

Antioxidant and antibacterial activities of mangosteen (*Garcinia mangostana* L.) pericarp extracted by ethanol and deep eutectic solvents (DES)

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Abstract

Mangosteen (*Garcinia mangostana* L.) is widely cultivated in Southeast Asia, and generates approximately 17,712 million tonnes of pericarp (MPE) annually. However, the pericarp is often discarded as waste despite being rich in bioactive compounds. With increasing environmental concerns over the use of synthetic solvents, and rising consumer demand for natural and safe food ingredients, there is an urge to explore sustainable extraction methods for recovering natural antioxidants and antimicrobials. The present work thus aimed to investigate the antioxidant and antibacterial activities of MPE using three extraction techniques: solvent extraction (SE), microwave-assisted extraction (MAE), and ultrasound-assisted extraction (UAE), in combination with ethanol and deep eutectic solvents (DES) as green alternatives. Extracts were analysed for total phenolic content (TPC), total flavonoid content (TFC), total monomeric anthocyanin content (TMAC), and antioxidant activities *via* DPPH and ABTS assays. Antibacterial activity was evaluated using the disc diffusion method against selected Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria. Among the methods, UAE yielded the highest TFC (694.83 mg QE/g), while DES-SE demonstrated the highest TPC (587.50 ± 41.45 mg GAE/g) and TMAC (333.98 ± 41.75 mg cyd-3-glu/g). Antioxidant assays revealed that ethanolic extracts exhibited stronger radical scavenging (46.4 - 60.5%), while DES extracts, particularly with SE, showed superior antibacterial effects against all bacteria tested, ranging from 18.5 - 32.50 mm, in some cases surpassing standard antibiotics. These findings highlighted the potential of combining DES with UAE and SE to enhance the recovery of bioactive compounds with both antioxidant and antibacterial properties, and support the valorisation of mangosteen pericarp as a natural ingredient in functional foods and natural preservatives, aligning with sustainable and health-conscious food production trends.

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Introduction

Mangosteen (*Garcinia mangostana* L.), often referred to as the "queen of tropical fruits," is a fruit-bearing tree native to Southeast Asia. This fruit is widely appreciated for its unique flavour and therapeutic potentials. Widely consumed in Thailand, Malaysia, Indonesia, and the Philippines, mangosteen has also been used in folk medicine for centuries to treat infections, wounds, diarrhoea, and skin disorders (Moongkarndi *et al.*, 2004; Pothitirat *et al.*, 2010). Botanically, a fully matured mangosteen fruit has dark red-purple skin, and typically measures 5 - 7 cm

in diameter, with a pericarp thickness ranging from 6 - 10 mm (Zadernowski *et al.*, 2009). Structurally, the fruit is made up of 31% pulp, 48% inner pericarp, 17% outer pericarp, and 4% cap or calyx (Ibrahim *et al.*, 2017).

Within the genus *Garcinia*, with over 300 species, numerous bioactive secondary metabolites have been identified. These include xanthenes, flavonoids, triterpenoids, anthocyanins, benzophenones, and tannins (Chin and Kinghorn, 2008). Notably, the mangosteen pericarp, often discarded as waste in fruit processing, is particularly rich in polyphenolic compounds that have been

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shown to demonstrate antioxidants, anti-inflammatory, anticancer, and neuroprotective activities (Aisha *et al.*, 2012; Oberholzer *et al.*, 2018). For instance, xanthenes such as α - and β -mangostin have shown promising pharmacological activities, including antibacterial, antifungal, and antiproliferative effects (Mughtaridi and Wijaya, 2017). These compounds have exhibited inhibitory effects against common bacterial pathogens like *Staphylococcus aureus*, *Salmonella Typhi*, and *Escherichia coli*, suggesting their potential use in natural antibacterial formulations (Jacob *et al.*, 2016).

Despite their therapeutic value, the efficient recovery of bioactive polyphenols from mangosteen pericarp is challenging due to the sensitivity of these compounds to oxidation, pH, and thermal degradation (de Souza *et al.*, 2015). Also, conventional solid-liquid extraction methods often require prolonged extraction times, and will consume large volumes of organic solvents, raising concerns about environmental and safety impacts. Due to these, green extraction technologies have gained significant attention. Deep eutectic solvents (DES), formed by the eutectic mixture of a hydrogen bond donor and acceptor, represent a new class of environmentally friendly solvents that offer advantages such as low volatility, biodegradability, and tunable polarity (Gullón *et al.*, 2020). Unlike ionic liquids, which pose concerns regarding cost, toxicity, and recyclability (Keskin *et al.*, 2007), DES is considered a safer and more sustainable alternative, and it has been successfully applied in the extraction of polyphenols, flavonoids, and plant proteins (Patra *et al.*, 2023).

In addition, advanced extraction techniques such as microwave-assisted extraction (MAE) and ultrasonic-assisted extraction (UAE) have been shown to enhance the yield and stability of thermolabile phytochemicals by reducing extraction time and solvent consumption (Nayak *et al.*, 2015; Yuvanatemiya *et al.*, 2022). These techniques, in conjunction with DES, offer promising strategies for maximising the recovery of bioactive constituents from agricultural by-products like mangosteen pericarp.

Previous studies highlighted the factors influencing the efficacy of bioactive compounds, including pre-treatments such as using freeze drying and hot air drying (Soontornwat *et al.*, 2024). It was found that freeze drying yielded the best recovery of α -mangostin. However, freeze drying consumes more energy and is possibly more expensive. Therefore,

considering the significant bioactive content and underutilised potential of mangosteen pericarp, the present work aimed to investigate its antioxidant and antibacterial properties using various extraction methods, including DES, UAE, and MAE. The findings would contribute to the valorisation of fruit waste into high-value natural products for use in nutraceuticals, food preservatives, or pharmaceutical applications.

Materials and methods

Chemicals

The chemicals used for extraction and analysis were 80% ethanol, distilled water, deep eutectic solvents, choline chloride, lactic acid, Folin-Ciocalteu phenol reagent, deionised water, 20% sodium carbonate (Na_2CO_3), distilled water, 10% aluminium chloride and potassium acetate, 10% vanillin, 8% hydrochloric acid (HCL), DPPH reagent (2,2-diphenyl-1-picryl-hydrazyl), 2,2'-Azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium solution (ABTS), and 2.45 mM potassium persulphate. The standards used for the calibration curve were gallic acid and quercetin, while Trolox was used as the standard antioxidant. Lastly, for the antibacterial activity test, MHA powder, distilled water, 0.5 McFarland standard, and streptomycin (STREP 10) were prepared.

Plant material and sample preparation

The mangosteen pericarp powder was purchased from a local supplier, and passed through a 250- μm laboratory sieve to obtain a consistent powder. Then, the powders were packed in airtight containers, and kept at room temperature (25°C) for subsequent analyses.

Deep eutectic solvent (DES) preparation

DES were prepared according to Hernández-Corroto *et al.* (2020) with some modifications. The mixture of choline chloride and lactic acid (ChCl:LA) at a molar ratio of 1:2 was first heated in a water bath at 80°C for 2 h, and later shaken using a magnetic stirrer. Then, the temperature was turned to 50°C until a clear liquid was produced overnight.

