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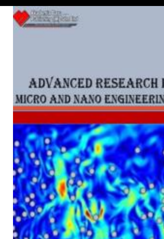

Insights into the Pharmacognostic Elucidation of Harumanis Mango (*Mangifera Indica* Linn.) Leaves Extracts as Therapeutic Agent

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ABSTRACT

Mangifera indica L. (mango) of the Anacardiaceae family is a fruit with many phytochemicals that are extensively used in various traditional systems of medicine to prevent and treat various diseases. In Malaysia, harumanis is a local variety of mango widely cultivated in the northern region, namely Perlis. However, the drawback of this harumanis variety of mango is that research and information on its therapeutics are scarce and limited. Therefore, localized harumanis leaves were introduced to a series of pharmacognostic studies to provide critical details explaining harumanis as a potential therapeutic agent. Initially, the harumanis leaves were introduced to quality analysis tests, unveiling that the raw material used abided by proper post-harvesting protocols. Then, the harumanis leaves were extracted using ultrasonic aided extraction (UAE) with 100% methanol (13.73±0.24 %), 50% methanol (14.47±0.19 %), 100% ethanol (10.35±0.19 %), 50% ethanol (12.61±0.26 %), and distilled water (8.09±0.21 %). Next, the initial phytochemical screening revealed the presence of flavonoids, tannins, and saponins in all extracts. Subsequently, the phenolic and flavonoid content were quantified with the 50% methanol extracts recorded the highest total phenolic content (5.76 ± 0.02 mg/g), 50% ethanol extracts had the highest total flavonoid content (4.38 ± 0.04 mg/g). Fingerprinting analysis via FTIR spectroscopy was used to identify phytochemicals confirming the presence of flavonoids and phenolics compounds in all extracts. The extracts were then introduced to a DPPH inhibition assay with the highest IC_{50} value obtained from 50% methanol with 75.2 ± 0.22 µg/ml. Next, the antimicrobial capabilities were tested using a disc diffusion study on *E. coli, B. subtilis*, and Face normal flora showing that the methanolic extract has superior abilities in microbial inhibitions with the value recorded at 13mm, 22mm, and 14mm respectively. Overall, we obtained valuable pharmacognostic data on the harumanis leaves extracts as potential antioxidant and antimicrobial agents.

1. Introduction

The pharmacognostic study is synonymous with the discovery and development of new drugs, as natural products have been a significant source of drugs for centuries. It involves identifying,

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isolating, and characterizing bioactive compounds present in natural sources that can be used to develop new drugs or enhance existing treatments. Many of the modern drugs that are used today, such as aspirin and morphine, were originally derived from natural sources. This is also supported since the public's interest in naturally sourced medicines is due to various factors, including the fact that are less expensive, have fewer side effects, and are mistakenly believed to be superior to manufactured products [1]. Studying natural products allows the researcher to understand their chemical composition and mechanism of action, which can lead to discovering new drug targets and treatments for various diseases.

Nowadays, the therapeutic potential of *Mangifera indica* has led to increased interest in studying its bioactive compounds for potential use in drug development. Commonly known as the mango tree, *Mangifera indica* is a tropical fruit tree native to South Asia. Beyond its culinary uses, the plant has a long history of use in traditional medicine to treat various ailments [2]. In the northern region of Malaysia, namely Perlis, harumanis mango is a premium mango cultivar well-known for its unique taste and aroma. Natural antioxidant consumption is inversely related to morbidity and mortality from degenerative diseases. This mango variety is highly valued for its sweet, juicy, and fibreless flesh, making it a popular fruit for consumption.

Although it is gaining interest as a desirable fruit, little is known about its therapeutic potential, particularly the leaves. The main challenges are the lack of research on the bioactive compounds present in the leaves. Most studies on harumanis mango have focused on the fruit, and little is known about the chemical composition of the leaves. Ethnomedicinal accounts of mango, in general, mention that in addition to the fruit, other parts, such as the leaves, are used to treat various ailments such as diarrhea, toothache, and diabetes. On the scientific side, there are details and evidence of the pharmacological activities of mango, such as anti-diabetes, anti-cancer and antioxidants. Although there are studies on the pharmacological properties of mango leaves, they do not reflect the variety of harumanis, because different varieties generally produce different functional therapeutic effects.

