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Research

Ultrasonic-Assisted Extraction of Phenolic Compound From Harumanis Mango Leaves (*Mangifera indica*) Using Green Natural Deep Eutectic Solvents (NADESs)

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ABSTRACT

Harumanis mangoes, known for their taste and nutrition, are a symbol of Perlis, Malaysia. Natural Deep Eutectic Solvents (NADESs) are being studied for eco-friendly extraction methods. There is limited information on green extraction from Harumanis mango leaves, and NADESs have not been used for this purpose. This study aims to evaluate the green extraction of phenolic compounds from Harumanis mango leaves using five NADESs systems, assess the phytochemical composition, and test their antimicrobial potential. These NADESs were prepared by heating and stirring their components until a clear solution was formed. The extraction yields of NADESs were compared with conventional solvents like 100% ethanol, 100% methanol, 50% ethanol, and 50% methanol for extracting phenolic compounds. The chosen NADES was processed with Ultrasonic Assisted Extraction (UAE). The total phenolic content (TPC) in the extracts was measured using a gallic acid standard curve and spectrophotometry. The extract with the highest TPC value from NADES extraction was evaluated for phytochemicals using FTIR and tested for antimicrobial activity with the disc diffusion method. Based on the screening of different NADESs, the highest phenolic content was recorded by lactic acid: glycerol system at 135.74 mg GAE g^{-1} dw. The lowest TPC value was recorded with sodium acetate: glycerol system, which was 32.76 mg GAE g^{-1} dw. Among the conventional solvents, the highest TPC value was recorded by 50% ethanol at 133.09 mg GAE g^{-1} dw, and the lowest TPC value was by 100% ethanol at 67.18 mg GAE g^{-1} dw. The use of UAE with lactic acid: glycerol NADES system yields the highest TPC value of 142.87 mg GAE g^{-1} dw. Harumanis extract using NADES-3 had saponins, phenols, glycosides, tannins, and antimicrobial activity on gram-positive and gram-negative bacteria. The results show that NADES effectively extracts phenolic compounds from Harumanis leaves, which have significant therapeutic potential.

Key words: Green extraction, harumanis, *Mangifera indica*, natural deep eutectic solvents (NADESs), phenolic compounds, phytochemicals

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INTRODUCTION

Green extraction methods aim to obtain bioactive compounds from plant sources while minimizing environmental impact and reducing energy consumption. These methods utilize solvents that are non-toxic, renewable, and biodegradable, such as ionic liquids (ILs), deep eutectic solvents (DESs), and supercritical fluids (SCFs). Ionic liquids (ILs) are a novel class of solvents used for environmentally friendly extraction of bioactive compounds, offering advantages such as broad miscibility, low vapor pressure, non-flammability, good thermal properties, and recyclability (Liu *et al*., 2015; Hidalgo & Almajano, 2017; Airouyuwa *et al*., 2022). However, they have drawbacks like high production costs, toxicity, poor water stability, purification challenges, and low biodegradability (Smith *et al*., 2014; Tang *et al*., 2015; Airouyuwa *et al*., 2022).

Recently, researchers have turned to deep eutectic

solvents (DESs) for bioactive compound extraction. DESs are similar to ILs but are more eco-friendly, boasting advantages such as biodegradability, water stability, lower toxicity, and cost-effectiveness (Hidalgo & Almajano, 2017; Freitas *et al*., 2022). This shift reflects a growing commitment to greener extraction methods. However, DESs differ from ILs in their raw material source and chemical formation process. They are created through a chemical reaction between a halide salt or another hydrogen bond acceptor (HBA) and a hydrogen bond donor (HBD).

Natural Deep Eutectic Solvents (NADESs), a subtype of DES, have been developed by combining primary metabolites with bio-renewable materials like sugars, alcohols, organic acids, and amino acids (Cannavacciuolo *et al*., 2022; Freitas *et al*., 2022). NADES could be highly advantageous for dissolving, storing, or transporting metabolites that are insoluble in water within living cells and organisms. Furthermore, NADESs alleviate physicochemical constraints on metabolite transport and cellular processes through the formation of liquid microenvironments (Durand *et al*., 2021).

Previous studies have demonstrated the efficacy of DESs in extracting phytochemicals from various plant species, including *Herba epimedii* (Guo *et al*., 2020), *Acanthopanax senticosus* (Shi *et al*., 2020), Olive leaves (Akli *et al*., 2022), and Lotus (Yang *et al*., 2023). The reliability of the method attracts more studies on the samples using the same method, one of them is on the Harumanis mango sample. Harumanis mango stands out as a prominent mango variety, renowned for its appealing appearance, delightful sweetness, high nutritional value, and status as an icon of Perlis, the northernmost state in Malaysia. Limited flowering and fruit-bearing seasons lead to higher demand than supply, resulting in premium pricing.

