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Optimization of Harumanis Mango Leaves Extract for Enhanced Pharmacognostic Profile Using Response Surface Methodology Approaches

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ABSTRACT

Harumanis mango (*Mangifera indica* var Harumanis) is renowned for its fruit; however, its leaves remain an underexplored resource for therapeutic potential. Although other mango varieties have been studied for their medicinal properties, limited research has focused on the bioactive profile of Harumanis mango leaves, creating a critical knowledge gap. Hence, this study aims to fill the gap by optimizing the extraction of bioactive compounds from Harumanis leaves using dynamic maceration with ethanol and methanol as solvents. Phytochemical screening identified the presence of polyphenols, which were further validated by FTIR spectroscopy. Quantitative analysis revealed high levels of flavonoid and phenolic contents in both ethanolic and methanolic extracts, comprising total phenolic contents of 60.58 ± 0.005 and 36.73 ± 0.003 µg/g, respectively, and total flavonoid contents of 58.73 ± 0.015 and 46.25 ± 0.003 µg/g, respectively. The antioxidant evaluation of the ethanolic extract showed IC_{50} values of 52.905 ± 1.12 µg/mL, while the methanolic extract showed 84.649 ± 0.87 µg/mL; additionally, antibacterial tests further supported their therapeutic potential. These findings highlight the promising potential of these extracts for the development of natural therapeutics. The high concentrations of polyphenols and their bioactivity underscore the medicinal value of Harumanis leaves, highlighting their potential for use in herbal medicine. Overall, this study

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fills a crucial research gap, showcasing the untapped potential of Harumanis leaves as a natural resource for alternative therapies and unlocking new possibilities for innovative applications in the industrial and pharmaceutical fields.

Keywords: Dynamic maceration, Harumanis leaves, pharmacognosy, phytochemicals, response surface methodology

INTRODUCTION

Pharmacognostic approaches focus on discovering and developing new medications from natural sources. This field involves identifying, isolating, and characterizing plant bioactive compounds to create new treatments or enhance the existing ones. The increasing global demand for plant-based bioactive compounds, particularly antioxidants and antibacterial agents, has underscored their growing importance in the pharmaceutical and nutraceutical industries. These compounds are valued for their natural origin, safety, and efficacy in combating oxidative stress and microbial infections, contributing significantly to health and disease prevention. This trend reflects a shift towards sustainable plant-derived alternatives to synthetic compounds (Narayanankutty et al., 2024). Public interest in natural medicines is driven by their affordability, perceived safety, and potential effectiveness compared with synthetic drugs (Ekor, 2014). Exploring these remedies could unveil novel therapeutic targets and foster innovative and holistic approaches to healthcare.

The therapeutic potential of *Mangifera indica* (mango tree) has garnered increasing attention owing to its abundant bioactive components, making it a promising candidate for drug development. Native to South Asia, this tropical fruit is renowned for its culinary appeal and its traditional medicinal uses (Thivagaran et al., 2023). In Malaysia's Perlis region, the Harumanis mango stands out for its exceptional sweetness and aroma, making it a prized fruit. Its high antioxidant content suggests potential benefits in lowering the risk of progressive health disorders (Liu et al., 2018). Although fruit garners much attention, the therapeutic potential of mango leaves, especially those from the Harumanis variety, remains largely unexplored. Research has primarily focused on the fruit, leaving a gap in our understanding of the bioactive compounds in the leaves. Traditionally, mango leaves have been used to treat ailments like diarrhea, toothaches, and diabetes (Ediriweera et al., 2017). Scientific evidence supports these uses, revealing antidiabetic, anticancer, and antioxidant properties (Ganogpichayagrai et al., 2017). However, studies have not specifically addressed the Harumanis variety, which may have distinct therapeutic effects compared with other mango types.

Moreover, the efficacy of natural products may differ depending on factors such as plant maturity, environmental conditions, and harvest methods (Tungmunnithum et al., 2020). The concentration and type of compounds a plant produces are influenced by its species,

genotype, and growth conditions (Isah, 2019). To maximize the benefits of mango leaves, optimizing the extraction methods to capture their full phytochemical potential is essential.

In the global pursuit of sustainable resource utilization, the exploration of underutilized biomass and innovative extraction techniques has gained momentum (Azhar et al., 2021; Fatt et al., 2021; Herman et al., 2021; Rohim et al., 2021). These studies have provided valuable insights into the identification of bioactive compounds. However, most of this research has primarily focused on characterizing chemical compositions rather than optimizing the extraction processes. This study addresses the gap by characterizing the bioactive profile of Harumanis mango leaves and optimizing extraction conditions to enhance yield and bioactivity. This approach highlights the importance of refining the extraction techniques to maximize the potential of plant-based resources for pharmaceutical and nutraceutical applications.

Recent studies have reported that Harumanis mango leaves contain notable phenolic and flavonoid compounds (Rahman et al., 2024). These compounds are known for their ability to neutralize free radicals, reduce oxidative stress, and exhibit antimicrobial activity, making them highly valuable in alternative medicine to promote overall health. Hence, the primary objective of this research is to screen and elucidate the active phytochemicals in Harumanis mango leaves using an optimized dynamic maceration technique. By addressing current knowledge gaps and challenges, this study aimed to highlight the medicinal potential of these leaves and explore their application in developing new nutraceutical and pharmaceutical products. Understanding the unique properties of Harumanis mango leaves and determining their optimal extraction conditions is crucial for leveraging their health benefits and advancing their use as phytomedicines. Ultimately, this study seeks to provide valuable insights into the potential of Harumanis mango leaves as a source of novel herbal drugs.

MATERIALS AND METHOD

This study used various analytical methods to explore the therapeutic potential of Harumanis leaves (Figure 1). This study aimed to identify the key bioactive compounds contributing to their therapeutic effects by carefully collecting and examining these data. Different analytical tools have provided a deeper and more detailed understanding of the pharmacological properties of these leaves.

