and phenolics, include water, methanol, ethanol, and acetone (Lezoul *et al*., 2020; Anuar *et al*., 2021). Solvents used for phytochemical extraction can be categorised into three main types: green solvents, organic solvents, and supercritical fluids (Kumar *et al*., 2023). Water is particularly advantageous due to its wide availability, environmental friendliness, and efficiency in extracting a broad range of polar compounds (Xu *et al*., 2017; Alqahtani *et al*., 2023). Moreover, water, methanol, and ethanol are consistent with the ethnobotanical usage of many medicinal plants, as they have traditionally been used in preparations such as decoctions and tinctures (Jahurul *et al*., 2019; Maling *et al*., 2024). The effective extraction and purification of antioxidant and phytochemical compounds from plant materials depend on various factors, including duration, temperature, solvent concentration, and polarity (Ng *et al*., 2020). These factors require optimisation for each plant source to maximise yield and compound quality.

Natural deep eutectic solvents (NADES) are a new class of green solvents that offer a sustainable alternative to conventional organic solvents (Li, 2022). Comprising a hydrogen bond acceptor (HBA), such as choline chloride, and a hydrogen bond donor (HBD), such as lactic acid, they effectively extract various phytochemicals (Prabhune & Dey, 2023). NADES are environmentally friendly and easy to prepare, making them an attractive option for obtaining bioactive compounds (Prabhune & Dey, 2023). Razboršek *et al*. (2020) found that NADES, composed of natural components, offered significant advantages over traditional solvents like methanol for extracting phenolic compounds from black chokeberry fruits. Another study highlighted the effectiveness of combining NADES with ultrasound-assisted extraction (UAE) to enhance the recovery of flavonoids and phenolics from date

Article History
Accepted: 21 February 2025
First version online: 27 March 2025
Cite This Article:
Mohd Rosdan, M.D.E., Awang, M.A., Benjamin, M.A.Z., Andrew, F.A., Saini, A., Mohd Amin, S.F. & Julmohammad, N. 2025. Natural Deep Eutectic Solvents vs. Conventional solvents: Effects on crude yield, mangiferin content, antioxidant activity, and toxicity in *Mangifera pajang* Kosterm. fruit extracts. Malaysian Applied Biology, 54(1): 87-97. https://doi.org/10.55230/mabjournal.v54i1.3212

fruits (Djaoudene *et al*., 2024). NADES made with choline chloride and lactic acid (1:2) proved to be an effective solvent for extracting phenolic compounds from *Lippia citriodora* leaves and exhibited higher extraction yields than methanol (Ivanović *et al*. 2018). Additionally, NADES have shown success in extracting phytochemicals from sources like mangosteen peel, outperforming other solvents (Plaza *et al*., 2021). Thus, NADES represent a more sustainable and efficient alternative to traditional organic solvents for extracting phytochemicals (Sakurai *et al*., 2024).

Mangifera pajang Kosterm. fruits, commonly known as 'Bambangan,' originate from Borneo Island, specifically in Sabah and Sarawak, Malaysia, and belong to the Anacardiaceae family (Tangah *et al*., 2017). According to Tangah *et al*. (2017), the *M. pajang* plant is distinguished from other *Mangifera* species by its rough, brown outer skin, resembling potato-brown at maturity (Figure 1). Common mangoes typically have smooth green, red, or yellow skin. The fruit is among the largest of the *Mangifera* species (Roslan *et al*., 2021), weighing approximately 0.5–1.0 kg or more and measuring about 20 cm in diameter. The skin can be peeled like that of a banana, and the fruit is broadly ovoid or globose in shape, fibrous, with a woody seed endocarp. *M. pajang* fruits are traditionally consumed pickled or freshly prepared due to their limited shelf life and have a fruiting season in Sabah, Malaysia. Also referred to as wild mangoes, they are a rich source of various bioactive compounds such as flavonoids, phenolics, and anthocyanins, and possess potent antioxidant activities (Benjamin *et al*., 2021). The pulp of *M. pajang* fruits is fibrous and juicy, with a distinctive aromatic flavour and strong scent, making it suitable for fresh consumption (Jahurul *et al*., 2019). Based on past studies (Roslan *et al*., 2020), *M. pajang* fruits show great potential for commercialisation as health-promoting products due to their high antioxidant and phytochemical content, as well as their rich Vitamin C and superior antioxidant properties, making them a valuable source for functional food and medicinal applications. The literature reports potential biological activities of *M. pajang* fruit extracts (MPFE), including antioxidant activity, anticancer effects, antibacterial activity, cytoprotective effects, cardiovascular benefits, and antidiabetic properties (Jahurul *et al*., 2019).

Plant Fruit

Fig. 1. Plant and fruit of *M. pajang*.

Although *M. pajang* fruits have shown promise for various beneficial effects, their practical utilisation remains constrained. Further investigation is needed to thoroughly examine the selection of suitable solvents for extraction, to identify environmentally responsible extraction techniques and to compare them to alternative solvent options. This study aimed to assess the extraction yield (EY), mangiferin content (MC), total flavonoids (TF), total phenolics (TP), antioxidant activity, and toxicity of MPFE using different solvents, including NADES, water, methanol, and ethanol. The results of this study may provide valuable guidance to researchers and industry professionals in selecting appropriate extraction solvents and conditions to enhance the recovery of yield and therapeutic compounds from *M. pajang* fruits.

MATERIALS AND METHODS

Plant materials

Mangifera pajang fruits were purchased from vendors at Anjung Kinabalu, Kota Kinabalu, Sabah, Malaysia. The fresh fruit pulp was sliced into pieces measuring 1 cm x 1 cm and then dried in an oven (ED 23, Binder, Tuttlingen, Germany) at 50°C for 6 hr until completely dehydrated, with minor adjustments. After drying, the samples were finely ground using a grinder (EBM-9182, Elba, Borso del Grappa, Italy), and the resulting fine powder was stored for future analyses.

Preparation of NADES

To prepare the NADES, a modified heating and stirring technique was employed according to a previous method (Ozkan, 2024). HBA and HBD were combined at a molar ratio of 1:2. The water content of the NADES was adjusted by adding deionised water. The NADES and deionised water were mixed in a glass beaker and heated to 70°C, with subsequent stirring at a constant velocity until a homogeneous and clear liquid formed within 30 min. The resulting NADES were then stored at room temperature for future use.