To date, several studies have been conducted on Harumanis mangoes to enhance their quality and production. For instance, research has explored various fields such as methods to improve postharvest quality (Aifaa & Suhanna, 2015), visible imaging-based characterization (Ibrahim *et al*., 2016), analyses of morphological variability (Yusuf *et al*., 2018a; Yusuf *et al*., 2018b; Yusuf *et al*., 2020), molecular DNA marker analyses (Rahman *et al*., 2018; Rahman *et al*., 2020; Rahman *et al*., 2024), and extraction of phytochemicals using water and other common solvents (Peron *et al*., 2024).

None of the previous studies have explored the use of NADESs for green extraction of phytochemicals, such as phenolic compounds, from the unique Harumanis mango variety. Several studies have focused on extracting bioactive compounds using NADESs from various parts of non-Harumanis mango varieties, with mango peel being the primary source (Pal & Jadeja, 2020; Chen *et al*., 2022; Lajenkar *et al*., 2022). Lesser-explored parts like leaves, kernels, and seeds (agricultural wastes) could be valuable targets for NADESs extraction, providing additional insights and data on bioactive compounds.

Mismanagement of agro-industrial waste from mangoes contributes to environmental issues around fruit industries (García-Mahecha *et al*., 2023). Utilizing leaves, kernels, and seeds for bioactive compound extraction not only helps in managing waste but also generates substances applicable in various industries like food, pharmaceuticals, and cosmetics.

The extraction of bioactive compounds from natural sources, such as Harumanis leaves, is crucial for advancing therapeutic and nutraceutical applications. Traditional extraction methods often rely on organic solvents that can be toxic, environmentally damaging, and less efficient. This research addresses a significant gap in the field by exploring the use of Natural Deep Eutectic Solvents (NADES) for extracting bioactive compounds from Harumanis leaves. NADES, composed of natural and biodegradable components, offer a more sustainable alternative to conventional solvents (Dai *et al*., 2013; Paiva *et al*., 2014).

The novelty of this research lies in utilizing NADES to enhance the extraction efficiency and yield of bioactive compounds from Harumanis leaves, which are recognized for their potential health benefits but have not been extensively studied with this green chemistry approach. By incorporating NADES, this study aims to overcome the limitations of traditional extraction methods and contribute to a more eco-friendly and effective approach to natural product extraction (Smith *et al*., 2014). The application of NADES represents a promising advancement in green chemistry, addressing both environmental concerns and efficiency in the extraction process (Liu *et al*., 2018; Zhang *et al*., 2020). Hence, this study aims to synthesize five different NADESs and assess their green extraction efficiency for total phenolic compounds from local Harumanis mango leaves.

MATERIALS AND METHODS

Biological sample

The Harumanis leaves samples were collected from the Department of Agriculture at Bukit Temiang (Perlis, Malaysia) and washed thoroughly with sterile distilled water to remove impurities like dirt from insects and soil. The leaves were carefully separated from the stems and petioles, focusing solely on

the leaf material. Following this, the samples were dried for 24 hr at 50°C in a circulating air-drying oven (Akli *et al*., 2022). Once adequately dried, the leaves were further processed by being cut into smaller, manageable pieces using a sterile scalpel. These leaf pieces were then ground into a powdered form using a microfine grinder. The powdered samples were carefully stored in polyethylene containers, which were kept away from light exposure to prevent potential degradation over time.

Preparation of Natural Deep Eutectic Solvents (NADESs)

To conduct a comparative study, five different NADESs were synthesized using glycerol, betaine, lactic acid, and sodium acetate purchased from Sigma-Aldrich, Germany. The synthesis process was done according to the method described by Pal and Jadeja (2020) and Yang *et al*. (2023) with slight modifications.

The mixtures were heated at 70°C while stirring at 500 rpm until a perfectly transparent liquid was formed, with the process taking between 60 to 120 min. The NADESs were then stored in sealed glass vials in the dark at room temperature. Their stability in terms of color (remaining clear & homogeneous) and potential crystal formation were monitored regularly over at least one month. Information about the prepared NADESs is detailed in Table 1.

Table 1. Information regarding the various DES systems utilized

Type of NADES	HBA	HBD	Mole Ratio	Water content
NADES 1	Sodium Acetate	Glycerol	1:3	20%
NADES 2	Sodium Acetate	Lactic Acid	1:3	20%
NADES 3	Lactic Acid	Glycerol	1:3	20%
NADES 4	Betaine	Glycerol	1:2	20%
NADES 5	Betaine	Lactic Acid	1:2	20%

[#] The prepared DES system was mixed with 20% distilled water

Extraction methods

Heating-stirring extraction

The extraction procedure was done as previously reported method with slight modifications (Pal & Jadeja, 2020; Yang *et al*., 2023). The dried mango leaves sample was added to a beaker along with various DES systems and placed on a magnetic stirrer at 500 rpm and 60°C for 90 min.

The liquid-to-solid ratio was maintained at 50:1 ($mL\,g^{-1}$) for each sample. Following each extraction cycle, the samples were filtered using Whatman paper No. 1, and the resulting clear supernatant was used for analyzing the total polyphenolic content (TPC) via the Folin–Ciocalteu method.