Leaves Sample Preparation

Approximately 1 kg of pruned Harumanis leaves (ID: BT-D-B9P16-MA128) were collected from the Agricultural Complex of Jabatan Pertanian Negeri Perlis located at Bukit Temiang, Perlis. Figure 2 shows the leaf preparation process used in this study.

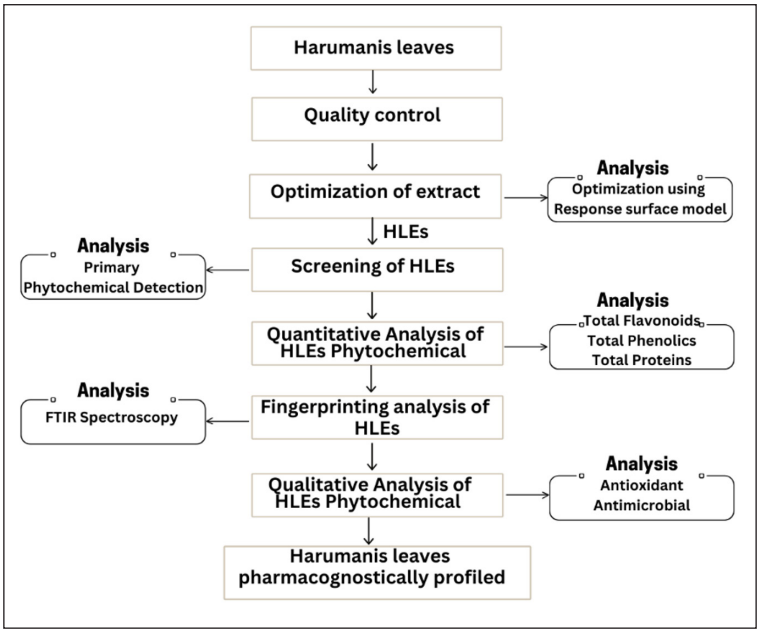

Figure 1. Research methodology overview

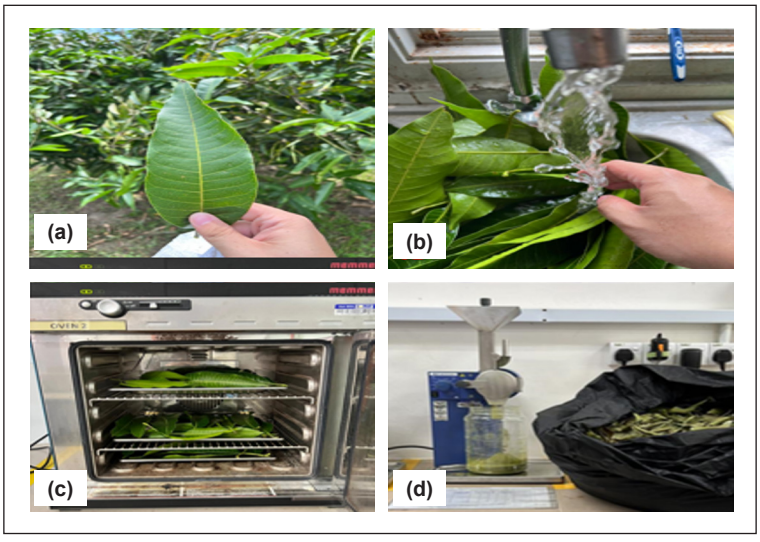

Figure 2. (a) Leaves collection; (b) washing and cleaning of leaves; (c) drying in oven; and (d) grinding

Foreign Particles Analysis

The leaves were initially weighed and spread to assess the presence of foreign particles in ground Harumanis leaves on a thin sheet. Any foreign material, such as small stones or insects, was identified and removed by visual inspection with a magnifying glass. Isolated

foreign particles were weighed and divided according to the total sample weight. The result was multiplied by 100 to determine the percentage of foreign particles in the sample (Sani et al., 2018).

Total Ash Content Analysis and Acid Insoluble Ash Analysis

The total ash content was determined by weighing 2 g of Harumanis leaf powder and placing it into a silica crucible of 50 mL. The sample was initially heated using a hot plate, followed by combustion in a furnace at 400 °C for 4 h. The percentage of total ash was determined by dividing the ash mass by the original mass of the powder and multiplying it by 100, as described by Ngadiarti et al. (2022). The resulting ash in the crucible was mixed with 5 M hydrochloric acid (25 mL) for the acid-insoluble ash. The mixture was heated for 5 min. Then, the mixture was rinsed using 5 mL of hot water, leaving behind insoluble ash. The insoluble ash was then filtered through Whatman No. 1 filter paper and neutralized with hot water. The insoluble ash residue was returned to the crucible, dried, and incinerated until a constant weight was achieved. After cooling, the residue was measured, and the percentage of acid-insoluble ash was determined by dividing the weight of the residue by the initial weight of the sample powder and multiplying the result by 100, following the method described by Pradhan et al. (2010).

Total Moisture Content Analysis

The moisture content of powdered Harumanis leaves was assessed using an A&D Hybrid Moisture Analyzer. Approximately 2 g of each sample was weighed and loaded into the device to calculate the moisture content. To ensure reliable results, the procedure was repeated thrice.

Preparation of Harumanis Leaves Extracts (HLEs)

Design of Experiment

Response surface methodology (RSM) was applied to optimize dynamic maceration extraction. Hence, different extraction times, extraction temperatures, and solvent ratios were studied to analyse the results and characteristics of the extracted products. The experimental design employed in this study was the Box-Behnken design using Design Expert Version 7 software developed by State-Ease Inc. (Minneapolis, MN, USA). The parameters were selected based on the study of Ramlee et al. (2024) with slight modifications. The Box-Behnken design consisted of three components, each with three levels, resulting in 22 experiments. The study incorporated three distinct independent variables: time (A: 15–45 min), temperature (B: 30–60 °C), and solvent concentration or ratio (C: 0–100% in distilled water). The dependent variable was yielding percentage

(%). The design was implemented to optimize the dependent variables. The model was validated by comparing the experimental and predicted values (Rohilla & Mahanta, 2021).