Extraction yield

Four solvents were used: NADES, water, methanol, and ethanol. Following the method by Awang *et al*. (2021) with some modifications, a ratio of 5 g powdered sample to 100 mL extraction solvent (1:20 g/mL) was employed for UAE. The UAE was performed for 15 min at 68% amplitude using an ultrasonic probe (Q500 Sonicator, QSonica, Newtown, CT, USA). Solid residues

were removed by filtering through filter paper. The filtered MPFE were further separated under reduced pressure at 70°C using a rotary evaporator (Laborota 4000, Heidolph, Schwabach, Germany), following the method by Stephenus *et al*. (2023) with slight modifications. Subsequently, the MPFE was dried in an oven for 24 hr at 50°C. For subsequent analyses, NADES and water samples were dissolved in deionised water, while methanol and ethanol samples were dissolved in methanol. The EY of MPFE from the different solvents was calculated using equation:

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

Mangiferin content

The determination of MC using high-performance liquid chromatography (HPLC) followed the method by Marinov *et al*. (2024) with slight alterations. Pure standard mangiferin (1 mg/mL) dissolved in methanol was used. Mangiferin detection employed an Agilent 1100 system (Agilent Technologies, Santa Clara, CA, USA) with an ultraviolet-visible (UV-Vis) detector and an InertSustain C_{18} column (5 µm, 150 × 4.6 mm). The mobile phases consisted of 0.1% formic acid in deionised water (mobile phase A) and acetonitrile (mobile phase B). Isocratic elution, a flow rate of 0.8 mL/min, a 15-min run, 20 µL injection volume, and a wavelength of 254 nm was used. Each sample underwent filtration using a 0.22 µm membrane filter before injection. The MC of MPFE was determined based on equation:

$$\text{MC (mg/g)} = \frac{\text{Mass of mangiferin (mg)}}{\text{Dried mass of crude extract (g)}}$$

Total flavonoids

The TF was determined using the aluminium colourimetric assay as described by Fakhrulddin *et al*. (2022) with slight modifications. A mixture of 1 mL diluted MPFE (1 mg/mL) and 1 mL of 2% aluminium chloride was incubated in the dark for 15 min, and absorbance was measured at 430 nm using a UV-Vis spectrophotometer (Lambda 25, PerkinElmer, Waltham, MA, USA). Rutin acted as the standard reference, and the TF of MPFE was expressed as mg RE/g dry extract in equation:

$$\text{TF (mg RE/g dry extract)} = \frac{c \times V}{m}$$

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

Total phenolics

The TP was determined using the Folin–Ciocalteu method, following Awang *et al*. (2023) with slight modifications. A mixture of 500 µL diluted MPFE (1 mg/mL), 500 µL Folin-Ciocalteu reagent, and 1.5 mL of 20% sodium carbonate was adjusted to 10 mL with water. After shaking, it was kept in the dark for 2 hr, and absorbance was measured at 765 nm. Gallic acid acted as the standard reference, and the TP of MPFE was expressed as mg GAE/g dry extract, as indicated in equation:

$$\text{TP (mg GAE/g dry extract)} = \frac{c \times V}{m}$$

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

DPPH assay

Using the method of Asfaw *et al*. (2024) with slight modifications, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) inhibition activity was estimated. A mixture of 1 mL diluted MPFE (1 mg/mL) and 1 mL of 0.1 mM DPPH was incubated in the dark for 30 min, and absorbance was measured at 517 nm. Blank DPPH solution acted as the negative control, and Trolox acted as the positive control. The DPPH inhibition activity of the MPFE was calculated using equation:

$$\text{DPPH inhibition activity (\%)} = \frac{Abs_c - Abs_s}{Abs_c} \times 100$$

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

ABTS assay

The 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) inhibition activity was assessed using a modified method by Hussen & Endalew (2023). ABTS was prepared by reacting 5 mL of 7 mM ABTS with 88 µL of 140 mM potassium persulphate (1:0.35 ratio) and incubating in the dark at room temperature for 24 hr. The solution was then diluted to an absorbance of 0.70 ± 0.02 at 734 nm and equilibrated to 30°C. Scavenging activity was measured by mixing 100 µL of diluted MPFE (1 mg/mL) with 100 µL of ABTS reagent in a 96-well plate, incubating for 5 min at room temperature, and recording the absorbance at 734 nm using a microplate reader (Multiskan SkyHigh, Thermo Fisher Scientific, Waltham, MA, USA). Blank ABTS solution served

as the negative control, and Trolox as the positive control. The ABTS inhibition activity of MPFE was calculated using equation:

$$\text{ABTS inhibition activity (\%)} = \frac{Abs_c - Abs_s}{Abs_c} \times 100$$

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

FRAP assay

The ferric-reducing antioxidant power (FRAP) assay was performed following a modified method by Russo *et al*. (2013). The FRAP reagent was prepared by mixing 38 mM sodium acetate (pH 3.6), 20 mM ferric chloride, and 10 mM TPTZ in 40 mM hydrochloric acid at a 10:1:1 ratio. Diluted MPFE (1 mg/mL, 20 µL) was combined with 180 µL of FRAP reagent in a 96-well plate and incubated at 37°C for 40 min in the dark. Absorbance was measured at 593 nm, with Trolox acting as the standard reference. Results were expressed as mg TE/g dry extract using equation:

$$\text{FRAP (mg TE/g dry extract)} = \frac{c \times V}{m}$$

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

Brine shrimp lethality assay

The brine shrimp lethality assay (BSLA) protocol was derived from the method outlined by Akano & Akinsomisoye (2024), with slight adjustments. Nauplii were hatched from brine shrimp eggs in an aerated aquarium filled with seawater over 48 hr. Once hatched, the actively moving nauplii were collected from the well-lit area and used for the assay. One hundred nauplii were meticulously transferred using a micropipette and glass capillary into a petri dish with 20 mL of seawater. Each concentration in the petri dish consisted of 2 mL of the different solvents and 20 mL of seawater containing nauplii, left at room temperature for 24 hr in the presence of light. Then, the remaining nauplii were enumerated using a magnifying glass. The experiment encompassed a potassium dichromate (positive control), seawater with nauplii (negative control), and different concentrations from MPFE (100, 300, 500, and 1000 µg/mL). The mortality was expressed as a percentage in equation:

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

Statistical analysis

All experiments were conducted in triplicate for accuracy with each replicate representing an independent experiment and results were reported as mean ± standard deviation (SD). Statistical analysis to assess significant differences between solvent extractions was performed using IBM SPSS Statistics (Version 28) with a one-way analysis of variance (ANOVA) followed by Tukey's Honestly Significant Difference (HSD) test.

RESULTS

Effect of different solvents on extraction yield

Figure 2 illustrates the EY of different solvents from MPFE. According to the results, the NADES extract exhibited the highest EY values (37.09 ± 2.34%), which were significantly higher compared to other solvents such as water, methanol, and ethanol extracts (p<0.05). The EY of water, methanol, and ethanol extracts were 22.82 ± 1.46%, 11.86 ± 1.33%, and 15.22 ± 3.05%, respectively. Therefore, the results indicate a ranking of EY as NADES > water > ethanol > methanol.