Conventional solvent extraction and ultrasonic-assisted extraction

The common solvent extraction technique was utilized to extract phenolic compounds from dried Harumanis mango leaf samples for a comparative study as described by Pal and Jadeja et al. (2020) and Yang et al. (2023) with modification. In this method, 100% ethanol, 100% methanol, 50% ethanol, and 50% methanol were poured into a beaker along with an appropriate amount of dried Harumanis mango leaves samples, maintaining a liquid-to-solid ratio of 50:1.

The mixture was then stirred at 60°C and 500 rpm for 90 min. Afterward, the sample was filtered through the Whatman No. 1 filter paper, and the resulting extracts were analyzed to assess the TPC. The NADES with the highest TPC reading was selected for the subsequent procedure, which involved utilizing the Ultrasonic-Assisted Extraction method. This step was conducted to promptly evaluate the initial effects of employing Ultrasonic-Assisted Extraction with the chosen NADES for the extraction of total phenolic compounds.

The dried Harumanis mango leaves sample was mixed with the selected NADES at a liquid-to-solid ratio of 1:50 in a centrifuge tube. The mixture was then subjected to extraction at 60°C with an ultrasonic frequency of 37 kHz for 60 min. The sample was filtered through the Whatman No. 1 filter paper, and the resulting extracts were analyzed to assess the TPC.

Determination of polyphenol by Total Phenolic Content (TPC)

The Folin-Ciocalteu method, with slight adjustments from Sutivisedsak *et al*. (2010), was used to measure the total phenolic content in the sample. The absorbance of the samples was measured at 765 nm using a UV-spectrophotometer. To quantify the total phenolic content, a standard curve with concentrations from 10 to 1000 mg/L of gallic acid was used.

The absorbance of known concentrations of this standard solution was recorded using a UV-spectrophotometer. Plotting absorbance against concentration yielded a strong correlation (Y = 0.0009x + 0.006; R^2= 0.9984). The total phenolic content in the extracts was expressed in mg gallic acid equivalent per gram of dry weight of samples (Lajenkar *et al*., 2022).

Screening of the phytochemical composition and antimicrobial potential

The phytochemical composition of the extract was analyzed using modified protocols based on Rao *et al*. (2016). For phenol detection, a small amount of Harumanis mango leaf extract was mixed with 1 mL of water and a few drops of Iron III chloride ($FeCl_3$). The appearance of blue, green, red, or purple color indicates the presence of phenols.

To detect glycosides, a sample of the extract was mixed with water and a few drops of aqueous NaOH. A yellow color indicates the presence of glycosides. For tannin detection, 5 mL of the extract was mixed with 2 mL of 5% $FeCl_3$ solution. A greenish-black precipitate indicates the presence of tannins. The xanthoproteic test was used to test for proteins. Concentrated nitric acid was added to the extract, and a yellow color indicated the presence of proteins.

Analysis using Fourier Transform Infrared (FTIR) spectroscopy determined the functional groups in the sample. About 3 mL of the extract was added to a cuvette and the absorbance was measured at 517 nm in the path of an IR source. The FTIR spectra were then analyzed to assess the sample's ability to absorb infrared energy at different wavelengths, indicating its molecular composition and structure. The spectra were compared to a database for identifying the functional groups.

For the preliminary antimicrobial screening, *Escherichia coli* (a gram-negative bacterium) and *Bacillus* sp. (a gram-positive bacterium) were used as target organisms. The Harumanis leaves sample, extracted with the selected NADES, was tested using the disc diffusion method. A series of extract dilutions, ranging from 300 µg to 0.015 µg, were tested, with distilled water and commercial ampicillin included as controls. The disc diffusion assay was conducted following standard protocols, where filter paper discs containing the test substances were placed on agar plates with the bacteria. After incubation, the zones of inhibition around the discs were measured to evaluate the antimicrobial activity.

Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics (version 27.0, IBM Corp., Armonk, NY, USA). Descriptive statistics were calculated to summarize the data, and results are presented as mean ± standard deviation (SD).

RESULTS

Biological samples and preparation of NADES

After drying at 50°C, the mango leaves maintained their structure but turned to a dried green-brown color, typical of dried foliage. This preserved their physical integrity and prevented damage. The dried leaves were then ground into a fine powder, enhancing their surface area for better extraction of bioactive compounds.

A transparent mixture was obtained when sodium acetate: glycerol (NADES-1), sodium acetate: lactic acid (NADES-2), lactic acid: glycerol (NADES-3), betaine: glycerol (NADES-4), and betaine: lactic acid (NADES-5) solvents were heated and stirred at 70°C for 60 to 120 min.

Table 2. The visual characteristics of the prepared NADESs (NADES-1, NADES-2, NADES-3, NADES-4, and NADES-5) after the heating and stirring process were observed

Type of NADES	Component of NADES	Visual Characteristics
NADES 1	Sodium Acetate-Glycerol	Transparent liquid
NADES 2	Sodium Acetate-Lactic Acid	Transparent liquid
NADES 3	Lactic Acid-Glycerol	Transparent liquid
NADES 4	Betaine-Glycerol	Transparent liquid
NADES 5	Betaine-Lactic Acid	Transparent liquid

Screening of NADESs for TPC extraction by heating-stirring method

This study utilized five different Natural Deep Eutectic Solvents (NADESs) to extract polyphenolic compounds from Harumanis mango leaves. Among these NADESs, NADES-3 (lactic acid: glycerol) yielded the highest TPC value of 135.74±2.1 $\mathrm{mg\ GAE\ g^{-1}\ dw}$, as depicted in Figure 1.