Extraction process

i) Solvent extraction (SE)

Briefly, mangosteen pericarp powder was mixed with 50 mL of ethanol at a ratio of 1:10 at 50°C

for 1 h. The obtained filtrate was subjected to a rotatory evaporator to remove the solvent. Then, the extracts were weighed and kept in a freezer at -20°C until subsequent analyses. The following steps were repeated using DES ($n = 3$).

ii) Oven extraction (OE)

The extraction started with 5 g mangosteen pericarp powder mixed with 50 mL of solvent at a ratio of 1:10. The extraction procedure was carried out in an oven for 1 h and 50°C. The filtrate was kept in a freezer at -20°C until subsequent analyses. These following steps were repeated using DES by replacing the filtration procedure with centrifugation, and the experiments were run two times for each solvent.

iii) Ultrasound-assisted extraction (UAE)

The UAE extraction process was replicated following the same steps as SE and OE, but with a modification in the equipment utilised, specifically employing a power sonic instrument. The optimum condition for the ultrasonic power process was described by Suryono *et al.* (2019) at a frequency of 40 kHz, a temperature of 50°C, and a sonication time of 1 h.

Chemical and physical analyses

i) Total phenolic content (TPC)

The TPC of the mangosteen pericarp extracts (MPE) was determined by the Folin-Ciocalteu colorimetric technique (Singleton and Rossi, 1965; Krisanti *et al.*, 2021). A standard solution of gallic acid was prepared, and the mixture was incubated for 2 h. A UV-vis spectrophotometer was used to detect the intensity at 760 nm once incubation was done. Results were expressed as mg GAE/g of crude extract. For a more reliable result, samples were performed in duplicate (each batch), and similar procedures were repeated using MPE extracted from DES.

ii) Total flavonoid content (TFC)

The methodology proposed by Krisanti *et al.* (2021) was used as a guide for the aluminium chloride colorimetric method for the determination of TFC. Quercetin served as a standard curve, and the final absorbance was measured at 400 - 500 nm using a UV-Vis spectrophotometer. Data were expressed as milligrams of quercetin equivalent (mg QE/g) for each gram of dried sample extract. Samples were

performed in triplicate, and similar procedures were repeated using MPE extracts from DES.

iii) Total monomeric anthocyanin content (TMAC)

The pH differential technique was used to determine the TMAC. The extracts from MPE were subjected to buffers of pH 1.0 and 4.5, respectively, and mixtures were allowed to stand for 20 min before being read by the UV-Vis spectrophotometer at 510 and 700 nm. Eq. 1 was then used to compute the TMAC (Ng and Cheok, 2020).

$$\text{TMA (mg/L)} = \frac{A \times \text{MW} \times \text{DF} \times 1000}{e \times l} \quad (\text{Eq. 1})$$

where, A = absorbance: (A510 - A700) pH 1.0 - (A510 - A700) pH 4.5; MW = molecular weight: 449.2 g/mole; DF = dilution factor; e = molar absorptivity coefficient: 26,900 L/mol/cm; and l = path length in cm.

Colour measurement

MP extracts were subjected to colorimetric evaluation or colour testing using a Hunter Lab Colorflex EZ (USA) colourimeter (Deylami *et al.*, 2016). The samples were transferred to opaque quartz after the instrument had been standardised with various colour tiles. The duplicates of the result from the quartz container were gathered. Using CIE Lab* coordinates, the colour values were defined (Deylami *et al.*, 2016). The quantitative characteristic of colour is known as chroma (C*) which was calculated using Eq. 2:

$$C^* = \sqrt{a^{*2} + b^{*2}} \quad (\text{Eq. 2})$$

pH measurement

The pH was determined using a pH meter. The pH meter needle moves once it indicated a settling location. The pH reading displayed by the pH meter needle was then noted (Astuti *et al.*, 2017).

Antioxidant activity

ABTS radical scavenging assay

The ABTS assay of MPE was conducted according to Fattahi *et al.* (2011) with slight modification. To produce the ABTS radical cation (ABTS^{•+}), 100 mL of potassium persulphate solution (2.45 mM) and 100 mL of ABTS^{•+} solution (7 mM) were combined and incubated in the dark for 24 h. Then, the ABTS solution was diluted using 80% ethanol, resulting in an absorbance value of 0.70 ±

0.05 at 734 nm. Next, 2 mL of ABTS•+ radical cation solution and 20 µL of the sample extract were mixed for 5 min before detecting the absorbance at 734 nm. Trolox served as the standard curve.

Antibacterial activity

The agar disk diffusion method was applied to test the antibacterial activity of MPE after the extraction process from SE, OE, and UAE against two categories of bacteria, namely Gram-positive (*Bacillus cereus* and *Staphylococcus aureus*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*, and *Proteus mirabilis*). The test bacteria were obtained from stock cultures of the Food Microbiology Laboratory, Universiti Malaysia Terengganu.

Preparation of McFarland turbidity standard

Under an established method, the McFarland standard was developed (Hudzicki, 2009). Microbial turbidity was adjusted to 0.5 McFarland standard. A UV spectrophotometer was employed. The UV spectrophotometer featured a 1 cm light path and corresponding cuvette, and the absorbance was evaluated at 625 nm, ranging from 0.08 to 0.13, to ensure that the density of the turbidity standard was appropriate.

Bacterial inoculation

Test bacteria were inoculated into MHB and incubated. Using a needle and an aseptic technique,

6.0 mm antimicrobial susceptibility discs (sterilised blank discs) containing 20 µL of MPE were applied onto the agar. As a negative control, distilled water was placed onto a blank disc. A positive control was additionally included for the bacterial strains, consisted of a 10-µg streptomycin (STREP 10) antibiotic disc. The diameter of the growth inhibitory zone was measured using a ruler, and reported in mm following incubation at 37°C for 16 - 24 h (Mun, 2017).

Statistical analysis

The quantitative dependent variable at multiple levels of two categorical independent variables was analysed using a Two-way analysis of variance (Two-way ANOVA), and the least significant difference (LSD) test was used to determine whether there was a significant difference at $p < 0.05$ using Minitab software version 21.

Results and discussion

Extraction yields

Figure 1 shows the percentage of MPE total yield (%) extracted using different solvents and extraction methods. There was no significant interaction between the two factors, which had a p -value of 0.052 ($p > 0.05$). However, when focusing on the type of solvent used to extract the bioactive compounds, a significant different was noted.