Additionally, the quality and quantity of natural products can vary depending on various factors such as the plant's age, environmental conditions, and harvesting techniques [3]. It is well established that the type and concentrations of metabolites produced by a plant are determined by the species, genotype, physiology, developmental stage, and environmental factors during growth [4]. In other words, different plant varieties provide various polyphenols and pharmacological activities.

Therefore, the objective and aim of this research is to identify and characterize the bioactive compounds in harumanis leaves and determine their therapeutic potential. Addressing these challenges will be crucial for fully understanding the therapeutic potential of harumanis leaves and unlocking their potential health benefits. Hence, this study was devised to obtain insight into the therapeutic potential of harumanis leaves as a potential nutraceutical and pharmaceutical product. Since one of the significant challenges in the pharmacognostic study is the complexity and variability of natural sources, a series of experimental protocols were applied to explain the harumanis leaves' abilities as potential herbal drugs.

2. Methodology

2.1 Plant Material Preparation

Approximately 1 kg of harumanis pruned leaves was collected from the Institute of Sustainable Agrotechnology (INSAT) at the Universiti Malaysia Perlis. The pruned leaves were washed thoroughly and dried overnight in a 50°C oven. Then, the dried leaves were grounded into a fine powder by using the electric grinder. For further use, the powder was kept in an airtight container.

2.2 Determination of Foreign Matters

Harumanis leaves were weighed and dispersed over a thin sheet layer to execute the foreign matters. After that, the foreign stuff on the leaves was separated and sorted using a magnifying glass and naked eyes. The foreign matters that were isolated were weighted. To calculate the percentage of foreign matter, the weight of foreign particles was divided by the weight of the sample and multiplied by 100 [5].

2.3 Determination of Total Ash Contents

The weight of 2 g of harumanis leaves powder was heated in a 50 ml silica crucible on a hot plate, then heated in a muffle furnace for four hours at 400°C. After it was cooled down, the percentage of total ash was obtained by dividing the weight of total ash content by the initial weight of the powdered sample and multiplying it by 100 [6].

2.4 Determination of Acid Insoluble Ash

The total ash obtained was mixed with 25 ml of 5 M hydrochloric acid for 5 minutes for acid-insoluble ash determination. Before placing the watch glass in the crucible, it was rinsed with 5 ml of hot water. After collecting the insoluble ashes on a piece of Whatman No. 1 filter paper, the filtrate was rinsed with hot water until neutral. The insoluble ashes were returned to the original crucible through filter paper, dried on a hotplate and burned to constant weight. After that, the residue was allowed to cool before being weighed as soon as possible. The percentage of acid-insoluble ash was calculated by dividing the weight of acid-insoluble ash by the initial weight of the sample powder and multiplying it by 100 [7].

2.5 Determination of Total Moisture Content

The total moisture content of harumanis powder was determined by using a moisture analyzer (A&D Hybrid Moisture Analyzer, Michigan). Approximately 2 g of harumanis sample powder was weighed and placed in the moisture analyzer. The moisture analyzer calculated the percentage of total moisture content. For the harumanis sample, the method was repeated three times to improve accuracy.

2.6 Preparation of Harumanis Leaves Extract

Exactly 5g of harumanis sample powder was weighed and placed in conical flasks. Five different solvents were used in ultrasonic-assisted extraction, which are 100% methanol, 50% methanol, 100% ethanol, and 50% ethanol and distilled water. In each conical flask, 100 ml of the respective solvent was added. After that, the solution was transferred to an ultrasonic bath with a temperature of 50°C and the amplitude was set at 25% for 20 minutes. After the extraction, the solution was cooled down, and the extract was separated using a centrifuge at 4000 rpm for 15 minutes. The extract was dried in an oven overnight at 65°C and stored at room temperature [8]. The percentage yield of the extract was calculated by dividing the weight of the crude extract by the weight of the sample powder and then multiplying the value by 100. The method was repeated in triplicate to improve accuracy.

2.7 Phytochemical Screening of Harumanis Leaves Extract
2.7.1 Sample preparations

Approximately 10 mg/ml of the stock solution sample extracts with different solvents, including 100% methanol, 50% methanol, 100% ethanol, 50% ethanol and distilled water extracts, were prepared. The extract was diluted with the solvent used to make a solution with a concentration of 10 mg/ml. The filtration using Whatman No.1 filter paper was performed to separate the residues and the solution. The solution was then subjected to a series of tests.