NADES-5 (betaine: lactic acid) showed the second-highest TPC value at 120.36±1.54 $\mathrm{mg\ GAE\ g^{-1}}$

dw, followed by NADES-2 (sodium acetate: glycerol) at 116.37±1.48 mg GAE g^{-1} dw and NADES-4 (betaine: glycerol) at 113.59±1.98 mg GAE g^{-1} dw. The lowest TPC value was recorded for NADES-1 (sodium acetate: glycerol) at 32.76±1.02 mg GAE g^{-1} dw. Consequently, the solvent with lactic acid: glycerol (NADES-3) was chosen as the preferred solvent for the subsequent extraction of phenolics from Harumanis mango leaves.

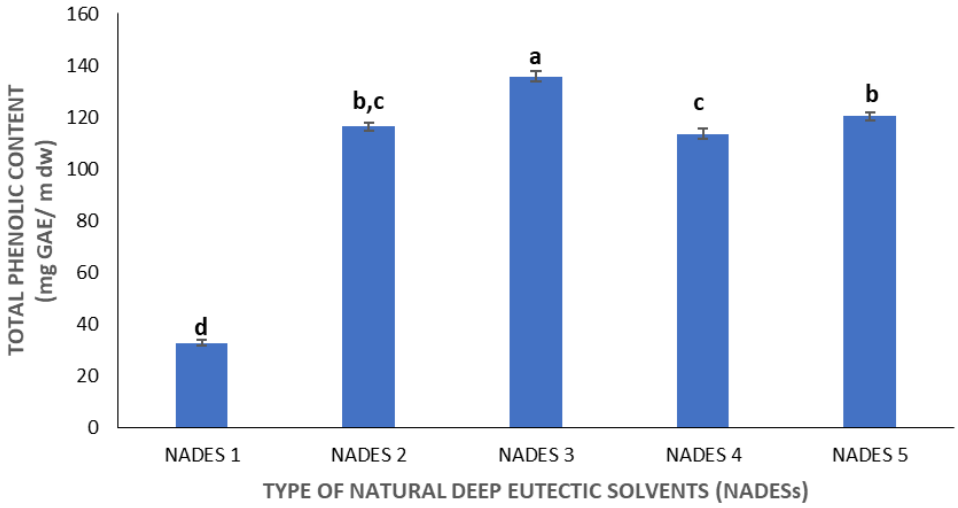

Fig. 1. Effect of different five NADES systems on TPC. NADES: Natural Deep Eutectic Solvent; NADES-1: sodium acetate: glycerol; NADES-2: sodium acetate: lactic acid; NADES-3: lactic acid: glycerol; NADES-4: betaine: glycerol; NADES-5: betaine: lactic acid; TPC: Total Phenolic Content; GAE: gallic acid equivalent. The different letters on the graph indicate statistically significant differences between the groups, with a $p<0.05$.

Evaluation of TPC by NADES-UAE and conventional solvents

The extraction of Harumanis mango leaves involving conventional solvents was conducted using a mixture of 50% ethanol, 50% methanol, 100% ethanol, and 100% methanol. These were carried out to compare the total phenolic content yield of deep eutectic solvents, which represent a new environmentally friendly technology, as opposed to conventional solvents. Table 3 illustrates that the highest TPC value recorded among conventional solvents is using 50% ethanol at 133.09±1.6 mg GAE g^{-1} dw. The second-highest TPC value is by 50% methanol at 105.25±1.9 mg GAE g^{-1} dw, followed by 100% methanol at 78.41±2.1 mg GAE g^{-1} dw. Meanwhile, 100% ethanol displays the lowest TPC value of 67.18±2.9 mg GAE g^{-1} dw.

NADES-3, composed of lactic acid: glycerol and demonstrating the highest TPC reading, was chosen for the subsequent extraction process. This selected solvent was used for Ultrasonic-Assisted Extraction to quickly evaluate its initial impact on extracting total phenolic compounds. Remarkably, the TPC value gained by NADES-3 in combination with UAE was the highest in this study, reaching 142.87 mg GAE g−1 dw.

Evaluation of the phytochemical composition

Table 3 presents the qualitative tests for the phytochemical composition of the extract. The presence of saponins was indicated by the foam layer observed when diluting one mL of Harumanis extract with distilled water. Adding iron III chloride ($FeCl_3$) to a portion of the extract resulted in a green color, indicating the presence of phenols.

Yellow coloration upon mixing the extract with aqueous NaOH indicated the presence of glycosides. The combination of the extract with a 5% $FeCl_3$ solution produced a greenish-black precipitate, indicating the presence of tannins. Lastly, the addition of concentrated nitric acid resulted in a yellow-colored solution, indicating the presence of protein in the Harumanis mango leaf extract. FTIR spectra were recorded for the Harumanis mango leaf extracts using the optimal extraction conditions, spanning from 3358.99 cm^{-1} to 741.89 cm^{-1} (Figure 3).