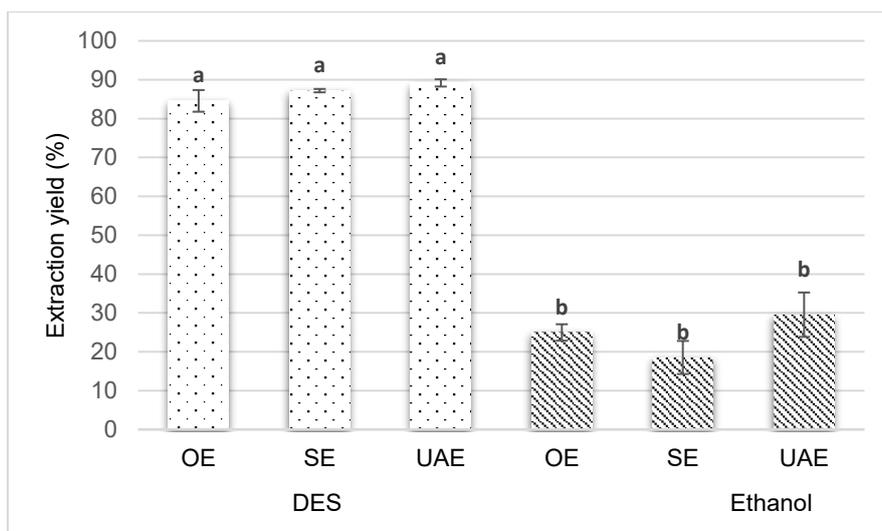


Figure 1. Total extraction yields of mangosteen (*Garcinia mangostana* L.) pericarp extracted using solvent, oven, and ultrasonic-assisted extraction method using ethanol and DES. Means with different lowercase letters are significantly different between solvents used ($p < 0.05$). OE: oven extraction; SE: solvent extraction; UAE: ultrasonic-assisted extraction; Dot bar: DES; and stripe bar: ethanol.

The choice of solvents significantly impacted extraction yields ($p < 0.05$), with DES showing the highest mean yield (86.97%), as compared to ethanol that only yielded 24.25% extraction. DES, formed by combining lactic acid and choline chloride, facilitated the breakdown of water-soluble and macromolecular substances in mangosteen pericarps (Saini *et al.*, 2022). Polar characteristics of DES also impacted extraction efficiency, particularly at high-temperature extraction, enhancing intermolecular interactions (Machmudah *et al.*, 2018). Another study also suggested that DES, when combined with water-based media, improved the extraction of water-soluble elements from mangosteen pericarps (Xu *et al.*, 2015). Meanwhile, Machmudah *et al.* (2018) found that extracting xanthone at temperatures ranging from 120 to 160°C using subcritical water extraction with 10% DES increased its yield significantly, reaching up to 59.14%. In the present work, the UAE extraction achieved the highest extraction yields (59.36%), followed by OE (54.76%) and SE (52.86%). The increased extraction yield with the UAE and SE methods may result from the intense ultrasound power and agitation, allowing for better interaction between solvents and samples during extraction. The versatility of the UAE in accepting alternative solvents may offer economic, environmental, and health benefits (Hiranrangsee *et al.*, 2016). Hence, extraction efficiency depends on various factors, including solvent properties, sample particle sizes, solvent-to-material ratios, extraction temperature, and procedure duration.

Phytochemical analysis of mangosteen pericarp extracted using solvent, oven, and ultrasonic-assisted extraction methods using ethanol and DES

i) Total phenolic content

Figure 2A shows the TPC of MPE extracted with different solvents and techniques. It was apparent that the TPC was significantly influenced by the combined effects of extraction methods and different solvents used ($p = 0.007$). DES-SE showed the highest TPC (587.50 ± 41.45 mg GAE/g), followed by DES-UAE (440.79 ± 10.53 mg GAE/g), and the obtained data aligned with the previous finding (Hiranrangsee *et al.*, 2016). As stated from previous research, the TPC of fresh mangosteen pericarp using solvent water shaking extraction DES-SE were 2493.56 ± 40.84 mg GAE/100 g, which was higher than the values obtained with UAE-DES,

which were 1975.00 ± 74.34 mg GAE/100 g (Hiranrangsee *et al.*, 2016). This result was due to the effective particle-solvent incorporation and increased surface area exposure contributed to elevated TPC values (Hiranrangsee *et al.*, 2016). DES with extraction methods generally yield higher phenolic extraction efficiency than ethanol, with ethanol extraction resulting in lower TPC values. However, ethanol-SE exhibited higher values than ethanol-UAE, indicating the effectiveness of SE and UAE in extracting phenolic compounds. Ethanol-OE yielded the lowest TPC (185.26 ± 18.95 mg GAE/100 g), like conventional extraction methods that yielded lower TPC values. This finding underscores the advantages of advanced extraction techniques like water-bath shaking and ultrasonic extraction over conventional methods. It is also noteworthy that the type of DES might have significant impact on the recovery of these bioactive compounds. As highlighted by Saini *et al.* (2022), acid-based DES, lactic acid in the present work, was effective in extracting polyphenols which could relate to the polarity of the DES.

ii) Total flavonoid content

Figure 2B shows no significant difference or interaction between the two independent variables ($p = 0.02$) on TFC of MPE. Although there was no correlation between these factors, each extraction method and solvent exhibited significant differences individually ($p < 0.05$). UAE yielded the highest TFC (694.83 mg QUE/g), followed by SE (633.17 mg QUE/g), while OE exhibited the lowest (435.67 mg QUE/g). Our data agreed with those of Ghasemzadeh *et al.* (2018), who showed a TFC of 279.19 ± 19.55 mg/100 g. Their study utilised optimised parameters for microwave-assisted extraction, yielding TFC higher than TPC. Similar results were observed in the present work, with higher TFC (587.89 mg QUE/g) than TPC (333.74 mg GAE/100 g) using various solvents and extraction methods. DES exhibited significantly higher extraction values than ethanol ($p < 0.05$) for TFC in MPE. DES yielded TFC of 770.78 mg QUE/g compared to ethanol (405.78 mg QUE/g). These results might indicate the importance of DES composition, which was acid-based, and had higher polarity than ethanol (Saini *et al.*, 2022). The low TFC by ethanolic extraction might possibly be due to their low polarity, contributed by the high number of methoxyl groups of flavonoids in the pericarp (Vo *et al.*, 2023).