2.7.2 Preliminary phytochemical screening

The presence of phytochemicals was determined in harumanis leaves extracts; using harumanis leaves powder. Hydrochloric acid, sodium hydroxide, and iron chloride are the chemicals that were used in the following standard methods. The color change caused by the reaction of chemicals and phytochemicals present in harumanis leaves samples powder enabled qualitative analysis of phytochemicals on the samples.

2.7.3 Test for flavonoids

A few 10% sodium hydroxide solution drops were added to 2 ml of aqueous extract. This results in yellow color. When weak hydrochloric acid was added, the color was changed from yellow to colorless, indicating the presence of flavonoids [9].

2.7.4 Test for tannins

A ferric chloride test was utilized to identify the presence of tannins in the harumanis leaves extract. 5 ml of distilled water was used to dissolve the extract (50 mg). After that, a few drops of a neutral ferric chloride solution of 5% ferric chloride were added. The presence of phenolic compounds was indicated by a dark green color [10].

2.7.5 Test for saponins

Distilled water was used to dilute 50 mg of harumanis leaves extract to make a total volume of 20 ml. Then, the suspension was shaken for 2 minutes. The formation of a 2 cm layer of foam indicates the presence of saponins [10].

2.8 Fingerprinting Analysis of Harumanis Leaves Extracts

Fingerprinting analysis of the harumanis leaves extracts was conducted via Fourier-Transform Infrared Spectroscopy (FTIR), Perkin Elmer. Approximately 1 ml of 1 mg/ml concentration extract solution was employed. The liquid sample was placed into an FTIR spectroscope with a scan range of 650 cm^{-1} to 4000 cm^{-1} and a 4 cm^{-1} resolution. The spectrum was then used to calculate peak values and the likely functional group for each sample.

2.9 Quantitative Analysis of Phytochemical Content
2.9.1 Total phenolic content

The standard calibration curve was created using gallic acid solutions in methanol with the concentration of 0.03125, 0.0625, 0.125, 0.5 and 1 mg/ml. 100 µl of each prepared gallic acid concentration was pipetted into a test tube, followed by 100 µl Folin-Ciocalteu reagent (10%) and 2 ml of 2% sodium carbonate (Na_2CO_3) solution. The color of the solution was changed to dark blue. After thoroughly mixing solutions, they were incubated in a dark room for about 30 minutes at room temperature. The absorption wavelength was measured at 720 nm. All measurements were made in triplicate, and the standard calibration curve was plotted. The absorbance for harumanis extract was measured using the same techniques to determine the total phenolic content of harumanis extract samples. The total phenolic content of the extracts was determined in mg of gallic acid equivalents (GAE) per gram of sample. All the tests were repeated in triplicate. Total phenolic content was calculated by multiplying the concentration by the calibration curve and volume of extract and dividing the value by the mass used [11].

2.9.2 Total flavonoids content

A standard calibration curve was created using quercetin concentrations of 0.03125, 0.0625, 0.125, 0.25, 0.5 and 1.0 mg/ml. 250 µl quercetin was mixed with 150 µl aluminum chloride solution ($AlCl_3$). After 5 minutes, 0.5 ml of 1 M sodium hydroxide (NaOH) and 575 µl deionized water were added to the mixture. The color of the solution was changed to yellow. After that, the absorbance was measured with a UV-Vis Spectrophotometer set at 510 nm. A calibration curve was created based on the absorbance obtained. The total flavonoid content of extract samples was determined using the same procedures, and the absorbance was recorded for each extract [12]. All the tests were repeated in triplicate. The total flavonoid content was calculated by multiplying the concentration from the calibration curve and the volume of extract used and dividing the value by the mass of extract used.