Dynamic Maceration Preparation of HLEs

Approximately 5 g of powdered Harumanis leaf samples were measured and transferred into conical flasks. Subsequently, 50 mL of the solvent was introduced into each flask to aid the extraction process of the plant material. This methodology was replicated using different solvent concentrations, including distilled water, ethanol, a blend of ethanol and distilled water, methanol, and a blend of methanol and distilled water, each in a separate conical flask. The flasks were then subjected to controlled temperature conditions within a water bath to facilitate extraction through maceration, and the solution obtained after the extraction process was cooled and filtered using Whatman No. 1 filter paper. Next, the collected filtrate was placed in an oven and fully dried at 65 °C (Pereira et al., 2018). The extract yield was determined by dividing the weight of the crude extract by the weight of the powdered sample and multiplying the result by 100. This process was conducted thrice to enhance the accuracy.

Screening of HLEs

Preparation of Samples

A stock solution of approximately 10 mg/mL was prepared from the extract with the parameters that gives the highest yield. This stock solution was diluted with the appropriate solvent to achieve a final concentration of 10 mg/mL. The solution was filtered through Whatman No. 1 filter paper to remove any residues before proceeding with further tests.

Primary Phytochemical Detection

The phytochemicals in the HLEs were identified using standard qualitative methods, including hydrochloric acid, sodium hydroxide, and iron chloride. The presence of specific phytochemicals was indicated by the colour change that results from chemical reactions.

Flavonoids Detection

A few drops of a 10% sodium hydroxide solution were added to 2 mL of the aqueous extract. The addition of dilute hydrochloric acid caused the solution's colour to change from yellow to colourless, confirming the presence of flavonoids (Hassali et al., 2022).

Phenols Detection

Ellargic's test was performed to determine the presence of phenols. A few drops of 5% sodium nitrate ($NaNO_3$) and 5% gallic acid were added to the optimized ethanol and methanol HLEs. A brown precipitation indicated a positive result for phenols in the extracts (Jamil et al., 2023).

Saponins Detection

The presence of saponins was determined using foam tests. A 50 mg sample of HLEs was diluted with distilled water to a final volume of 20 mL and vigorously shaken for 2 min. The appearance of a 2 cm foam layer indicated the presence of saponins (Rai et al., 2023).

Protein Detection

The Biuret method was used to determine the presence of proteins. Approximately 1 mL of 4% NaOH solution and a few drops of 1% $CuSO_4$ were added to the optimized ethanol and methanol extracts of HLEs. A change in the colour of the extract solution to purple indicated a positive result in the protein test.

Quantitative Analysis of Phytochemical

Total Phenolic Content Analysis

Initially, a standard calibration curve using gallic acid was created at 31.25, 62.5, 125, 500, and 1000 ppm concentrations. For each concentration, 100 µL of gallic acid solution was mixed with 100 µL of 10% Folin-Ciocalteu reagent and 2 mL of 2% sodium carbonate (Na_2CO_3) solution. The mixture turned dark blue. After thorough mixing, the solutions were incubated for 30 min in the dark at room temperature. Absorbance was measured at 720 nm. This process was repeated thrice, and the calibration curve was plotted. The same procedure was followed to determine the total phenolic content in HLEs. The absorbance of the HLEs was measured and compared with the calibration curve. Total phenolic content was expressed as milligrams of gallic acid equivalents (GAE) per gram of sample. The final calculation involved multiplying the concentration from the calibration curve by the extract volume and dividing it by the mass of the sample. All tests were conducted in triplicate (Nadzir et al., 2020).

Total Flavonoids Content Analysis

A calibration curve was constructed using quercetin at 31.25, 62.5, 125, 250, 500, and 1000 ppm concentrations. To determine flavonoid content, 250 µL of quercetin was mixed with 150 µL of 10% (w/v) aluminium chloride solution. After 5 min, 0.5 mL of 1 M sodium hydroxide and 575 µL of deionized water were added to the solution, resulting in a yellow solution. The absorbance was measured at 430 nm using a UV-Vis spectrophotometer. The total flavonoid content in the HLEs was determined by comparing their absorbance with the calibration curve, and the results were expressed as the concentration of flavonoids per unit mass of the extract (Bouyahya et al., 2018). All analyses were performed in triplicates.

Fingerprinting Analysis of HLEs

Fourier-Transform Infrared Spectroscopy (FTIR) using a Perkin Elmer instrument was employed for fingerprinting analysis of HLEs. Approximately 1 mL of 1 mg/mL HLE solution was analysed, with the FTIR spectrometer set to scan from 650 cm^{-1} to 4000 cm^{-1} at a resolution of 4 cm^{-1}. The resulting spectrum was examined to identify the key peak values and corresponding functional groups. The observed FTIR peaks were interpreted in relation to known bioactive compounds by correlating the identified functional groups with those typically found in specific bioactive molecules.

Qualitative Analysis of Harumanis Leaves

Determination of Antioxidant Activity

The antioxidant activity was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, as described by Bakar et al. (2019). A calibration curve was established with ascorbic acid concentrations ranging from 31.25 to 2000 ppm. The DPPH solution was prepared by dissolving 3.2 mg of DPPH in 100 mL methanol. Then, 200 µL of ascorbic acid was added to 2.8 mL DPPH solution. The mixture was then shaken and incubated at room temperature for 1 h. Absorbance was measured at 517 nm using a UV/Vis spectrophotometer. DPPH radical scavenging activity (%) was calculated by subtracting the absorbance of the extract from that of the control, dividing by the control absorbance, and multiplying by 100. The IC_{50} values were determined from these results. All tests were performed in triplicates.