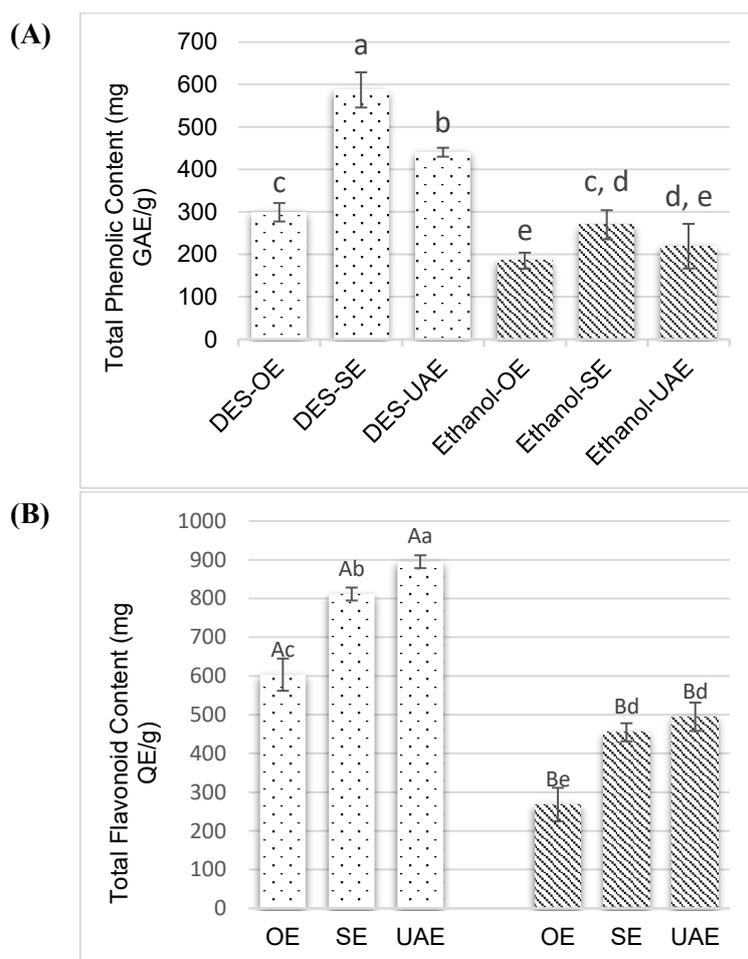


Figure 2. Total phenolic contents (A) and total flavonoid contents (B) of mangosteen (*Garcinia mangostana* L.) pericarp extracted using solvent, oven, and ultrasonic-assisted extraction method using ethanol and DES. Means with different lowercase letters are significantly different between solvents used ($p < 0.05$). OE: oven extraction; SE: solvent extraction; UAE: ultrasonic-assisted extraction; Dot bar: DES; and stripe bar: ethanol.

iii) Total monomeric anthocyanin content

Figure 3 shows the total TMAC of MPE obtained using different solvents and extraction methods. The correlation between solvents and extraction methods had a significant impact ($p = 0.001$). DES-SE yielded the highest TMAC (333.98 ± 41.75 mg cyd-3-glu/g), followed by DES-UAE (229.61 ± 20.87 mg cyd-3-glu/g), while both ethanol-SE and ethanol-OE yielded 125 ± 25.05 mg cyd-3-glu/g, and the lowest was from ethanol-UAE (66.80 ± 16.70 mg cyd-3-glu/g). Acidified solvents have been explored for anthocyanin extraction in other research due to their ability to break down cell tissue membranes (Pham *et al.*, 2022). Various studies have confirmed the utilisation of citric, acetic, hydrochloric, and formic acids in this process (Joshi and Devi, 2015; Bosiljkov *et al.*, 2017; Noola *et al.*, 2022; Pham *et al.*, 2022). Additionally, acid-based DES, which are known for their proton donation and

electron acceptance capabilities, exhibit outstanding dissolution properties, thus making them suitable for extracting polar compounds such as anthocyanins (Bosiljkov *et al.*, 2017). While ethanol showed lower efficacy compared to DES, no significant difference was observed between DES-OE, ethanol-SE, and ethanol-OE, possibly due to weak cooperation between solvents and extraction methods in oven extraction. Ethanol-UAE yielded the lowest TMAC (66.80 ± 16.70 mg cyd-3-glu/g), contrary to previous studies, indicating higher extraction values with UAE. The lower TMAC obtained with UAE may result from less homogenisation between the solvent and sample compared to HAE. Additionally, the results suggested that anthocyanidin and sugar molecules within anthocyanins in the pericarp might have influenced the UAE process (Albuquerque *et al.*, 2023).

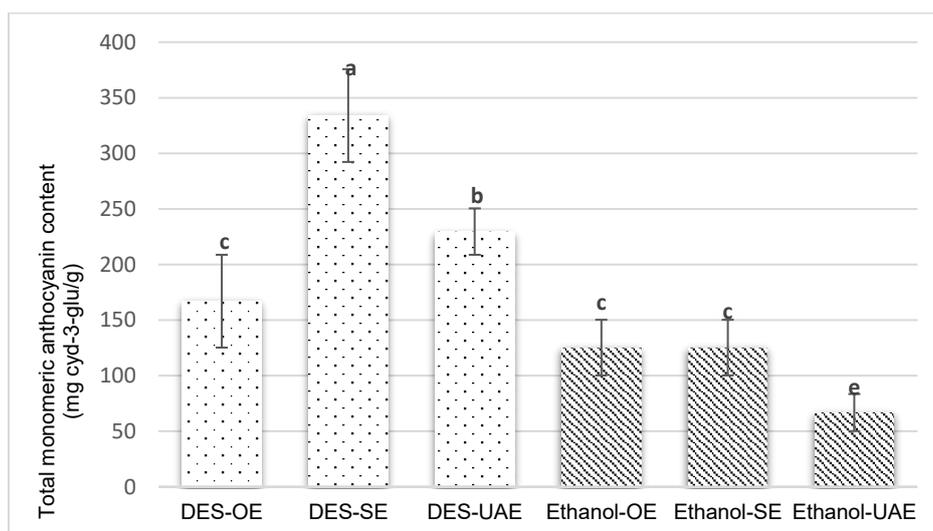


Figure 3. Total monomeric anthocyanin content of mangosteen (*Garcinia mangostana* L.) pericarp extracted using solvent, oven, and ultrasonic-assisted extraction method using ethanol and DES. Means with different lowercase letters are significantly different between solvents used ($p < 0.05$). OE: oven extraction; SE: solvent extraction; UAE: ultrasonic-assisted extraction; Dot bar: DES; and stripe bar: ethanol.

Antioxidant activity analysis

ABTS radical scavenging assay

Figure 4 shows the antioxidant capacity of MPE, assessed by calculating the proportion of the ABTS•+ radical that it inhibited (inhibition %) and the total concentration of ABTS presented in the MPE using different solvents and extraction methods. Significant variations emerged among different solvents ($p < 0.05$), with ethanol exhibiting the highest ABTS content and inhibition values of 592.28 mg TE/g and 54.45% inhibition. Ethanol-UAE yielded the highest values (659.500 ± 121.67 mg TE/g and $59.76 \pm 9.70\%$), followed by ethanol-OE (622.83 ± 95.00 mg TE/g and $56.84 \pm 7.57\%$), and ethanol-SE (494.50 ± 8.82 mg TE/g and $46.75 \pm 0.66\%$). However, DES showed lower efficacy, with a mean ABTS value of 341.500 mg TE/g and 17.962% inhibition. The DES-UAE combination exhibited total ABTS and inhibition values of 356.42 ± 6.66 mg TE/g and $18.69 \pm 0.13\%$, followed by DES-OE (342.50 ± 2.08 mg TE/g and $17.96 \pm 0.07\%$) and DES-SE (319.58 ± 8.33 mg TE/g and $17.24 \pm 0.26\%$). The lower antioxidant activity of DES extraction may be attributed to the high viscosity of the DES that might hinder the actual transfer of antioxidant compounds to the ABTS radical tested. Moreover, the reduced antioxidant activity of DES extraction may be due to thermodynamic factors governing solvent-solute interactions (Abarca-

Vargas *et al.*, 2016). Moreover, altering the molar ratio of DES constituents ran the risk of reducing the antioxidant activity of mangosteen pericarp due to increased viscosity, as studies indicated that increasing DES water content by 10 - 20% may yield a roughly 4.18% increase in antioxidant activity (Ling *et al.*, 2020). These findings emphasised the significance of optimising extraction parameters and the type of solvent to achieve extracts with maximum antioxidant activity.