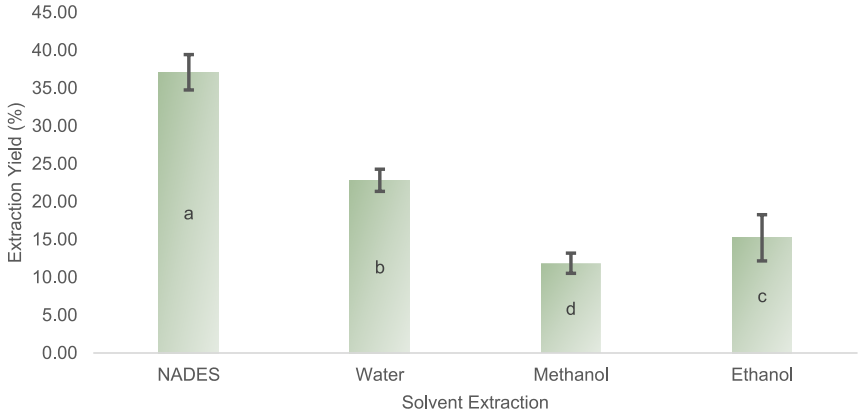

Fig. 2. EY of MPFE at different solvents.

Effect of different solvents on mangiferin content

Figure 3 illustrates the MC of MPFE at different solvents. The NADES extract showed the highest MC values (0.032 ± 0.000 mg/g), significantly exceeding those of other solvents such as water, methanol, and ethanol extracts (p<0.05). Water, methanol, and ethanol extracts exhibited MC values of 0.026 ± 0.000 mg/g, 0.010 ± 0.000 mg/g, and 0.012 ± 0.000 mg/g, respectively. Thus, the results suggest a ranking of MC as NADES > water > ethanol > methanol.

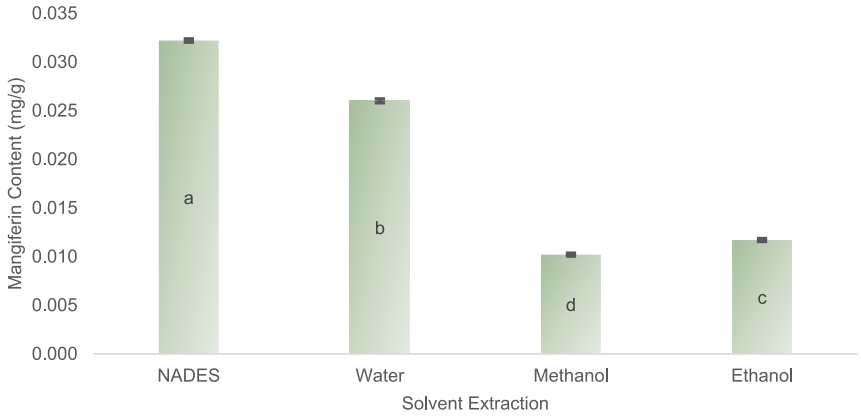

Fig. 3. MC of MPFE at different solvents.

Effect of different solvents on total flavonoids

Figure 4 presents the TF of MPFE using different solvents. Based on the findings, the NADES extract exhibited significantly higher TF levels (0.80 ± 0.01 mg RE/g dry extract) compared to water, methanol, and ethanol extracts (p<0.05). Water, methanol, and ethanol extracts showed TF values of 0.43 ± 0.02 mg RE/g dry extract, 0.41 ± 0.01 mg RE/g dry extract, and 0.42 ± 0.01 mg RE/g dry extract, respectively. Thus, the TF ranking is NADES > water > ethanol > methanol.

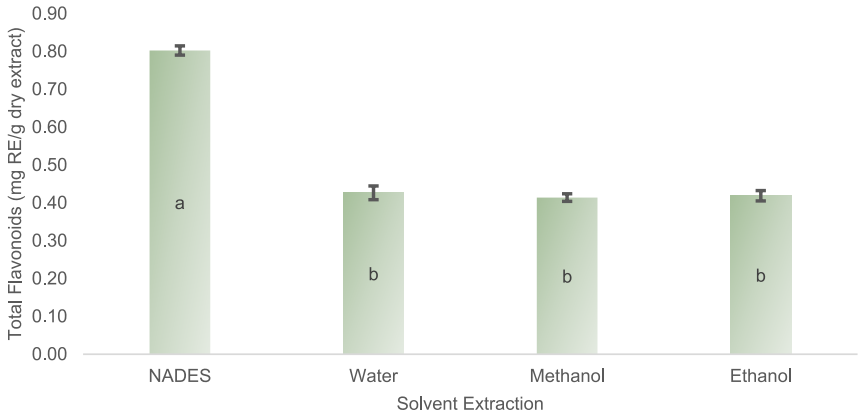

Fig. 4. TF of MPFE at different solvents.

Effect of different solvents on total phenolics

Figure 5 shows the TP of different solvents from MPFE. The results indicate that the NADES extract exhibited significantly higher levels of TP (14.94 ± 1.74 mg GAE/g dry extract) compared to water, methanol, and ethanol extracts (p<0.05). The TP values were 11.15 ± 1.13 mg GAE/g dry extract for the water extract, 4.48 ± 0.64 mg GAE/g dry extract for the methanol extract, and 6.52 ± 0.55 mg GAE/g dry extract for the ethanol extract. The ascending order for TP is NADES > water > ethanol > methanol.

Effect of different solvents on antioxidant activity

Figure 6 shows the DPPH inhibition activity of MPFE at different solvents. The results indicate that the NADES extract exhibited the highest antioxidant activity at 82.38 ± 0.24%, compared to Trolox at 99.03 ± 0.93%. In contrast, the antioxidant activities of water (77.65 ± 0.54%), methanol (66.89 ± 0.20%), and ethanol (74.72 ± 0.21%) extracts were slightly lower and significantly different (p<0.05). These differences emphasise the superior extraction efficiency of the NADES extract in recovering valuable antioxidant compounds from *M. pajang* fruits.

Figure 7 shows the ABTS inhibition activity for MPFE with different solvents. The findings indicate that the NADES extract exhibited the highest antioxidant activity at 86.37 ± 0.03%, compared to Trolox at 98.99 ± 0.09%. In contrast, the antioxidant activities of ethanol (86.07 ± 0.07%), water (85.80 ± 0.07%), and methanol (85.54 ± 0.09%) extracts were slightly lower and significantly different (p<0.05). These differences highlight the superior extraction efficiency of the NADES extract in recovering valuable antioxidant compounds from *M. pajang* fruits.