Determination of Antimicrobial Activity

The HLE's antimicrobial activities were conducted using a disc diffusion method against *Escherichia coli, Pseudomonas aeruginosa,* and *Bacillus cereus*. The agar plates were divided into four sections: two for the controls (2 mg/mL ciprofloxacin as a positive control and methanol as a negative control) and two for the extract solutions. The bacterial suspension was adjusted to a McFarland standard of 0.4. Sterile paper discs were impregnated with a 2 mg/mL extract solution and placed on agar plates. After 24 h of incubation at 35 °C, the inhibition zones were measured to assess antimicrobial activity.

RESULTS AND DISCUSSION

Quality Control Analysis

Quality control is critical to ensure the consistency, safety, and efficacy of herbal-derived drug preparations (Wang et al., 2023). The term "quality" refers to a drug's characteristics, including its identity, chemical and physical properties, purity, composition, and biological features. This analysis includes determining the total ash content, acid-insoluble ash, foreign

matter, and total moisture content of Harumanis leaves as raw materials for developing herbal products. Animal-derived substances, including insects and microscopic microbial organisms, which may produce harmful toxins, are among the potential herbal contaminants (Kunle et al., 2012). Therefore, foreign matters, including molds, insects, spider webs, sands, and stones, were discarded from the leaves sample. In this study, the percentage of foreign matter in the Harumanis leaf sample was 2.28 ± 0.18%, which is considered low. This indicates that the percentage of foreign matter in the plant sample is relatively acceptable as it is less than 10% under pharmacopeial standards of herbal material. The total ash content was determined by measuring the total amount of material remaining after the burning. This includes "physiological ash", derived from the plant sample itself, and "non-physiological" ash, which is the residue of the extraneous matter adhering to the plant surface caused by environmental contamination. As the results obtained, the percentage of total ash content in plant raw materials was 9.02 ± 0.27%. This value is acceptable, as it is less than 14%, the maximum acceptable limit of total ash content recommended by the European Pharmacopoeia (Abdu et al., 2015).

Acid-insoluble ash is a component of total ash that indicates the presence of silica in the samples, most notably in sand and siliceous soil. Moreover, these values represent the concentrations of oxalates, carbonates, phosphates, oxides, and silicates (Shen et al., 2023). Therefore, these values are indicators of the quality of herbal remedies. According to the results, the percentage of acid-insoluble ash in the Harumanis leaves sample was 1.44 ± 0.25%, which is low and below 10%. This indicates the high purity of the herbal medicines. However, the presence of soil, for example, causes contamination that results in a high percentage of acid-insoluble ash that eventually disturbs the quality of the products.

To develop a high-quality herbal product, the percentage of total moisture content should be low because a higher moisture content attracts microbes to grow, which eventually contaminates the whole product. Therefore, it is important to control the moisture content of the raw material to ensure the product is of good quality. Based on the results, the total moisture content of the raw material was 8.93 ± 0.31%, which is considered low. This proves that Harumanis leaves have the potential to be developed into high-quality herbal products. Figure 3 illustrates the overall results of the quality control analysis of Harumanis leaves.

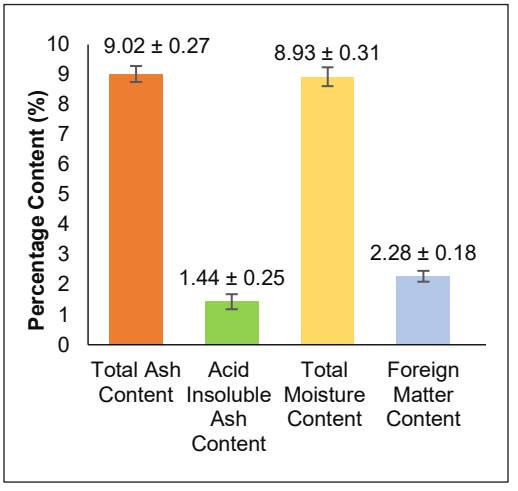

Figure 3. Percentage content for quality control analysis

Optimization of Dynamic Maceration Extraction

Dynamic maceration was optimized via RSM Box and–Behnken design to evaluate the best conditions for extracting bioactive compounds from Harumanis leaves. This approach was tested at various times, temperatures, and solvent ratios to determine the optimal extraction parameters. For ethanol extracts, the optimal conditions were 25 min of extraction time, 45 °C temperature, and a solvent ratio of 42% ethanol in 58% distilled water. This setup yielded a predicted extraction result of 22.13 ± 0.48%. In practice, the yield was slightly higher at 24.74 ± 0.32%. For extracts using methanol, the best conditions were an extraction time of 37 min, a temperature of 55 °C, and a solvent ratio of 44% methanol in 56% distilled water. This provided a predicted yield of 23.77 ± 0.26%, with an actual yield of 26.80 ± 0.13%. The interactions between different parameters and the extraction yield were recorded and are shown in Figure 4.

These optimized conditions (Table 1) were selected because they align with the goal of achieving an efficient and environmentally friendly extraction. The slightly higher actual yields compared to the predictions demonstrate that these conditions are effective and energy efficient. These results suggest that combining water with organic solvents, such as ethanol or methanol, enhances the extraction of soluble compounds, making it a better choice than using pure solvents alone. Similar findings have been reported in other studies, highlighting the effectiveness of aqueous methanol in plant extraction (Sultana et al., 2009).