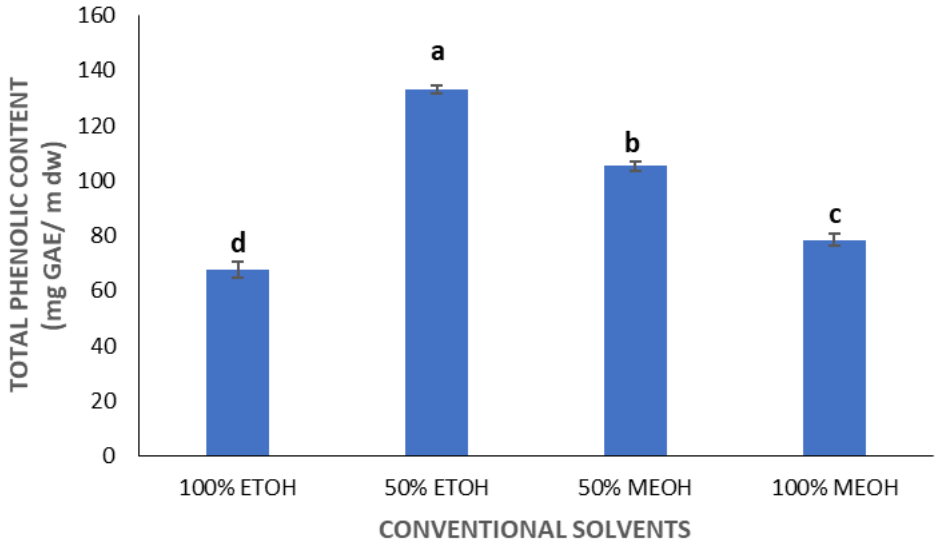

Fig. 2. Effect of different conventional solvents including 100% ethanol, 50% ethanol, 50% methanol, and 100% methanol on TPC yield. TPC: Total Phenolic Content; GAE: gallic acid equivalent. The different letters on the graph indicate statistically significant differences between the groups, with a $p<0.05$.

Table 3. Qualitative Test of Phytochemicals including saponins, phenols, glycosides, tannins, and protein in the Harumanis Leaf Extract Using NADES-3

Qualitative Test	Result
Saponins	Positive
Phenols	Positive
Glycosides	Positive
Tannins	Positive
Protein	Positive

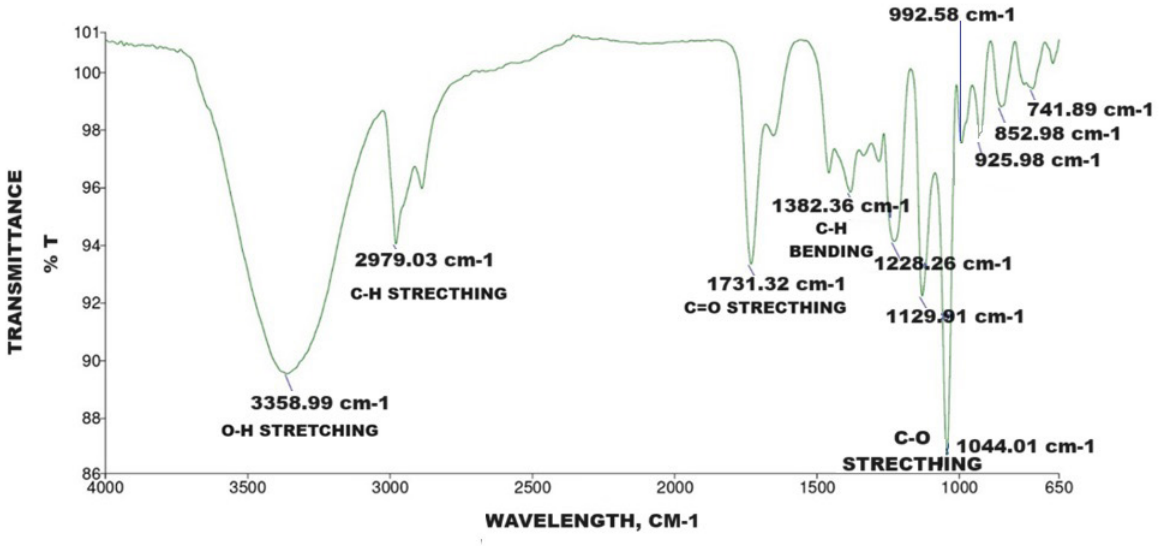

Fig. 3. FTIR Spectrum of the extract with the highest TPC value extracted by NADES-2 (lactic acid: glycerol) with UAE

The FTIR spectrum of the sample exhibits key absorption peaks at specific wavenumbers, indicating the presence of various functional groups. A broad peak at 3358.99 cm^{-1} corresponds to O-H stretching vibrations, suggesting the presence of hydroxyl groups, likely from alcohols or phenols. The peak at 2979.03 cm^{-1} is attributed to C-H stretching in alkanes, indicative of methyl or methylene groups. A distinct peak at 1731.32 cm^{-1} is characteristic of C=O stretching, which is commonly found in carbonyl compounds such as esters, aldehydes, or carboxylic acids.