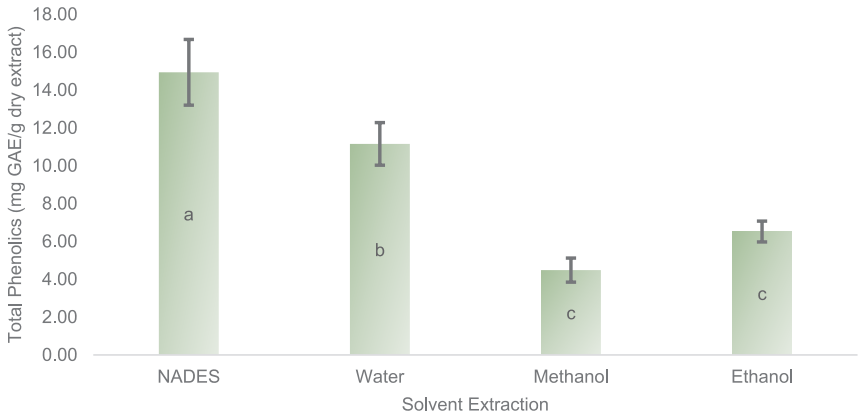

Fig. 5. TP of MPFE at different solvents.

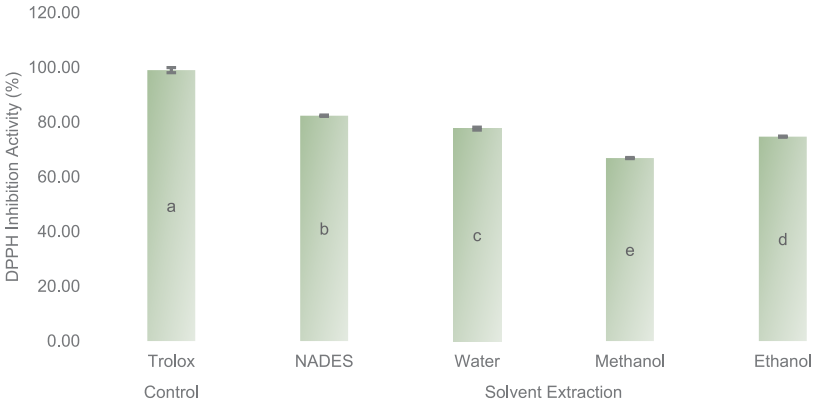

Fig. 6. DPPH inhibition activity of MPFE at different solvents.

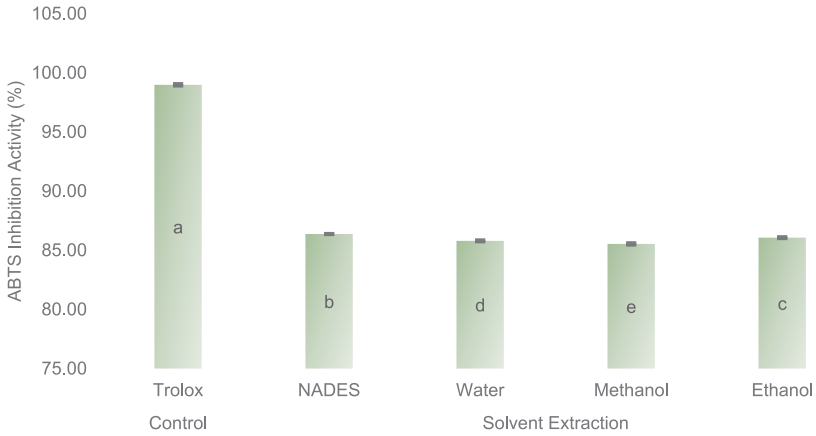

Fig. 7. ABTS inhibition activity of MPFE at different solvents.

Figure 8 shows the FRAP assay results for MPFE with different solvents. The NADES extract exhibited significantly higher FRAP levels (304.57 ± 5.24 mg TE/g dry extract) compared to ethanol, methanol, and water extracts (p<0.05). The FRAP values were 188.00 ± 1.57 mg TE/g dry extract for ethanol, 181.67 ± 1.37 mg TE/g dry extract for methanol, and 173.14 ± 0.52 mg TE/g dry extract for water. The ascending order for the FRAP is NADES > ethanol > methanol > water.

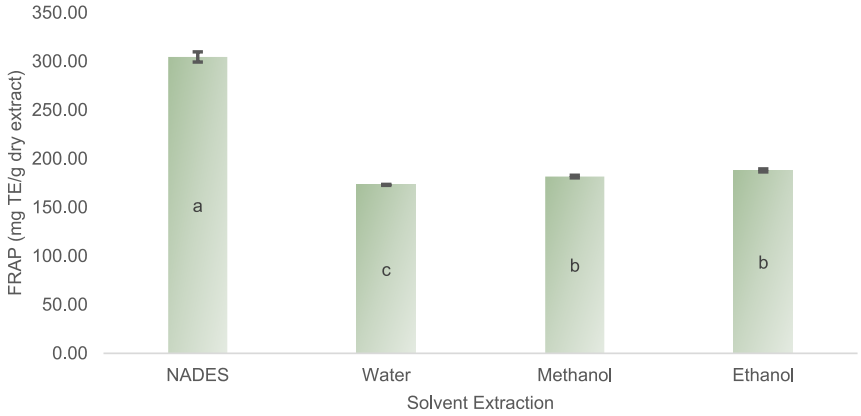

Fig. 8. FRAP assay of MPFE at different solvents

Effect of different solvents on BSLA

Figure 9 presents the toxicity of MPFE using the BSLA with different solvents. Based on the findings, NADES and water extracts exhibited an LC_{50} value > 1000 µg/mL, indicating that concentrations above this threshold had a non-toxic impact on the test organisms. In contrast, ethanol showed significantly lower LC_{50} values of 116.02 µg/mL, indicating toxicity. Methanol also had the lowest LC_{50} value of 68.50 µg/mL, indicating toxicity compared to the other solvents. These results highlight the varied toxic effects of different solvents on the brine shrimp.