Physical analysis

Colour and pH analysis

The colour saturation or intensity of MPE is shown in Figure 5A. The type of solvent showed a significant difference ($p < 0.05$), and had a different chroma value for both DES and ethanolic extracts.

The chroma values varied significantly between DES and ethanolic extracts, with DES extraction yielding higher values. DES-SE had the highest chroma (8.355 ± 1.03), followed by DES-UAE (7.20 ± 0.06). However, DES-OE (4.33 ± 0.25) showed no significant difference with all the ethanolic extracts, namely ethanol-SE (3.60 ± 0.52), ethanol-UAE (3.45 ± 1.02), and ethanol-OE (4.03 ± 0.11). DES may facilitate the targeted extraction of bioactive components and pigments, thereby increasing chroma values. SE and UAE exhibited higher chroma values than OE in DES, indicating

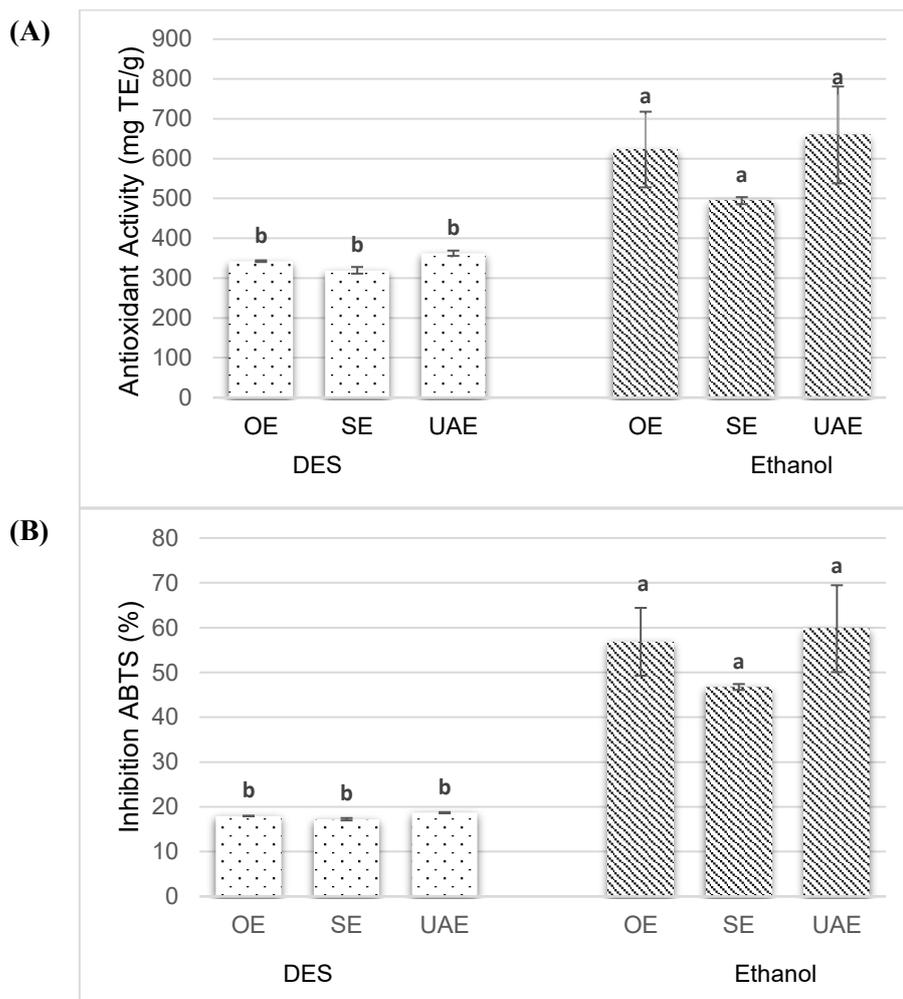


Figure 4. Total antioxidant activities (A) and percentage of ABTS inhibition (B) of mangosteen (*Garcinia mangostana* L.) pericarp extracted using solvent, oven, and ultrasonic-assisted extraction method using ethanol and DES. Means with different lowercase letters are significantly different between solvents used ($p < 0.05$). OE: oven extraction; SE: solvent extraction; UAE: ultrasonic-assisted extraction; Dot bar: DES; and stripe bar: ethanol.

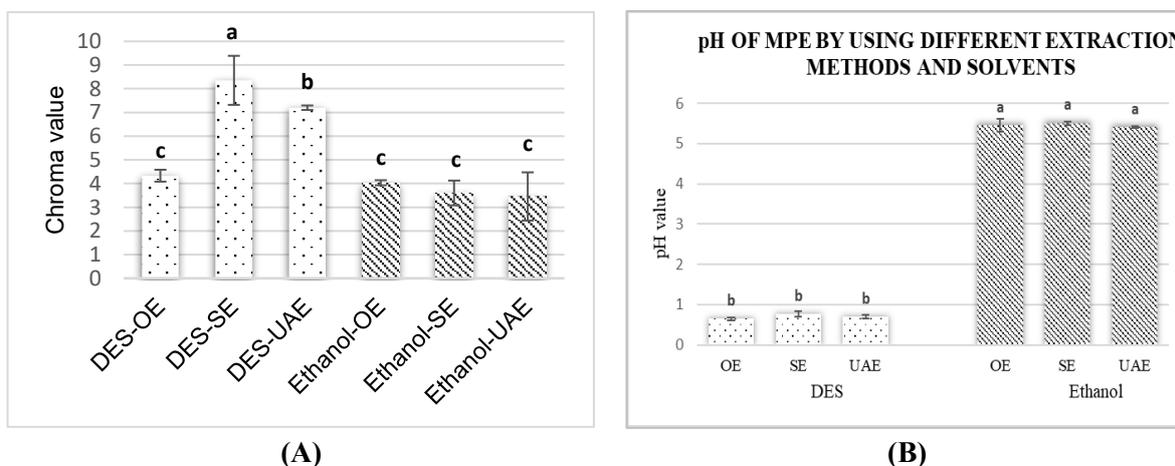


Figure 5. Chroma values (A) and pH values (B) of mangosteen (*Garcinia mangostana* L.) pericarp extracted using solvent, oven, and ultrasonic-assisted extraction method using ethanol and DES. Means with different lowercase letters are significantly different between solvents used ($p < 0.05$). OE: oven extraction; SE: solvent extraction; UAE: ultrasonic-assisted extraction; Dot bar: DES; and stripe bar: ethanol.

more intense colouration. Varying solvent polarities can influence both the extraction process and the stability of isolated chemical compounds, thus impacting the final extract intensity. Additionally, the efficiency of sample-solvent interactions plays a crucial role in determining extract colour intensity (Altemimi *et al.*, 2017). One study has shown that extraction techniques, such as using acetone, could affect pigment properties, and had stronger extracting effect than ethanol, indicating the importance of solvent choice in determining fruit peel colour and pigment structure (Kurniawan *et al.*, 2020).