Additionally, the spectrum shows peaks at 1382.36 cm^{-1} and 1228.26 cm^{-1}, corresponding to C-H bending and C-O stretching vibrations, respectively, suggesting the presence of methyl groups and ether or ester linkages. Further peaks at 1129.91 cm^{-1} and 1044.01 cm^{-1} reinforce the presence of C-O stretching, while the peaks in the fingerprint region (992.58 cm^{-1}, 925.98 cm^{-1}, 852.98 cm^{-1} & 741.89 cm^{-1}) may be associated with C-H bending or specific aromatic ring structures. These functional groups indicate a complex organic composition, consistent with the components typically found in natural deep eutectic solvents (NADES).

Table 4 shows the results of the antimicrobial screening conducted for *Escherichia coli* (*E. coli*) and *Bacillus sp*. bacteria. The positive control ampicillin at a concentration of 20 µg exhibited an inhibition zone of 15.3 mm for *E. coli* and 13.6 mm for Bacillus sp. In contrast, the water control showed no inhibition against either bacterial strain.

Table 4. Preliminary antimicrobial test of the Harumanis Leaves Extract Using NADES-3 by disc diffusion method

Tested bacteria	Ampicillin 20 µg	Water	300 µg	150 µg	15 µg	1.5 µg	0.15 µg	0.015 µg
	Inhibition zone (mm)							
E. coli	15.3	-	18.0	12.7	10.3	-	-	-
Bacillus sp.	13.6	-	25.0	16.3	10.7	-	-	-

The extract's inhibitory effects were concentration-dependent, with the highest concentration (300 µg) resulting in inhibition zones of 18.0 mm for *E. coli* and 25 mm for Bacillus sp. At lower concentrations (e.g., 1.5 µg & below), the extract showed minimal to no inhibition against both bacterial strains. These findings highlight the antimicrobial potential of the extract, particularly at higher concentrations, against the tested bacterial strains.

DISCUSSIONS

Assessment of extraction performance: total phenolic content using five prepared NADESs

NADESs are formed by combining a hydrogen bond donor and acceptor, resulting in natural compounds made from renewable materials and exhibiting varying strengths of hydrogen bonding forces. Researchers are increasingly exploring diverse combinations of these components, resulting in a growing range of NADESs developed (Dai *et al*., 2013; Garcia *et al*., 2016).

Four substances were chosen as hydrogen bond acceptors: sodium acetate, lactic acid, and betaine. The selection of hydrogen bond donors included glycerol and lactic acid, depending on their combination of chemicals (Table 1). The properties of these materials have been evaluated in previous studies for synthesizing NADESs under specific applications and conditions (Bakirtzi *et al*., 2016; Pal & Jadeja, 2020; Makris & Lalas, 2020; Yang *et al*., 2023).

All the synthesized NADESs formed transparent liquid mixtures after the completion of the reaction. This characteristic was also observed in most of the previously reported synthesized NADESs, including combinations such as betaine: glycerol, betaine: lactic acid, lactic acid: sodium acetate, sodium acetate: glycerol (Bakirtzi *et al*., 2016; Pal & Jadeja, 2019; Yang *et al*., 2023). The transparent, clear appearance of NADESs arises from the arrangement and interaction of their molecules.

Total phenolic compound (TPC) was measured to directly compare the performance of the synthesized NADESs, reflecting their extraction efficiency and potential bioactivity. Previous studies have also utilized TPC as a key measurement, especially for early screening of various solvents in extracting phenolic compounds from plant sources such as Kesar mango (Pal & Jadedja, 2020), olive leaves (Siamandoura & Tzia, 2020), broccoli leaves (Cao *et al*., 2023), and onion leaves (Aquino *et al*., 2023).

Out of all NADESs, NADES-3, comprising lactic acid and glycerol, produced the most favorable results with 135.74 mg GAE g^{-1} dw (Figure 1). Yang *et al*. (2023) reported the highest TPC value recorded by the same system among 19 different lactic acid-based NADESs during the initial screening. The solvent containing lactic acid and glycerol achieved a TPC value exceeding 110 mg/g for the extraction of *Nelumbo nucifera Gaertn* leaves. Thus, NADES-3 was selected as the preferred solvent

for subsequent phenolic extraction from Harumanis mango leaves samples.

The NADES system that recorded the second-highest TPC value in this study is NADES-5 containing betaine and lactic acid, which is 120.36 mg GAE g^{-1} dw. Falani *et al*. (2021) also used betaine and lactic acid as NADES, with the addition of UAE, to extract phenolic compounds from hazelnut skin, yielding results with a similar trend to ours, not reaching the highest category but still significant and not among the lowest, indicating its promising performances. In their study, the TPC value for NADES-14 containing betaine and lactic acid ranges between 10 to 12 g GAE per 100 g of skin, compared to the highest value recorded by NADES-1 (ChCl: Urea), which is 13.98 g GAE per 100 g of skin, and the lowest value by NADES-4 (ChCl: Xylitol), which is 7.14 g GAE per 100 g of skin (Falani *et al*., 2021).