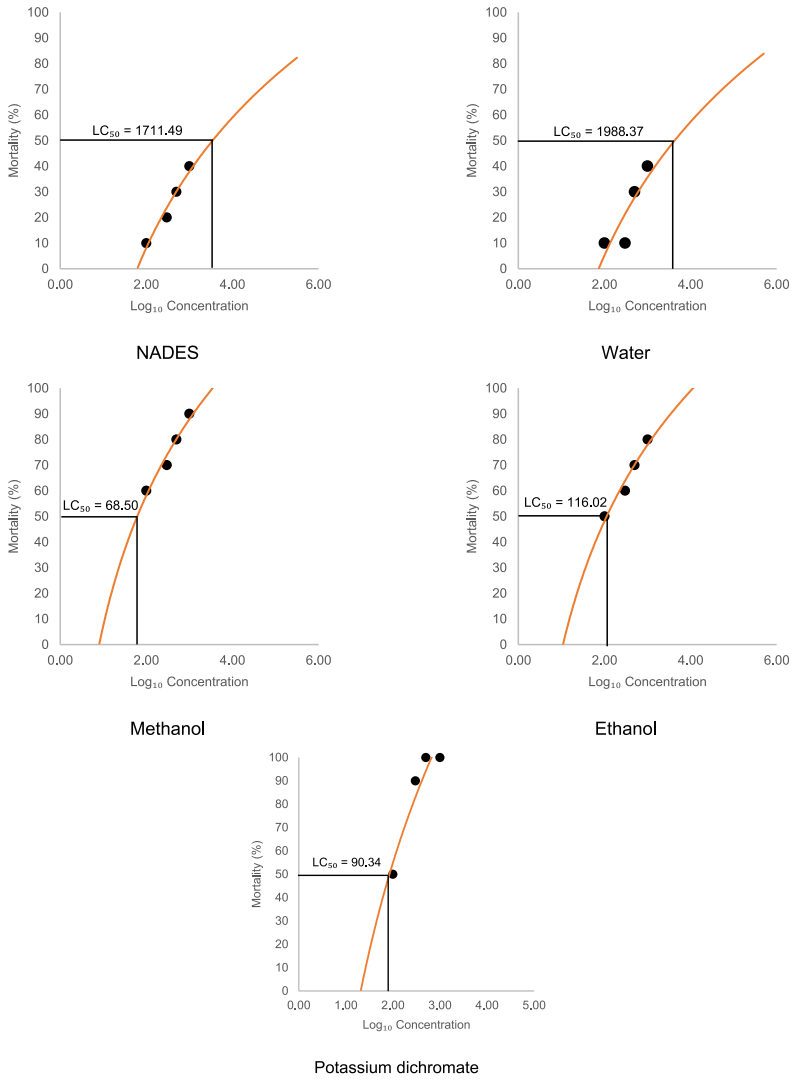

Fig. 9. BSLA of MPFE at different solvents.

DISCUSSION

The high EY and MC observed with the NADES extract can be attributed to its ability to effectively penetrate cell walls and solubilise target phytochemicals (Hikmawanti *et al*., 2021). Furthermore, the use of NADES as a green solvent offers additional advantages, such as biodegradability, low toxicity, and reduced environmental impact, making it a more sustainable choice for extracting bioactive compounds from *M. pajang* fruits, particularly the mangiferin compound (Chemat *et al*., 2019; Yang *et al*., 2019; Benvenutti *et al*., 2020). Mangiferin belongs to the xanthone group, which is one of the major compounds in *M. pajang* fruits and has demonstrated various therapeutic properties, such as hypolipidemic, anti-HIV, anti-tumour, immunomodulatory, and antioxidant activities (Hassan *et al*., 2011). In *Mangifera* species, MC in the pulp ranges from 0.002 ± 0.000 mg/g to 0.20 ± 0.070 mg/g (Luo *et al*., 2012). Recent studies have shown that NADES containing acidic HBD exhibit higher EY due to their lower pH and greater polarity, which enhance interactions with phenolic compounds, leading to increased extraction efficiencies compared to conventional solvents (Socas-Rodríguez *et al*., 2021). Furthermore, the results of MC showed the highest values in NADES due to dilution with deionised water, which could effectively smooth and facilitate the chromatographic analysis of this bioactive compound (Alañón *et al*., 2020). Thus, this dilution could reduce the high viscosity of NADES, thereby enhancing flow characteristics and mass transfer, leading to more accurate and reliable quantitative results from HPLC analysis.

NADES is more efficient in extracting TF and TP than other solvents due to its unique properties, such as polarity, viscosity, and the ability to form strong hydrogen bonds, which help preserve target compounds from degradation (Plaza *et al*., 2021). Plaza *et al*. (2021) also evaluated seven types of NADES with different HBDs, including glycerol, ethylene glycol, urea, sorbitol, lactic acid, citric acid, and formic acid, as well as varying molar ratios, and found that choline chloride (as HBA) and lactic acid (as HBD) were the most effective for antioxidant extraction. Barbieri *et al*. (2020) supported this, demonstrating that NADES could improve the extraction efficiency of phenolic compounds from rosemary (*Rosmarinus officinalis*) by stabilising the extracted compounds through hydrogen bonds with NADES. These intermolecular interactions can reduce oxidative degradation by limiting the movement of solute molecules and minimising their contact with oxygen. Similarly, Alves *et al*. (2022) employed NADES to extract polyphenols from green tea for use in food packaging. Researchers utilised NADES as a solvent for extracting flavonoids and phenolics because NADES has emerged as a promising alternative to traditional organic solvents for extracting bioactive compounds from natural sources (Radošević *et al*., 2016; Obluchinskaya *et al*., 2021).

The results align with previous studies indicating that NADES extracts demonstrate superior antioxidant activity against DPPH compared to conventional solvents. He *et al*. (2020) showed that NADES extracts of *Salvia miltiorrhiza* exhibited significant quenching effects on the DPPH radical, with quenching ratios ranging from 85.9% to 90.6% at a concentration of 10 mg SM/mL, which surpassed the results for water (85.7%) and methanol (65.6%) extracts. Additionally, the antioxidant activity of NADES extracts was found to be higher than that of organic solvent extracts from *Pluchea indica* leaves (Hikmawanti *et al*., 2024). A study by Mansinhos *et al*. (2021) found that the NADES extract of *Lavandula pedunculata* subsp. *lusitanica* exhibited an antioxidant capacity of 72.13 mg TE/g DW via the ABTS radical, which was significantly higher than the methanol extract (34.67 mg TE/g DW) and ethanol extract (46.31 mg TE/g DW). The ABTS radical also showed that NADES extracts exhibited higher antioxidant capacity values compared to the water, methanol, and ethanol extracts from *Aronia melanocarpa* fruit (Zor, 2024). Moreover, Krgović *et al*. (2025) reported that NADES extracts from black raspberry pomace exhibited superior FRAP values, ranging from 688.76 µmoL Fe^{2+}/g DW to 1745.14 µmoL Fe^{2+}/g DW, surpassing the water extract (488.23 µmoL Fe^{2+}/g DW) and the 70% ethanol extract (779.09 µmoL Fe^{2+}/g DW). Similarly, Martinović *et al*. (2022) evaluated the FRAP activity of bilberry fruit, bilberry leaves, and green tea leaves and found that NADES extracts exhibited higher antioxidant activity compared to both water and ethanol extracts. Thus, the choice of solvent is critical for extracting bioactive compounds from plants (Abubakar & Haque, 2020).