Figure 5B indicates no significant difference between extraction methods, but the solvent type had a notable effect on the pH analysis ($p < 0.05$) of MPE. Ethanolic extracts displayed slightly acidic pH values, with ethanol-SE at 5.51 ± 0.05 , ethanol-OE at 5.36 ± 0.16 , and ethanol-UAE at 5.40 ± 0.02 . The decrease in pH from the original ethanol pH of 7 may be attributed to phenolic compounds in the extracts. As indicated in another study, orange peel and horchata by-products showed similar acidity levels in ethanolic extracts, which ranged from 4.91 ± 0.00 to 5.97 ± 0.01 (Montero-Calderon *et al.*, 2019). Conversely, DES, containing lactic acid, lowered pH significantly as DES-SE at 0.765 ± 0.065 , DES-UAE at 0.705 ± 0.045 , and DES-OE at 0.645 ± 0.035 . Variations in pH can impact extraction efficiency and stability, particularly anthocyanins that have unique molecular structures at various pH values, thus affecting final coloration and antioxidant properties (Can-Cauich *et al.*, 2017; Munekata *et al.*, 2023). Therefore, pH levels are crucial factors influencing bioactive substance behaviour and stability in plant

material extraction.

Antimicrobial activity analysis

The agar disk diffusion method assessed the antibacterial activity of MPE using SE, MAE, and UAE against Gram-positive and Gram-negative bacteria. The results of the inhibition zones (mm) are shown in Table 1. There was no observed direct correlation between solvent type and extraction method. Solvent types emerged as the critical variable, exhibiting notable antibacterial efficacy of MPE rather than the type of extraction methods employed. In comparison with the positive control streptomycin, this finding highlighted the superiority of DES, particularly in inhibiting both Gram-negative and Gram-positive bacteria. DES extraction exhibited stronger inhibition effects compared to streptomycin, with average inhibition values of 24.3 mm for *Escherichia coli*, 29.3 mm for *Pseudomonas aeruginosa*, 27 mm for *Proteus mirabilis*, 21 mm for *Staphylococcus aureus*, and 29.3 mm for *Bacillus cereus*. Prior research validated the superior antimicrobial potency of DES, attributed to their acidic nature, primarily lactic acid content, creating an unfavourable environment for bacterial growth and survival (Swebocki *et al.*, 2023). Interestingly, in comparison with the negative control, there was no inhibition zones appeared for all bacteria tested. The present work showed that the antibacterial effect observed was solely based on the bioactive compounds found in MPE.

Oliveira *et al.* (2021) showed that the antibacterial abilities of DES extracts from *Curcuma longa* L. leaves had the inhibition of 23.70 mm

Table 1. Antibacterial activities of mangosteen (*Garcinia mangostana* L.) pericarp extracted using solvent, oven, and ultrasonic-assisted extraction method using ethanol and DES.

Extract	Inhibition zone (mm)				
	Gram-negative bacteria			Gram-positive bacteria	
	<i>E. coli</i>	<i>P. florescence</i>	<i>P. mirabilis</i>	<i>S. aureus</i>	<i>B. cereus</i>
SE (ethanol)	7.00 ± 0.00	7.00 ± 0.00	7.50 ± 0.15	NI	NI
OE (ethanol)	7.00 ± 0.00	7.00 ± 0.00	7.75 ± 0.36	NI	NI
UAE (ethanol)	7.00 ± 0.00	7.00 ± 0.00	8.00 ± 0.20	NI	NI
SE (DES)	22.00 ± 2.80	27.50 ± 2.12	25.00 ± 2.80	18.50 ± 2.12	29.00 ± 1.41
OE (DES)	24.50 ± 2.12	29.00 ± 1.41	26.00 ± 1.41	22.50 ± 2.12	26.50 ± 0.71
UAE (DES)	26.50 ± 0.71	31.50 ± 2.12	30.00 ± 2.20	22.00 ± 1.41	32.50 ± 0.71
Positive control (streptomycin 10 µg)	15.50 ± 0.72	14.50 ± 2.12	18.50 ± 0.71	16.50 ± 1.41	24.50 ± 0.71
Negative control (DES and ethanol)	7.00 ± 0.00	7.05 ± 0.10	7.00 ± 0.00	NI	NI

Values are mean ± standard deviation of two replications ($n = 2$). Low inhibition: 0 - 15 mm; moderate inhibition: 16 - 20 mm; high inhibition: > 21 mm. NI: no inhibition.

against *Listeria monocytogenes*, 18 mm against *Clostridium perfringens*, 21 mm against *Staphylococcus aureus* (Gram-positive bacteria), whereas Gram-negative bacteria like *Escherichia coli* had inhibition zone of 15.1, and 18.8 mm for *Salmonella* sp. These findings supported the fact that DES derived from lactic acid could stabilise medicinal substances, and enhance their antibacterial effectiveness, such as β -lactam antibiotics (Olivares et al., 2018). The water content of DES also influenced antibacterial effects, with higher acid concentrations promoting greater inhibition as reported by Radošević et al. (2018). In the present work, the DES was synthesised using a molar ratio of 2:1 for lactic acid to choline chloride, resulting in a more acidic solvent. This increased acidity is anticipated to improve antibacterial activity by creating a more unfavourable environment for bacterial growth, thereby enhancing the inhibitory properties.

In contrast, ethanolic extracts from the three extraction methods showed minimal antibacterial effects, with an average inhibition zone of approximately 7.5 mm for Gram-negative bacteria, less effective than the positive control (streptomycin), and particularly ineffective against all Gram-positive bacteria. Ethanolic extraction methods exhibited no significant differences in antibacterial effects, as demonstrated by previous findings, which indicated an inhibition zone of 6.5 mm for *E. coli*, while *S. Enteritidis* exhibited no inhibition area (Pohan and Rahmawati, 2022). From this assay, it was clear that the MP extracted from DES exhibited superior antibacterial activity as opposed to the ethanolic extract, suggesting a potential application as a natural antibacterial agent. However, it is worth mentioning that other assays, such as minimum inhibitory/bactericidal concentration assay (MIC/MBC), could be conducted in the future, which could be more suitable for assessing both hydrophilic and hydrophobic compounds present in DES and ethanolic extracts.

Conclusion

The present work investigated the antioxidant and antibacterial activities of mangosteen (*Garcinia mangostana* L.) pericarp extracts using ethanol and deep eutectic solvents (DES) across various extraction techniques. Statistical analysis revealed

significant interactions between solvent types and extraction methods in relation to total phenolic content (TPC), total monomeric anthocyanin content (TMAC), and colour parameters. Notably, the combination of SE with DES was found to achieve the highest TPC, TMAC, and chroma values, indicating effective extraction of anthocyanins and phenolic compounds. DES also demonstrated superior extraction efficiency, and yielded lower pH levels compared to ethanol. For TFC, UAE methods exhibited significantly higher extraction yields. Furthermore, DES-based extracts showed the highest antioxidant activity in the ABTS assay, and exhibited greater antibacterial efficacy than ethanolic extracts. In summary, the present work showcased notable variations in antioxidant activity, highlighting the effectiveness of DES across three extraction methods in chemicals and antimicrobial analysis.