The lowest TPC value recorded in our study was observed in NADES-1, composed of sodium acetate and glycerol, with 32.76 mg GAE g^{-1} dw. This result differs slightly from the findings of Pal and Jadeja (2020), where NADES containing sodium acetate and glycerol exhibited the second-highest TPC readings, ranging between 60 to 80 mg GAE g^{-1} dw. Despite both studies employing similar screening methods, primarily heating and stirring, and utilizing mango samples, the observed differences in TPC values suggest potential variations in extraction efficiency or compound composition.

The difference in TPC values between our study and Pal and Jadeja (2020) highlights the complexity of phenolic compound extraction, which is influenced by factors such as solvent composition, extraction methods, and sample characteristics. Although our methods were similar, minor variations in conditions or sample types may have contributed to the differing results. Our study focuses on Harumanis mangoes and leaves, in contrast with their research which mainly on Kaesar mangoes and peels. This difference emphasizes the importance of testing NADESs on different mango varieties to enhance biopharmaceutical applications in the future.

Lactic acid-glycerol based NADES in combination with ultrasonic-assisted extraction and the comparison with conventional solvents

The highest total phenolic content (TPC) value obtained from extraction using conventional solvent was recorded by 50% ethanol, which is 130.91 mg GAE g^{-1} dw. This value is still lower than the extraction yield achieved using NADES-3 (lactic acid & glycerol) at 135.74 mg GAE g^{-1} dw and more importantly, NADES is a more economical- and environmentally-friendly option. This result demonstrates that NADES-3 can significantly enhance TPC value compared to all the tested solvents. NADES-3 was then selected for the extraction of phenolic compounds from Harumanis mango leaves using the green technique of UAE.

In this study, the use of lactic acid: glycerol NADES system in combination with UAE increased the TPC value by 5.3% at 142.87 mg GAE g−1 dw. In a previous study, Mansinhos *et al*. (2021) observed an increase in TPC values when using NADES-UAE for recovering phenolic compounds from *Lavandula pedunculata subsp. lusitanica*, compared to maceration methods with conventional solvents and constant heat that yielded TPC values ranging from 18.22 to 50.05 mg GAE g^{-1} dw across different solvents.

Jeong *et al*. (2018) and Nam *et al*. (2015) investigated that UAE achieved higher extraction efficiency than the other conventional methods used such as stirring, heating, heating with stirring, and UAE for the extraction of monoterpenes and phenolic compounds from *Mentha piperita* L. and flavonoids from *Flos sophorae*, respectively. Their studies showed that this finding emphasizes the potential of NADES-UAE as a more effective approach for phenolic compound extraction.

The TPC value of 142.87 mg GAE g−1 dw achieved through NADES and UAE extraction in our study presents an interesting perspective when compared to findings in the existing literature. While it slightly falls below the TPC value of 155.28 mg GAE g−1 dw reported by Pal & Jadeja (2022) using glycerol: sodium acetate (3:1) with 20% water alongside microwave-assisted extraction (MAE), it significantly outperforms the TPC values documented by Lanjekar *et al*. (2022) for Alphonso mango using UAE with lactic acid: glucose (5:1) and 20% water (TPC of 69.85 mg GAE/g).

Our TPC value of the extracts of NADES-3 with UAE also surpasses those reported by Rojas *et al*. (2018) for Ataulfo variety mango peel using ethanol solvent, which measured 72.61 mg g^{-1}, Umamahesh *et al*. (2016) for Sindhura mango with aqueous extracts at 87.38 mg GAE g^{-1} dw, and Ajila *et al*. (2010) for Badami mango peels employing 80% acetone as solvent, yielding 54.67 mg g^{-1}. It is important to note that variations in extraction techniques and sample processing methodologies across studies may contribute to these differences.

The TPC value using NADES and UAE extraction reported in our study highlights its efficacy in extracting phenolic compounds from mangoes, showcasing its potential as a robust approach for

achieving substantial phenolic content compared to both traditional and advanced extraction techniques outlined in the literature. This highlights the importance of adopting innovative extraction methods to maximize the yield and quality of bioactive compounds.

The environmental impact of using Natural Deep Eutectic Solvents (NADESs) is increasingly recognized as a critical factor in their adoption of green chemistry applications. NADESs are composed of natural components like sugars, amino acids, and organic acids, which contribute to their low toxicity and high biodegradability. This contrasts with traditional organic solvents, which are often derived from petroleum, can be toxic, and may pose significant environmental risks due to their persistence and potential for bioaccumulation.

Research has shown that NADESs typically exhibit lower toxicity profiles compared to conventional solvents. For example, Dai *et al*. (2013) highlighted that the components of NADESs are generally recognized as safe (GRAS) and are biodegradable, reducing the ecological impact when these solvents are released into the environment. Similarly, Paiva *et al*. (2014) demonstrated that NADESs can be broken down by natural processes, thus minimizing their long-term environmental footprint.