According to a recent study by Benjamin *et al*. (2022), the categorisation of toxicity indicators shows that plant extracts with LC_{50} values above 1000 µg/mL are considered non-toxic. Those with values between 500 µg/mL and 1000 µg/mL display mild toxicity, while extracts with LC_{50} values below 500 µg/mL are classified as toxic. This study supported these findings, showing the toxicity levels of different solvents based on LC_{50} values from MPFE as follows: Water < NADES < ethanol < methanol. A prior study by Meena *et al*. (2020) found that ethanol is moderately toxic, methanol is slightly toxic, and water is non-toxic when extracted from *Terminalia arjuna*. Dash *et al*. (2022) reported that the methanol extract of *Combretum roxburghii* exhibited high cytotoxicity, causing mortality in all brine shrimp at concentrations ranging from 100 to 500 µg/mL. These findings suggest that methanol and ethanol may not be the most effective solvents for extracting bioactive compounds from this fruit. In contrast, NADES solvents demonstrate potential suitability as alternative options that could be utilised in various applications without posing significant harm to organisms (Koh *et al*., 2023). Thus, understanding the toxicity profiles of solvent extracts is essential to ensure safe usage in fruit extraction and crucial for making informed decisions in research development.

CONCLUSION

Overall, NADES emerges as the superior solvent for extracting *M. pajang* fruits. It yields the highest amounts of EY, MC, TF, and TP, along with potent antioxidant activity, likely due to its ability to control DPPH, ABTS, and FRAP reactivity kinetics. Moreover, NADES demonstrates exceptional non-toxicity towards brine shrimp in the BSLA. These findings highlight the promising potential of NADES in advancing the therapeutic utilisation of the medicinal properties of underutilised *M. pajang* fruits. Future research could focus on utilising the most effective solvent to further explore the biological activities of *M. pajang* fruits, particularly their anti-diabetic and anti-obesity properties.

ACKNOWLEDGEMENTS

The authors extend their thanks to the Faculty of Food Science and Nutrition at Universiti Malaysia Sabah for providing the facilities and financial backing. This research was supported by Universiti Malaysia Sabah through the Skim Geran Inovasi (SGI0166) and Skim Pensyarah Lantikan Baru (SLB2234).

ETHICAL STATEMENT
Not applicable

CONFLICT OF INTEREST
The authors declare no conflict of interest.

REFERENCES
Abubakar, A.R. & Haque, M. 2020. Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. Journal of Pharmacy and Bioallied Sciences, 12(1): 1-10. https://doi.org/10.4103/jpbs.JPBS_175_19

Akano, O.P. & Akinsomisoye, O.S. 2024. Brine shrimp bioactivity and cytotoxic potential of *Irvingia gabonensis* (Aubry-Lecomte ex O'Rorke) Baill seed. Jundishapur Journal of Health Sciences, 16(1): e142215. https://doi.org/10.5812/jjhs-142215

Alañón, M.E., Ivanović, M., Gómez-Caravaca, A.M., Arráez-Román, D. & Segura-Carretero, A. 2020. Choline chloride derivative-based deep eutectic liquids as novel green alternative solvents for extraction of phenolic compounds from olive leaf. Arabian Journal of Chemistry, 13:1685-1701. https://doi.org/10.1016/j.arabjc.2018.01.003

Alqahtani, N.K., Mohamed, H.A., Moawad, M.E., Younis, N.S. & Mohamed, M.E. 2023. The hepatoprotective effect of two date palm fruit cultivars' extracts: Green optimization of the extraction process. Foods, 12(6): 1229. https://doi.org/10.3390/foods12061229

Alves, T.F.P., Teixeira, N., Vieira, J., Vicente, A.A., Mateus, N., de Freitas, V. & Souza, H.K.S. 2022. Sustainable chitosan packaging films: Green tea polyphenolic extraction strategies using deep eutectic solvents. Journal of Cleaner Production, 372: 133589. https://doi.org/10.1016/j.jclepro.2022.133589

Anuar, A., Awang, M.A. & Tan, H.F. 2021. Impact of solvent selection on the extraction of total phenolic content and total flavonoid content from kaffir lime leaves: Ultrasonic assisted extraction (UAE) and microwave assisted extraction (MAE). AIP Conference Proceedings, 2347: 020020. https://doi.org/10.1063/5.0051934

Asfaw, T.B., Tadesse, M.G., Tessema, F.B., Woldemariam, H.W., Chinchkar, A. V., Singh, A., Upadhyay, A. & Mehari, B. 2024. Ultrasonic-assisted extraction and UHPLC determination of ascorbic acid, polyphenols, and half-maximum effective concentration in *Citrus medica* and *Ziziphus spina-christi* fruits using multivariate experimental design. Food Chemistry: X, 22: 101310. https://doi.org/10.1016/j.fochx.2024.101310

Awang, M.A., Benjamin, M.A.Z., Anuar, A., Ismail, M.F., Ramaiya, S.D. & Mohd Hashim, S.N.A. 2023. Dataset of gallic acid quantification and their antioxidant and anti-inflammatory activities of different solvent extractions from kacip fatimah (*Labisia pumila* Benth. & Hook. f.) leaves. Data in Brief, 51: 109644. https://doi.org/10.1016/j.dib.2023.109644

Awang, M.A., Chua, L.S., Abdullah, L.C. & Pin, K.Y. 2021. Drying kinetics and optimization of quercetrin extraction from *Melastoma malabathricum* leaves. Chemical Engineering and Technology, 44(7): 1214-1220. https://doi.org/10.1002/ceat.202100007

Barbieri, J.B., Goltz, C., Cavalheiro, F.B., Toci, A.T., Igarashi-Mafra, L. & Mafra, M.R. 2020. Deep eutectic solvents applied in the extraction and stabilization of rosemary (*Rosmarinus officinalis* L.) phenolic compounds. Industrial Crops and Products, 144: 112049. https://doi.org/10.1016/j.indcrop.2019.112049

Benjamin, M.A.Z., Abu Bakar, M.F., Abu Bakar, F.I., Sabran, S.F., Kormin, F. & Mohamad Fuzi, S.F.Z. 2021. Development of bambangan (*Mangifera pajang*) carbonated drink. IOP Conference Series: Earth and Environmental Science, 736(1): 012010. https://doi.org/10.1088/1755-1315/736/1/012010

Benjamin, M.A.Z., Ng, S.Y., Saikim, F.H. & Rusdi, N.A. 2022. The effects of drying techniques on phytochemical contents and biological activities on selected bamboo leaves. Molecules, 27(19): 6458. https://doi.org/10.3390/molecules27196458

Benvenutti, L., Sanchez-Camargo, A. del P., Zielinski, A.A.F. & Ferreira, S.R.S. 2020. NADES as potential solvents for anthocyanin and pectin extraction from *Myrciaria cauliflora* fruit by-product: *In silico* and experimental approaches for solvent selection. Journal of Molecular Liquids, 315: 113761. https://doi.org/10.1016/j.molliq.2020.113761

Chemat, F., Vian, M.A., Ravi, H.K., Khadhraoui, B., Hilali, S., Perino, S. & Tixier, A.-S.F. 2019. Review of alternative solvents for green extraction of food and natural products: Panorama, principles, applications and prospects. Molecules, 24(16): 3007. https://doi.org/10.3390/molecules24163007

Dash, H.B., Kumar, S., Saha, M., Devi, R.S. & Biswal, S.K. 2022. Estimation of secondary metabolites and toxicity to *Artemia salina* L. of selected plants of family Combretaceae: An approach for screening of herbal preventive agents. International Journal of Pharmaceutical Sciences and Research, 13(2): 969-976.