Despite these promising findings, the present work had certain limitations. The chemical composition of DES was limited to a specific formulation, and possibly, challenging in scaling as DES is highly viscous. Moreover, as the scope of bioactive compounds was focused primarily on phenolics, flavonoids, and anthocyanins. Future research should explore a broader range of DES formulations to optimise the selectivity for targeted bioactive compounds. Investigating further antioxidant and antimicrobial activities using other assays in understanding the mechanistic pathways is also recommended. Finally, the scalability assessment will be critical to advance the practical application of DES-based extraction in valorising mangosteen pericarp.

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References

- Abarca-Vargas, R., Pena Malacara, C. F. and Petricevich, V. L. 2016. Characterization of chemical compounds with antioxidant and

- cytotoxic activities in *Bougainvillea x buttiana* Holtum and Standl, (var. Rose) extracts. *Antioxidants* 5(4): 45-56.
- Aisha, A. F., Abu-Salah, K. M., Ismail, Z. and Majid, A. M. 2012. *In vitro* and *in vivo* anti-colon cancer effects of *Garcinia mangostana* xanthenes extract. *BMC Complementary Medicine and Therapies* 12(1): 1-10.
- Albuquerque, B. R., Dias, M. I., Pinela, J., Calhelha, R. C., Pires, T. C., Alves, M. J., ... and Barros, L. 2023. Insights into the chemical composition and *in vitro* bioactive properties of mangosteen (*Garcinia mangostana* L.) pericarp. *Foods* 12(5): 994-1008.
- Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D. G. and Lightfoot, D. A. 2017. Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. *Plants* 6(4): 42-64.
- Astuti, K. W., Wijayanti, N. P. A. D., and Prasetya I. G. N. J. A. 2017. Development of gel dosage form of ethyl acetate extract of mangosteen rind (*Garcinia mangostana* L.). *Journal of Health Sciences and Medicine* 28(1): 28-32.
- Bosiljkov, T., Dujmić, F., Bubalo, M. C., Hribar, J., Vidrih, R., Brnčić, M., ... and Jokić, S. 2017. Natural deep eutectic solvents and ultrasound-assisted extraction: Green approaches for extraction of wine lees anthocyanins. *Food and Bioproducts Processing* 102: 195-203.
- Can-Cauich, C. A., Sauri-Duch, E., Betancur-Ancona, D., Chel-Guerrero, L., González-Aguilar, G. A., Cuevas-Glory, L. F., ... and Moo-Huchin, V. M. 2017. Tropical fruit peel powders as functional ingredients: Evaluation of their bioactive compounds and antioxidant activity. *Journal of Functional Foods* 37: 501-506.
- Chin, Y. W. and Kinghorn, A. D. 2008. Structural characterization, biological effects, and synthetic studies on xanthenes from mangosteen (*Garcinia mangostana*), a popular botanical dietary supplement. *Mini-Reviews in Organic Chemistry* 5(4): 355-364.
- de Souza, V. B., Thomazini, M., de Carvalho Balieiro, J. C. and Fávares-Trindade, C. S. 2015. Effect of spray drying on the physicochemical properties and color stability of the powdered pigment obtained from vinification byproducts of the Bordo grape (*Vitis labrusca*). *Food and Bioproducts Processing* 93: 39-50.
- Deylami, M. Z., Rahman, R. A., Tan, C. P., Bakar, J. and Olusegun, L. 2016. Effect of blanching on enzyme activity, color changes, anthocyanin stability and extractability of mangosteen pericarp: A kinetic study. *Journal of Food Engineering* 178: 12-19.
- Fattahi, J., Hamidoghli, Y., Fotouhi, R., Ghasemnejad, M. and Bakhshi, D. 2011. Assessment of fruit quality and antioxidant activity of three citrus species during ripening. *Journal of Horticulture Biology and Environment* 2(2): 113-128.
- Ghasemzadeh, A., Jaafar, H. Z., Baghdadi, A. and Tayebi-Meigooni, A. 2018. Alpha-mangostin-rich extracts from mangosteen pericarp: Optimization of green extraction protocol and evaluation of biological activity. *Molecules* 23(8): 1852-1867.
- Gullón, P., Gullón, B., Romani, A., Rocchetti, G. and Lorenzo, J. M. 2020. Smart advanced solvents for bioactive compounds recovery from agri-food by-products: A review. *Trends in Food Science and Technology* 101: 182-197.
- Hernández-Corroto, E., Plaza, M., Marina, M. L. and García, M. C. 2020. Sustainable extraction of proteins and bioactive substances from pomegranate peel (*Punica granatum* L.) using pressurized liquids and deep eutectic solvents. *Innovative Food Science and Emerging Technologies* 60: 102314.
- Hiranrangsee, L., Kumaree, K. K., Sadiq, M. B. and Anal, A. K. 2016. Extraction of anthocyanins from pericarp and lipids from seeds of mangosteen (*Garcinia mangostana* L.) by ultrasound-assisted extraction (UAE) and evaluation of pericarp extract enriched functional ice-cream. *Journal of Food Science and Technology* 53: 3806-3813.
- Hudzicki, J. 2009. Kirby-Bauer disk diffusion susceptibility test protocol. *American Society for Microbiology* 15(1): 1-23.
- Ibrahim, U. K., Kamarrudin, N., Suzihaque, M. U. H. and Abd Hashib, S. 2017. Local fruit wastes as a potential source of natural antioxidant: An overview. *IOP Conference Series - Materials Science and Engineering* 206(1): 012040.
- Jacob, D., Vignasini, N. and Iyer, P. R. 2016. Antibacterial activity of mangosteen (*Garcinia*