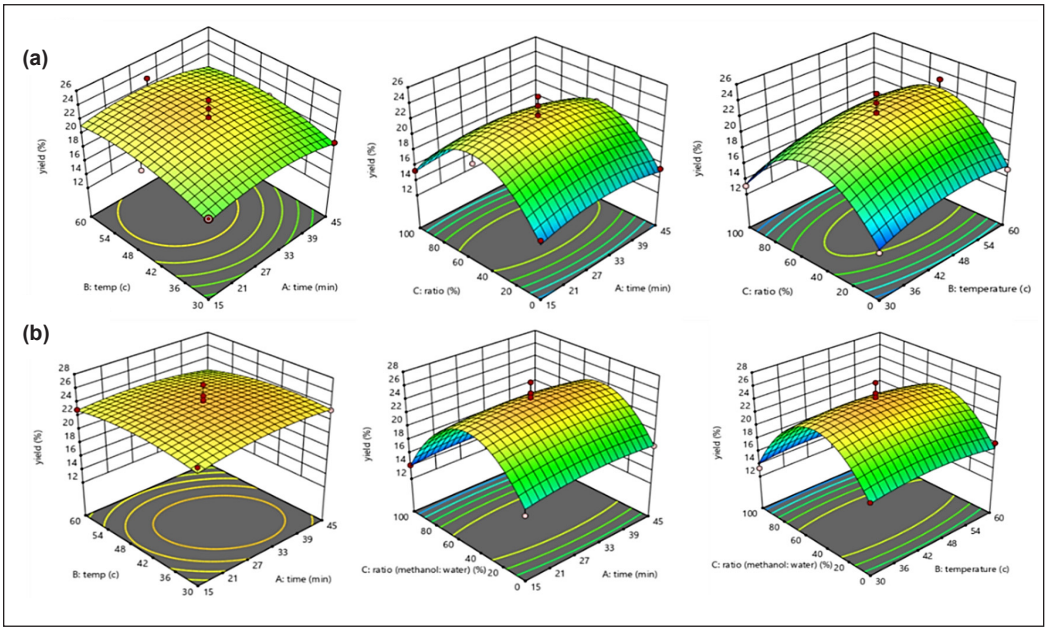

Figure 4. 3D contour for interactions of different parameters against yield of extract produced for extraction optimisation: (a) optimized ethanolic extraction conditions; and (b) optimized methanolic extraction condition

Table 1
Optimized parameters for dynamic maceration

Parameters	Ethanolic Leaves Extract	Methanolic Leaves Extract
Ratio (Solvent %)	42%	44%
Time (Min.)	25 Minutes	37 Minutes
Temperature (°C)	45 °C	55 °C
Yield (w/w %)	22.13 ± 0.48%	23.77 ± 0.26%

Primary Detection of Phytochemicals

Preliminary analysis of the phytochemical contents of HLEs was performed using four different types of tests (Biuret test, alkaline reagent test, Ellargic's test, and foaming test). Overall, these results suggest that HLEs contain a variety of phytochemicals, including flavonoids, proteins, phenols, and saponins, which may contribute to their therapeutic potential. Table 2 summarises the phytochemical tests, observations, and conclusions regarding the phytochemicals available in the optimized HLEs.

Table 2
Primary phytochemical screening for Harumanis leaves extracts (HLEs)

Test	Observation	Conclusion
Flavonoids Test	Yellow colour changed to colourless	Presence of flavonoids
Biuret Test	Purple hue formed	Presence of proteins
Ellargic's Test	Brown precipitation formed	Presence of phenols
Foaming Test (Saponins)	2 cm layer of foam formed above solution	Presence of saponins

Quantitative Control Analysis of Phytochemical

Total Phenolic and Flavonoids Contents of HLEs

The total phenolic content of HLEs was determined using the Folin-Ciocalteu method and gallic acid as the standard calibration (Figure 5).

Using UV-Vis spectroscopy, total phenolic content values were obtained from the standard calibration curve $y = 0.3262x + 0.0435$, where x is the absorbance and y is the phenolic concentration. The final concentration was measured in µg GAE/g in 5 mg extracts. According to the results obtained, the ethanolic extract has recorded the highest phenolic content with a value of 60.58 ± 0.005 µg/g, followed by methanolic extract (36.73 ± 0.003 µg/g). This study observed that the total phenolic content value was higher in ethanolic extracts than in methanolic extracts. This is because ethanol is a polar solvent with a strong capacity to dissolve a wide range of phenolic compounds (Jiménez-Moreno et al., 2019). The higher phenolic content in ethanolic extracts is attributed to the effectiveness

of ethanol in solubilizing phenolic compounds, including flavonoids and antioxidants, from plant materials (Dai & Mumper, 2010). Therefore, the choice of solvent plays a critical role in the extraction process, and ethanol is particularly effective in extracting phenolic compounds, contributing to the higher phenolic content. Polyphenols are often soluble in organic solvents that are less polar than water (Mehmood et al., 2022). This indicates that an aqueous mixture containing polar solvents, such as ethanol, is favourable for phenolic compound extraction from Harumanis leaves. Thus, ethanol is a good solvent for extracting polyphenols from HLEs.

The total flavonoid content of the HLEs was determined by an aluminium chloride colorimetric test using quercetin as a standard calibration (Figure 6).