Djaoudene, O., Bachir-Bey, M., Schisano, C., Djebari, S., Tenore, G.C. & Romano, A. 2024. A sustainable extraction approach of phytochemicals from date (*Phoenix dactylifera* L.) fruit cultivars using ultrasound-assisted deep eutectic solvent: A comprehensive study on bioactivity and phenolic variability. Antioxidants, 13(2): 181. https://doi.org/10.3390/antiox13020181

Fakhrulddin, I.M., Ramaiya, S.D., Muta Harah, Z., Nur Leena Wong, W.S., Awang, M.A. & Ismail, N.I.M. 2022. Effects of temperature on drying kinetics and biochemical composition of *Caulerpa lentillifera*. Food Research, 6(5): 168-173. https://doi.org/10.26656/fr.2017.6(5).637

Hassan, F.A., Ismail, A., Abdulhamid, A. & Azlan, A. 2011. Identification and quantification of phenolic compounds in bambangan (*Mangifera pajang* Kort.) peels and their free radical scavenging activity. Journal of Agricultural and Food Chemistry, 59(17): 9102-9111. https://doi.org/10.1021/jf201270n

He, X., Yang, J., Huang, Y., Zhang, Y., Wan, H. & Li, C. 2020. Green and efficient ultrasonic-assisted extraction of bioactive components from *Salvia miltiorrhiza* by natural deep eutectic solvents. Molecules, 25(1): 140. https://doi.org/10.3390/molecules25010140

Hikmawanti, N.P.E., Ramadon, D., Jantan, I. & Mun'im, A. 2021. Natural deep eutectic solvents (NADES): Phytochemical extraction performance enhancer for pharmaceutical and nutraceutical product development. Plants, 10(10): 2091. https://doi.org/10.3390/plants10102091

Hikmawanti, N.P.E., Saputri, F.C., Yanuar, A., Jantan, I., Ningrum, R.A., Juanssilfero, A.B. & Mun'im, A. 2024. Choline chloride-urea-based natural deep eutectic solvent for highly efficient extraction of polyphenolic antioxidants from *Pluchea indica* (L.)

Less leaves. Arabian Journal of Chemistry, 17(2):105537. https://doi.org/10.1016/j.arabjc.2023.105537

Hussen, E.M. & Endalew, S.A. 2023. In vitro antioxidant and free-radical scavenging activities of polar leaf extracts of *Vernonia amygdalina*. BMC Complementary Medicine and Therapies, 23(1): 146. https://doi.org/10.1186/s12906-023-03923-y

Ivanović, M., Alañón, M.E., Arráez-Román, D. & Segura-Carretero, A. 2018. Enhanced and green extraction of bioactive compounds from *Lippia citriodora* by tailor-made natural deep eutectic solvents. Food Research International, 111: 67-76. https://doi.org/10.1016/j.foodres.2018.05.014

Jahurul, M.H.A., Zaidul, I.S.M., Beh, L., Sharifudin, M.S., Siddiquee, S., Hasmadi, M., Sahena, F., Mansoor, A.H., Lee, J.S. & Jinap, S. 2019. Valuable components of bambangan fruit (*Mangifera pajang*) and its co-products: A review. Food Research International, 115: 105-115. https://doi.org/10.1016/j.foodres.2018.08.017

Koh, Q.Q., Kua, Y.L., Gan, S., Tan, K.W., Lee, T.Z.E., Cheng, W.K. & Lau, H.L.N. 2023. Sugar-based natural deep eutectic solvent (NADES): Physicochemical properties, antimicrobial activity, toxicity, biodegradability and potential use as green extraction media for phytonutrients. Sustainable Chemistry and Pharmacy, 35: 101218. https://doi.org/10.1016/j.scp.2023.101218

Krgović, N., Jovanović, M.S., Nedeljković, S.K., Šavikin, K., Lješković, N.J., Ilić, M., Živković, J. & Menković, N. 2025. Natural deep eutectic solvents extraction of anthocyanins–effective method for valorisation of black raspberry (*Rubus occidentalis* L.) pomace. Industrial Crops and Products, 223: 120237. https://doi.org/10.1016/j.indcrop.2024.120237

Kumar, A., Nirmal, P., Kumar, M., Jose, A., Tomer, V., Oz, E., Proestos, C., Zeng, M., Elobeid, T., Sneha, K. & Oz, F. 2023. Major phytochemicals: Recent advances in health benefits and extraction method. Molecules, 28(2): 887. https://doi.org/10.3390/molecules28020887

Lezoul, N.E.H., Belkadi, M., Fariborz, H. & Guillén, F. 2020. Extraction processes with several solvents on total bioactive compounds in different organs of three medicinal plants. Molecules, 25(20): 4672. https://doi.org/10.3390/molecules25204672

Li, D. 2022. Natural deep eutectic solvents in phytonutrient extraction and other applications. Frontiers in Plant Science, 13: 1004332. https://doi.org/10.3389/fpls.2022.1004332

Luo, F., Lv, Q., Zhao, Y., Hu, G., Huang, G., Zhang, J., Sun, C., Li, X. & Chen, K. 2012. Quantification and purification of mangiferin from Chinese mango (*Mangifera indica* L.) cultivars and its protective effect on human umbilical vein endothelial cells under H_2O_2-induced stress. International Journal of Molecular Sciences, 13(9): 11260-11274. https://doi.org/10.3390/ijms130911260

Maling, S., Kabakyenga, J., Muchunguzi, C., Olet, E.A., Namaganda, M., Kahwa, I. & Alele, P.E. 2024. Medicinal plants used by traditional medicine practitioners in treatment of alcohol-related disorders in Bushenyi District, southwestern Uganda. Frontiers in Pharmacology, 15: 1407104. https://doi.org/10.3389/fphar.2024.1407104

Mansinhos, I., Gonçalves, S., Rodríguez-Solana, R., Ordóñez-Díaz, J.L., Moreno-Rojas, J.M. & Romano, A. 2021. Ultrasonic-assisted extraction and natural deep eutectic solvents combination: A green strategy to improve the recovery of phenolic compounds from *Lavandula pedunculata* subsp. lusitanica (Chaytor) Franco. Antioxidants, 10(4): 582. https://doi.org/10.3390/antiox10040582

Marinov, T., Kokanova-Nedialkova, Z. & Nedialkov, P. 2024. UHPLC-HRMS-based profiling and simultaneous quantification of the hydrophilic phenolic compounds from the aerial parts of *Hypericum aucheri* Jaub. & Spach (Hypericaceae). Pharmacia, 71: 1-11. https://doi.org/10.3897/pharmacia.71.e122436