- mangostana*) pericarp. World Journal of Pharmaceutical Research 5(9): 1797-1802.
- Joshi, V. K. and Devi, M. P. 2015. Optimization of extraction treatment and concentration of extract on yield and quality of anthocyanins from plum var. 'Santa Rosa'. Indian Journal of Natural Products and Resources 5(2): 171-175.
- Keskin, S., Kayrak-Talay, D., Akman, U. and Hortaçsu, Ö. 2007. A review of ionic liquids towards supercritical fluid applications. The Journal of Supercritical Fluids 43(1): 150-180.
- Krisanti, E. A., Farizal, A. N. and Mulia, K. 2021. *Garcinia mangostana* L. fruit rind extract in ethyl acetate, *n*-butanol and water fractions: Phytochemical analysis, antioxidant assay and cytotoxicity assay. IOP Conference Series - Materials Science and Engineering 1053(1): 012040.
- Kurniawan, Y. S., Fahmi, M. R. G. and Yuliati, L. 2020. Isolation and optical properties of natural pigments from purple mangosteen peels. IOP Conference Series - Materials Science and Engineering 833(1): 012018.
- Ling, J. K. U., Chan, Y. S. and Nandong, J. 2020. Extraction of antioxidant compounds from the wastes of *Mangifera pajang* fruit: A comparative study using aqueous ethanol and deep eutectic solvent. SN Applied Sciences 2(8): 1365-1376.
- Machmudah, S., Lestari, S. D., Kanda, H., Winardi, S. and Goto, M. 2018. Subcritical water extraction enhancement by adding deep eutectic solvent for extracting xanthone from mangosteen pericarps. The Journal of Supercritical Fluids 133: 615-624.
- Montero-Calderon, A., Cortes, C., Zulueta, A., Frigola, A. and Esteve, M. J. 2019. Green solvents and ultrasound-assisted extraction of bioactive orange (*Citrus sinensis*) peel compounds. Scientific Reports 9(1): 16120.
- Moongkarndi, P., Kosem, N., Kaslungka, S., Luanratana, O., Pongpan, N. and Neungton, N. 2004. Antiproliferation, antioxidation and induction of apoptosis by *Garcinia mangostana* (mangosteen) on SKBR3 human breast cancer cell line. Journal of Ethnopharmacology 90(1): 161-166.
- Muchtaridi and Wijaya, C. A. 2017. Anticancer potential of α -mangostin. Asian Journal of Pharmaceutical and Clinical Research 10(12): 440-445.
- Mun, Y. M. 2017. Chemical constituents and bioactivity of selected Malaysian plants. Malaysia: Monash University, PhD thesis.
- Munekata, P. E., Pateiro, M., Domínguez, R., Nieto, G., Kumar, M., Dhama, K. and Lorenzo, J. M. 2023. Bioactive compounds from fruits as preservatives. Foods 12(2): 343-364.
- Nayak, B., Dahmoune, F., Moussi, K., Remini, H., Dairi, S., Aoun, O. and Khodir, M. 2015. Comparison of microwave, ultrasound and accelerated-assisted solvent extraction for recovery of polyphenols from citrus sinensis peels. Food Chemistry 187: 507-516.
- Ng, K. L. and Cheok, C. Y. 2020. Evaluation of thermal degradation kinetic order of anthocyanins extracted from *Garcinia mangostana* L. rind. Progress in Energy and Environment 13: 16-25.
- Noola, N., Gomez, S., Pathrose, B., Joseph, M. and Kuruvila, B. 2022. Comparison of extraction methods on anthocyanin pigment attributes from mangosteen (*Garcinia mangostana* L.) fruit rind as potential food colourant. Food Chemistry Advances 4: 100559.
- Oberholzer, I., Möller, M., Holland, B., Dean, O. M., Berk, M. and Harvey, B. H. 2018. *Garcinia mangostana* Linn displays antidepressant-like and pro-cognitive effects in a genetic animal model of depression: A bio-behavioral study in the flinders sensitive line rat. Metabolic Brain Disease 33: 467-480.
- Olivares, B., Martínez, F., Rivas, L., Calderón, C., Munita, J. M. and Campodonico, P. R. 2018. A natural deep eutectic solvent formulated to stabilize β -lactam antibiotics. Scientific Reports (8): 14900.
- Oliveira, G., Marques, C., de Oliveira, A., dos Santos, A. D. A., do Amaral, W., Ineu, R. P., ... and Mafra, M. R. 2021. Extraction of bioactive compounds from *Curcuma longa* L. using deep eutectic solvents: *In vitro* and *in vivo* biological activities. Innovative Food Science and Emerging Technologies 70: 102697.
- Patra, A., Prasath, V. A. and Pandiselvam, R. 2023. Deep eutectic solvent: An emerging trend for extraction of plant proteins. Journal of Molecular Liquids 389: 122887.
- Pham, T. N., Le, X. T. and Le, H. T. 2022. Effects of process parameters in microwave-assisted extraction on the anthocyanin-enriched extract from *Rhodomyrtus tomentosa* (Ait.) Hassk and

- its storage conditions on the kinetic degradation of anthocyanins in the extract. *Heliyon* 8(6): 1-8.
- Pohan, D. J. and Rahmawati, F. 2022. The effect of mangosteen pericarp (*Garcinia mangostana* Linn) extract on inhibits the growth of bacteria *Escherichia coli* ATCC 25922 and bacteria *Staphylococcus aureus* ATCC 25923. *International Journal of Research in Pharmacy and Pharmaceutical Sciences* 7(2): 29-38.
- Pothitirat, W., Chomnawang, M. T., Supabphol, R. and Gritsanapan, W. 2010. Free radical scavenging and anti-acne activities of mangosteen fruit rind extracts prepared by different extraction methods. *Pharmaceutical Biology* 48(2): 182-186.
- Radošević, K., Čanak, I., Panić, M., Markov, K., Bubalo, M. C., Frece, J., ... and Redovniković, I. R. 2018. Antimicrobial, cytotoxic and antioxidative evaluation of natural deep eutectic solvents. *Environmental Science and Pollution Research* 25: 14188-14196.
- Saini, A., Kumar, A., Panesar, P. S. and Thakur, A. 2022. Potential of deep eutectic solvents in the extraction of value-added compounds from agro-industrial by-products. *Applied Food Research* 2: 100211.
- Singleton, V. L. and Rossi, J. A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture* 16(3): 144-158.
- Soontornwat, A., Pongsuttiyakorn, T., Rakmae, S., Sritham, E., Sirisomboon, P., Pun, U. K., ... and Pornchaloempong, P. 2024. Mangosteen pericarp processing technology to create economic value and reduce biowaste. *Foods* 13(14): 2286.
- Suryono, S., Hadiyanto, H., Yasin, M., Widyowati, R. and Muniroh, M. 2019. Effect of frequency, temperature, and time of sonication on xanton content of mangosteen (*Garcinia mangostana* L.) peel extract through ultrasound assisted extraction. *E3S Web of Conferences* 125: 25006.
- Swebocki, T., Barras, A., Abderrahmani, A., Haddadi, K. and Boukherroub, R. 2023. Deep eutectic solvents comprising organic acids and their application in (bio)medicine. *International Journal of Molecular Sciences* 24(10): 8492-8512.
- Vo, T. P., Pham, N. D., Pham, T. V., Nguyen, H. Y., Tran, T. N. H., Tran, T. N. and Nguyen, D. Q. 2023. Green extraction of total phenolic and flavonoid contents from mangosteen (*Garcinia mangostana* L.) rind using natural deep eutectic solvents. *Heliyon* 9(4): 1-13.
- Xu, J., Wang, W., Liang, H., Zhang, Q. and Li, Q. 2015. Optimization of ionic liquid based ultrasonic assisted extraction of antioxidant compounds from *Curcuma longa* L. using response surface methodology. *Industrial Crops and Products* 76: 487-493.
- Yuvanatemiya, V., Srean, P., Klangbud, W. K., Venkatachalam, K., Wongsa, J., Parametthanuwat, T. and Charoenphun, N. 2022. A review of the influence of various extraction techniques and the biological effects of the xanthenes from mangosteen (*Garcinia mangostana* L.) pericarps. *Molecules* 27(24): 8775-8793.
- Zadernowski, R., Czaplicki, S. and Naczka, M. 2009. Phenolic acid profiles of mangosteen fruits (*Garcinia mangostana*). *Food Chemistry* 112(3): 685-689.