UV-Vis spectroscopy obtained TFC values from the calibration curve y= 2.7169x - 0.1484, where x is the absorbance and y is the flavonoid concentration. The final concentration was measured in µg QE/g in 5 mg extracts. According to the results obtained, ethanolic extracts possessed the highest flavonoid content with a value of 58.73 ± 0.015 µg/g, followed by methanolic extracts with 46.25 ± 0.003 µg/g. This means that the polarity of the solvent affects the TFC values, and the optimum value of TFC was obtained using ethanol as a solvent for extraction. This higher flavonoid content in ethanolic extracts is attributed to the effectiveness of ethanol in solubilizing flavonoids, a subgroup of phenolic compounds from plant materials. The polarity of ethanol allows it to extract a broader spectrum of flavonoids, contributing to the higher flavonoid content observed in ethanolic plant extracts than in methanolic extracts (Ekin et al., 2017). Ethanol proved to be the most reliable solvent for extracting flavonoid content. This is because ethanol is more advantageous than water and

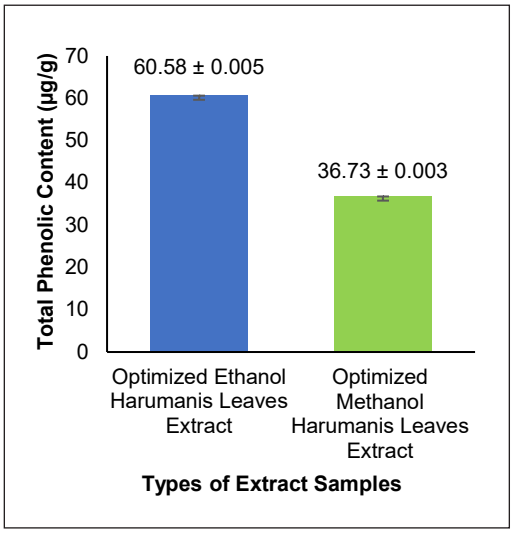

Figure 5. Total phenolic content of optimized ethanolic and methanolic Harumanis leaves extracts (HLEs)

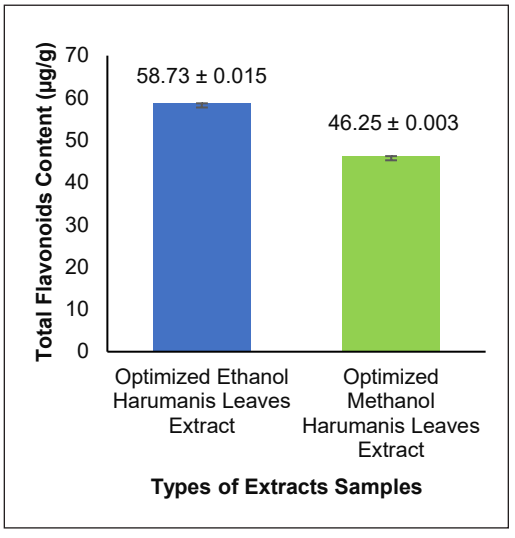

Figure 6. Total flavonoids content of ethanolic leaves and methanolic Harumanis leaves extracts (HLEs)

methanol regarding traceability (Abdu et al., 2015; Dai & Mumper, 2010). A study reported that maceration of mango leaves with a 1:10 (w/v) ethanol ratio also produced significant results in the expression of flavonoids (Sari et al., 2022). Hence, it can be concluded that an organic solvent, such as ethanol, was particularly effective in extracting the flavonoid content of Harumanis leaves.

Fourier Transform Infrared Spectrometry (FTIR) Analysis of HLEs

Figure 7 shows the FTIR analysis of the ethanolic and methanolic extracts of HLEs, highlighting the extracts' functional groups and chemical composition.

The FTIR spectrum of ethanolic HLE revealed a complex chemical composition indicative of the diverse natural products present. The broad absorption band at 3384.51 cm^{-1} corresponds to O-H stretching vibrations, suggesting the presence of hydroxyl