Martinović, M., Krgović, N., Nešić, I., Žugić, A. & Tadić, V.M. 2022. Conventional vs. green extraction using natural deep eutectic solvents-differences in the composition of soluble unbound phenolic compounds and antioxidant activity. Antioxidants, 11(11): 2295. https://doi.org/10.3390/antiox11112295

Meena, D.K., Sahoo, A.K., Swain, H.S., Borah, S., Srivastava, P.P., Sahu, N.P. & Das, B.K. 2020. Prospects and perspectives of virtual in-vitro toxicity studies on herbal extracts of *Terminalia arjuna* with enhanced stratagem in *Artemia salina* model: A panacea to explicit the credence of solvent system in brine shrimp lethality bioassay. Emirates Journal of Food and Agriculture, 32(1): 25-37. https://doi.org/10.9755/ejfa.2020.v32.i1.2055

Ng, Z.X., Samsuri, S.N. & Yong, P.H. 2020. The antioxidant index and chemometric analysis of tannin, flavonoid, and total phenolic extracted from medicinal plant foods with the solvents of different polarities. Journal of Food Processing and Preservation, 44(9): e14680. https://doi.org/10.1111/jfpp.14680

Obluchinskaya, E.D., Pozharitskaya, O.N., Zakharova, L. V., Daurtseva, A. V., Flisyuk, E. V. & Shikov, A.N. 2021. Efficacy of natural deep eutectic solvents for extraction of hydrophilic and lipophilic compounds from Fucus vesiculosus. Molecules, 26(14): 4198. https://doi.org/10.3390/molecules26144198

Ozkan, G. 2024. Valorization of artichoke outer petals by using ultrasound-assisted extraction and natural deep eutectic solvents (NADES) for the recovery of phenolic compounds. Journal of the Science of Food and Agriculture, 104(5): 2744-2749. https://doi.org/10.1002/jsfa.13158

Plaza, M., Domínguez-Rodríguez, G., Sahelices, C. & Marina, M.L. 2021. A sustainable approach for extracting non-extractable phenolic compounds from mangosteen peel using ultrasound-assisted extraction and natural deep eutectic solvents. Applied Sciences, 11(12): 5652. https://doi.org/10.3390/app11125625

Prabhune, A. & Dey, R. 2023. Green and sustainable solvents of the future: Deep eutectic solvents. Journal of Molecular Liquids, 379: 121676. https://doi.org/10.1016/j.molliq.2023.121676

Radošević, K., Ćurko, N., Gaurina Srček, V., Cvjetko Bubalo, M., Tomašević, M., Kovačević Ganić, K. & Radojčić Redovniković, I. 2016. Natural deep eutectic solvents as beneficial extractants for enhancement of plant extracts bioactivity. LWT, 73: 45-51. https://doi.org/10.1016/j.lwt.2016.05.037

Razboršek, M.I., Ivanović, M., Krajnc, P. & Kolar, M. 2020. Choline chloride based natural deep eutectic solvents as extraction media for extracting phenolic compounds from chokeberry (*Aronia melanocarpa*). Molecules, 25(7): 1619. https://doi.org/10.3390/molecules25071619

Rodrigues, R.P., Sousa, A.M., Gando-Ferreira, L.M. & Quina, M.J. 2023. Grape pomace as a natural source of phenolic compounds: Solvent screening and extraction optimization. Molecules, 28(6): 2715. https://doi.org/10.3390/molecules28062715

Roslan, J., Boniface, B.A., Ling, H.C., Shya, L.J., Akanda, M.J.H., Saallah, S. & Nor, M.Z.M. 2021. Effect of heat treatment on

physicochemical properties of bambangan (*Mangifera pajang*) fruit juice. Transactions on Science and Technology, 8(3-3): 545-551.

Roslan, J., Ling, H.C., Sintang, M.D. & Saallah, S. 2020. Effect of heat treatment on rheological properties of bambangan (*Mangifera pajang* Kosterm) fruit juice. Advances in Agricultural and Food Research Journal, 1(2): a0000115. https://doi.org/10.36877/aafrj.a0000115

Russo, D., Kenny, O., Smyth, T.J., Milella, L., Hossain, M.B., Diop, M.S., Rai, D.K. & Brunton, N.P. 2013. Profiling of phytochemicals in tissues from *Sclerocarya birrea* by HPLC-MS and their link with antioxidant activity. ISRN Chromatography, 2013: 283462. https://doi.org/10.1155/2013/283462

Sakurai, Y.C.N., Pires, I.V., Ferreira, N.R., Moreira, S.G.C., Silva, L.H.M. da & Rodrigues, A.M. da C. 2024. Preparation and characterization of natural deep eutectic solvents (NADESs): Application in the extraction of phenolic compounds from araza pulp (*Eugenia stipitata*). Foods, 13(13): 1983. https://doi.org/10.3390/foods13131983

Socas-Rodríguez, B., Torres-Cornejo, M.V., Álvarez-Rivera, G. & Mendiola, J.A. 2021. Deep eutectic solvents for the extraction of bioactive compounds from natural sources and agricultural by-products. Applied Sciences, 11(11): 4897. https://doi.org/10.3390/app11114897

Stephenus, F.N., Benjamin, M.A.Z., Anuar, A. & Awang, M.A. 2023. Effect of temperatures on drying kinetics, extraction yield, phenolics, flavonoids, and antioxidant activity of *Phaleria macrocarpa* (Scheff.) Boerl. (mahkota dewa) fruits. Foods, 12(15): 2859. https://doi.org/10.3390/foods12152859

Tangah, J., Bajau, F.E., Jilimin, W., Chan, H.T., Wong, S.K. & Chan, E.W.C. 2017. Phytochemistry and pharmacology of *Mangifera pajang*: An iconic fruit of Sabah, Malaysia. Systematic Reviews in Pharmacy, 8(1): 86-91. https://doi.org/10.5530/srp.2017.1.15

Xu, D.-P., Li, Y., Meng, X., Zhou, T., Zhou, Y., Zheng, J., Zhang, J.-J. & Li, H.-B. 2017. Natural antioxidants in foods and medicinal plants: Extraction, assessment and resources. International Journal of Molecular Sciences, 18(1): 96. https://doi.org/10.3390/ijms18010096

Yang, L., Li, L., Hu, H., Wan, J. & Li, P. 2019. Natural deep eutectic solvents for simultaneous extraction of multi-bioactive components from jinqi jiangtang preparations. Pharmaceutics, 11(1): 18. https://doi.org/10.3390/pharmaceutics11010018

Zor, M. 2024. Investigation of using natural deep eutectic solvents (NADES) for the extraction of bioactive compounds from *Aronia melanocarpa* fruit. Turkish Journal of Agriculture and Forestry, 48(4): 567-579. https://doi.org/10.55730/1300-011X.3202