Moreover, the biodegradability of NADESs is a significant advantage over traditional solvents. Traditional organic solvents, such as chloroform or methanol, can persist in the environment, leading to soil and water contamination. In contrast, the natural origin and easy degradation of NADESs make them less likely to accumulate in ecosystems, thus reducing their ecological footprint (Smith *et al*., 2014). This characteristic is particularly beneficial in applications where large volumes of solvents are used, such as in industrial-scale extractions or chemical reactions.

In terms of overall ecological footprint, NADESs are also superior due to their non-volatile nature and lower energy requirements. Unlike volatile organic compounds (VOCs), which can contribute to air pollution and climate change, NADESs do not easily evaporate, thereby reducing emissions and the associated environmental impact. Additionally, the production and recycling of NADESs generally require less energy compared to traditional solvents, further enhancing their sustainability profile (Liu *et al*., 2018).

A comprehensive comparison between NADESs and traditional solvents underscores the environmental benefits of NADESs, particularly regarding toxicity, biodegradability, and overall ecological impact. This analysis not only highlights the potential of NADESs as a greener alternative but also encourages further exploration into their applications across various industries where sustainability is a growing concern.

Phytochemicals composition and their potential for therapeutic application

Based on the findings from FTIR analysis, the extract of Harumanis mango leaves in NADES-3 (specifically, a mixture of lactic acid & glycerol) was observed to contain alcohols, phenols, alkanes, alkyl amines, carboxyl, and carbonyl groups. These phytochemical constituents are known for their potential therapeutic properties. Further phytochemical screening revealed the presence of saponins, phenols, glycosides, and tannins in the extracts, reinforcing their potential medicinal values.

The preliminary antimicrobial screening of the extract revealed its ability to inhibit the growth of *E. coli* and Bacillus sp. bacteria. The inhibitory effect increased with higher concentrations of the extract, leading to larger inhibition zones. At 300 µg concentration, the extract showed significant antimicrobial activity, resulting in substantial inhibition of bacterial growth. The positive control, ampicillin at 20 µg concentration, also displayed prominent inhibition, with inhibition zones of 15.3 mm for *E. coli* and 13.6 mm for Bacillus sp. In contrast, the negative control with water showed no inhibition, indicated by 0 mm inhibition zones for both bacteria. The range of inhibition zones varied with extract concentration, with the highest concentration (300 µg) showing inhibition zones of 18.0 mm for *E. coli* and 25 mm for Bacillus sp. These results emphasize the extract's potential as an antimicrobial agent, highlighting the need for optimal concentration in further studies.

In a previous study, a comprehensive phytochemical analysis of mango leaves revealed a diverse array of bioactive compounds. These compounds include phenolics, alkaloids, saponins, glycosides, terpenes, steroids, tannins, flavonoids, and phenols, all of which contribute to the leaves' antimicrobial, antioxidant, and antibacterial properties (Kumar *et al*., 2021). Mango is globally recognized for its rich content of bioactive compounds such as polyphenols, carotenoids, vitamins, and minerals, and has been utilized for centuries for its medicinal properties, including antioxidant (Freitas *et al*., 2022), anti-inflammatory (Gomes *et al*., 2021), anti-cancer (Mirza *et al*., 2020), and anti-diabetic (Ahmad *et al*., 2023) activities.

The adoption of deep eutectic solvents in the extraction process of Harumanis mango leaves shows potential, likely enhancing the observed high total phenolic content (TPC) value. The identification of

these phytochemicals highlights the extract's promise for therapeutic applications, as corroborated by previous studies. These findings present an encouraging path for exploring the medicinal advantages of Harumanis mango leaf extracts and their potential utility in the pharmaceutical and healthcare sectors.

CONCLUSION

This study highlights the potential of Natural Deep Eutectic Solvents (NADESs) for extracting phenolic compounds from Harumanis mango leaves in an eco-friendly manner. NADESs, especially lactic acid: glycerol NADES system, showed better results than conventional solvents in terms of total phenolic content (TPC). Ultrasonic Assisted Extraction (UAE) further improved the extraction process. The phytochemical analysis confirmed the presence of beneficial compounds like saponins, phenols, glycosides, and tannins in the NADES-extracted Harumanis extract. Moreover, the extract demonstrated significant antimicrobial activity against both gram-positive and gram-negative bacteria, indicating its potential therapeutic use. Overall, this study suggests that NADES-based extraction methods offer a sustainable approach to obtaining bioactive compounds from Harumanis mango leaves with potential health benefits. This research highlights the significant potential of NADES in extracting bioactive compounds from Harumanis leaves. The innovative use of NADES addresses the existing research gap related to the environmental and efficiency limitations of traditional organic solvents (Dai *et al*., 2013; Paiva *et al*., 2014). By adopting NADES, our study improves the yield and quality of extracted compounds while promoting a more sustainable and eco-friendly extraction method (Smith *et al*., 2014). This advancement sets a new standard in the field, offering a greener alternative that aligns with current environmental and efficiency goals. The successful implementation of NADES in this research opens new avenues for further exploration and development of sustainable extraction techniques (Liu *et al*., 2018; Grodonova *et al*., 2020).

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ETHICAL STATEMENT

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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