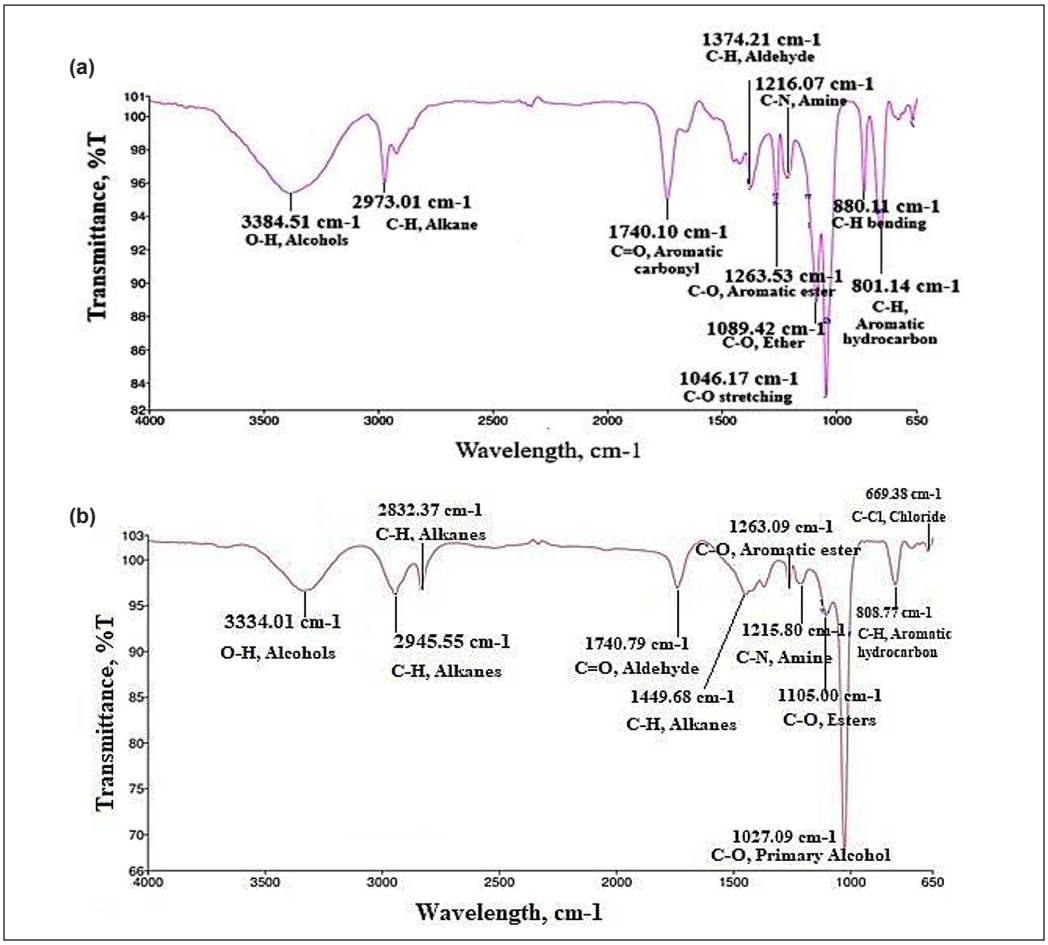

Figure 7. Fourier-Transform Infrared Spectroscopy (FTIR) spectra of Harumanis leaves extracts (HLEs): (a) ethanolic HLE; and (b) methanolic HLE

groups typically found in alcohols, a common feature in many plant-derived compounds. The peak at 2973.01 cm^{-1}, associated with C-H stretching, points to alkyl groups, which are prevalent in various organic molecules. The strong band at 1740.10 cm^{-1}, characteristic of C=O stretching in aromatic carbonyl compounds, indicates the presence of carbonyl functionalities, likely from aldehydes or ketones. Further, the band at 1374.21 cm^{-1} suggests aldehydic C-H bending, reinforcing the presence of aldehyde groups. The peaks at 1263.53 and 1216.07 cm^{-1}, corresponding to C-O and C-N stretching, respectively, imply the presence of aromatic esters and amine groups, typical of complex plant secondary metabolites. Additionally, the absorption bands at 1098.42 and 1046.17 cm^{-1} indicate C-O stretching, suggesting ether linkages within the extract. Lastly, the bands at 880.11 and 801.14 cm^{-1} confirmed the presence of aromatic hydrocarbons through out-of-plane C-H bending, highlighting the presence of aromatic rings.

Next, the FTIR spectrum of the methanolic HLE displayed several prominent peaks that corresponded to functional groups commonly associated with various phytochemicals. A broad band observed around 3334 cm^{-1} indicates the presence of O-H stretching vibrations characteristic of alcohols and phenolic compounds. The peaks at 2945 and 2832 cm^{-1} are associated with the C-H stretching vibrations of alkanes, suggesting the presence of long-chain hydrocarbons. The band at 1740 cm^{-1} corresponds to aldehydes' carbonyl (C=O) stretching, while the peak at 1449 cm^{-1} suggests C-H bending, further confirming the presence of alkanes. The sharp peak at 1263 cm^{-1} can be attributed to the C-O stretching of aromatic esters, indicating esterified compounds in the leaf extract. The C-N stretching was observed at 1215 cm^{-1}, suggesting the presence of amines, which may be linked to alkaloids or amino acids. Additionally, the peak at 1027 cm^{-1} corresponds to the C-O stretching of primary alcohols, while the band at 1105 cm^{-1} represents ester C-O stretching, suggesting the presence of various esters. The smaller peaks around 669 and 808 cm^{-1} indicate the presence of chlorides (C-Cl stretching) and aromatic hydrocarbons, respectively. Together, these spectra demonstrated the diverse chemical composition of HLEs, featuring structures of alcohols, esters, aldehydes, alkanes, and aromatic compounds, which are characteristic of phenolic and flavonoid compounds, contributing to their bioactivity and underscoring their potential therapeutic benefits.

Antioxidant Assay

Table 3 presents the DPPH scavenging activities of the HLEs. The study revealed that the ethanolic extract exhibited remarkable antioxidant activity, boasting the lowest IC_{50} value of 52.905 ± 1.12 μg/mL. In comparison, methanolic leaves showed a slightly higher IC_{50} value of 84.649 ± 0.87 μg/mL. A lower IC_{50} value indicates that a smaller concentration is required for 50% inhibition, highlighting the potent antioxidant capability of the ethanolic extract (Hassanpour & Doroudi, 2023). As a polar solvent, ethanol effectively extracts a

Table 3
DPPH scavenging activity of Harumanis leaves extracts (HLEs)

Extracts	DPPH Scavenging Activity Value (IC_{50})
Ascorbic acid (standard calibration)	0.7029 ± 0.97 µg/mL
Optimized ethanolic HLE	52.905 ± 1.12 µg/mL
Optimized methanolic HLE	84.649 ± 0.87 µg/mL

wide range of phenolic compounds, including flavonoids and antioxidants, from mango leaves. These bioactive compounds are known for their ability to scavenge free radicals, such as DPPH, which is crucial for combating oxidative stress.

The ethanolic extract has a higher phenolic content, enhanced DPPH radical scavenging activity, and superior antioxidant effects than the methanolic extract (Baliyan et al., 2022; Gondi & Rao, 2015; Kingne et al., 2019). This efficacy is attributed to the ability of ethanol as a polar solvent to extract a richer spectrum of antioxidant agents, including potent phenolic acids (Koirala et al., 2024). In contrast, the lower radical-scavenging activity of the methanolic extract reflects the reduced concentration of these valuable antioxidants. This study underscores ethanol as the optimal solvent for maximizing the extraction of antioxidant compounds from HLEs, with promising enhanced therapeutic benefits against oxidative stress.

Anti-Bacterial Assay

The antibacterial activity of the HLEs was assessed using the disc diffusion method against three diverse bacteria: *Escherichia coli, Pseudomonas aeruginosa*, and *Bacillus cereus*. This method visualizes the inhibitory zones around filter paper discs after incubation, reflecting the antibacterial effects of the extracts. The diameters of these zones directly correlate with the potency of the extracts against bacteria. To benchmark their efficacy, the samples were compared against ciprofloxacin, a broad-spectrum antibiotic, glycerin (glycerol), known to enhance plant extracts' antimicrobial activity, and Dettol, a renowned hand sanitizer. This comparative study aimed to ascertain the effectiveness of HLEs in inhibiting microbial growth, highlighting their potential as natural antibacterial agents. Based on Table 4, it can be seen that the HLEs in different extracts possess antimicrobial activity against all three tested microbes. Ethanolic extracts, especially ethanol with distilled water, exhibited the largest inhibition diameter against *E. coli* (31 mm) and consistent inhibition against *P. aeruginosa* (13 mm) and *B. cereus* (18 mm). Whereby its counterpart of ethanolic leaves in glycerin exhibits an inhibition diameter of 29 mm against *B. cereus*, 27 mm against *P. aeruginosa* and 13 mm against *E. coli*. Antibacterial activity is categorized based on the diameter of the inhibition zone: less than 5 mm is considered weak, 5–9 mm is moderate, 10–19 mm is strong, and over 20 mm is extremely strong (Gulcin & Alwasel, 2023). This classification reflects the antibacterial efficacy of the ethanolic leaf extracts against various

microorganisms. This indicates that the antibacterial properties of ethanolic leaves against *E. coli* are powerful when mixed with distilled water. When combined with glycerin, the growth inhibition of *P. aeruginosa* and *B. cereus* was very strong. This also shows that ethanolic leaves have the highest antimicrobial properties.

This finding also correlates with previous results where the highest antimicrobial properties were observed in ethanolic leaves, as the extract showed the highest antioxidant activity based on the lowest IC_{50} value. In the microbial environment, the higher content of phenols and flavonoids interacts with microbial cells at a molecular level, disrupting cellular processes and structures (Huang et al., 2022). They can penetrate microbial cell walls, leading to cell lysis and death. Furthermore, these compounds can interfere with microbial communication systems, affecting microbial colonization and survival. The antimicrobial activity of phenols and flavonoids in the environment is crucial for combating microbial infections and controlling their populations (Omidfar et al., 2023). However, methanolic leaves in glycerin also demonstrated promising results, as they strongly inhibited the growth of *P. aeruginosa* (30 mm) and *B. cereus* (27 mm). Both ethanolic and methanolic leaf

Table 4
Summary of antimicrobial tests for Harumanis leaves extracts (HLEs)

Test organism	**Sample**	**Diameter of inhibition zone (mm)**
E. coli	Ciproflaxacin	23
	Glycerin	18
	Dettol Hand Sanitizer	9
	Ethanolic Leaves + Glycerin	13
	Ethanolic Leaves + Distilled Water	31
	Methanolic Leaves + Glycerin	16
	Methanolic Leaves + Distilled Water	19
P. aeruginosa	Ciproflaxacin	33
	Glycerin	31
	Dettol Hand Sanitizer	9
	Ethanolic Leaves + Glycerin	27
	Ethanolic Leaves + Distilled Water	13
	Methanolic Leaves + Glycerin	30
	Methanolic Leaves + Distilled Water	21
B. cereus	Ciproflaxacin	35
	Glycerin	32
	Dettol Hand Sanitizer	6
	Ethanolic Leaves + Glycerin	29
	Ethanolic Leaves + Distilled Water	18
	Methanolic Leaves + Glycerin	27
	Methanolic Leaves + Distilled Water	19

extracts were better antibacterial agents than Dettol hand sanitizer. This may be because the product is commonly used for sanitization to lower concentrations of microbes on the skin's surface. Thus, the product exhibited mediocre antibacterial properties when tested via the disc diffusion method (Ecevit et al., 2022). Overall, the results showed that both HLEs had potent antimicrobial properties, whereas ethanolic leaves showed better antimicrobial properties, as supported by previous tests. Therefore, these results support using HLEs as potential medicinal herbal products.

Collectively, HLEs hold potential as natural alternatives to synthetic antioxidants and antibacterial agents for pharmaceutical applications. These extracts, along with compounds such as phenolic acids and flavonoids, exhibit potent antioxidant and antibacterial properties, effectively neutralize free radicals, and inhibit bacterial growth. Notably, HLEs have demonstrated robust free radical scavenging activity, consistent with similar findings for other mango varieties, including Tommy Atkins, Alphonso, and Kent (Sousa et al., 2023). With increasing consumer demand for plant-based and eco-friendly products, HLEs offer a viable alternative to synthetic compounds in pharmaceutical formulations, addressing therapeutic needs while minimizing environmental impacts.

CONCLUSION

This study highlights the therapeutic potential of Harumanis mango leaves, which contain a rich polyphenol composition. The leaves were confirmed to be of high quality and exhibited promising medicinal properties. Ethanolic HLEs stood out, showing the highest levels of total phenolic and flavonoid contents, positioning them as strong candidates for therapeutic use. FTIR analysis further identified various phytochemicals, such as phenols, alkanes, alkenes, and alcohols, contributing to the antioxidant capabilities of the extracts.

The superior antioxidant and antibacterial properties of ethanolic HLEs make it a promising candidate for natural therapeutic development. Their demonstrated efficacy highlights their potential applications in managing oxidative stress-related conditions and microbial infections, providing a sustainable and eco-friendly alternative to synthetic agents. HLEs offer the potential to develop innovative herbal formulations that promote human health while meeting the growing demand for environmentally sustainable solutions. Their commercialization could also drive economic growth by enabling the production of high-demand, sustainable products. Advancing research in this area is essential to bridge the gap between laboratory studies and practical applications, thus paving the way for the widespread adoption of HLEs as effective therapeutic agents. By supporting the development of eco-friendly alternatives to synthetic compounds, the commercialization of HLEs contributes to environmental sustainability and provides substantial economic benefits. These efforts will firmly establish HLEs as a valuable resource in natural therapeutics, advancing health outcomes and sustainability.

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