2.10 Determination of Antioxidant Activity via DPPH Pathway

The DPPH test, according to a method by Bakar *et al*., [13], was used to determine the antioxidant activity. The standard calibration curve comprised 31.25 ppm, 62.5 ppm, 125 ppm, 250 ppm, 500 ppm, 1000 ppm, and 2000 ppm of ascorbic acid in methanol. DPPH solution was prepared by dissolving 3.2 mg in 100 ml methanol. 200 µl of each concentration of ascorbic acid was added to 2.8 ml of DPPH solution. The solutions were shaken well and incubated at room temperature for 1 h before being analyzed with a UV/Vis spectrophotometer at 517 nm. Methanol was used as blank, while 0.2 ml of methanol and 2.8 ml of DPPH solution were used as control. The obtained absorbance was used to create a calibration curve. The same procedures were used to determine extract samples' antioxidant activity, and each extract's absorbance was recorded. By subtracting the absorbance of the extract from the absorbance of the control, the value is then divided by the absorbance of the control, and then the value obtained is multiplied by 100, thus, the DPPH radical scavenging activity (%) was determined. All the tests were performed in triplicate.

2.11 Determination of Antimicrobial Activity

The antimicrobial activity of harumanis leaves extract was tested using a disc diffusion test against three bacteria, *E. coli, B. subtilis*, and face microbes. The zone of inhibition of bacterial growth against the concentration was observed to determine the antibacterial activity of harumanis leaves extract. Four portions of the lid of the MHA plate were marked. Two portions were for control, 2 mg/ml concentration of Ciprofloxacin (broad-spectrum antibiotic) as the positive control and methanol as the negative control, while two other portions were for the extract's solution. The organism concentration was calibrated to a McFarland standard of 0.4. A sterile paper disc was soaked with a 2 mg/ml concentration of the leaves extract solution. The paper discs were immersed in the correct location on the petri dish using sterile forceps. To establish complete contact with the MHA's plate surface, the discs were gently pressed down on them. The plates were incubated for 24 hours at 35°C. After a 24-hour incubation period, a clear zone of inhibition was identified [14].

3. Results
3.1 Quality Control Analysis

Raw material quality is of utmost importance in plant pharmacognosy studies. The raw material's quality directly affects the final product's quality and efficacy. It is essential to ensure that the raw materials used in plant pharmacognosy are of the highest quality, free from contaminants and impurities. Poor quality raw materials can lead to inaccurate results in pharmacological and toxicological studies and negative effects on human health. Therefore, it is essential to obtain raw materials from reliable sources and conduct quality control tests to ensure they meet the necessary standards. Only by paying close attention to raw materials' quality can researchers confidently explore the potential benefits of plants in pharmacological and medicinal fields. In this study, the harumanis leaves quality was assessed using several methods: foreign matter, total ash, acid-insoluble ash, and moisture.

The total foreign matter analysis is a quality control test conducted on raw materials used in the pharmaceutical and herbal industries [15]. It involves determining the foreign matter in a given raw material sample. Foreign matter can be defined as any extraneous material that is not part of the plant or herb, such as soil, stones, metals, or insects. The presence of foreign matter in raw materials can affect the quality and purity of the final product and safety for human consumption. Therefore, total foreign matter analysis is essential in assessing the quality of raw materials used to produce herbal and pharmaceutical products. This test is typically performed by visually inspecting molds, insects, spider webs, sediments, and stones on the raw materials. It is a crucial step in ensuring the safety and efficacy of the final product. The results showed that the harumanis leaves sample foreign matter is minimal at 2.66±0.05 %, which meets herbal pharmacopeial criteria if the plant sample's foreign matter proportion is less than 10%.

The next analysis was the total and acid insoluble ash, two commonly performed quality control tests in the pharmaceutical and herbal industries. Total ash analysis determines the total amount of inorganic material present in a raw material sample. It indicates the purity of the raw material and its mineral content. On the other hand, the acid insoluble ash analysis is the determination of the amount of inorganic material in a sample that is not soluble in hydrochloric acid, most notably as sand, siliceous soil, oxalates, carbonates, phosphates, oxides, and silicate concentrations [16]. Acid insoluble ash analysis indicates the quality of the raw material and the level of impurities present. This study recorded the harumanis leaves ash value at 12.05±0.05% total ash and 4.194±0.12 % of

acid insoluble ash, abiding the European Pharmacopoeia [17] recommended maximum total ash level of 14%, hence portraying that the raw material used was in immaculate conditions.

Total moisture content is an important quality control test in pharmacognosy studies. It involves determining the amount of moisture present in a sample of raw material, which can affect the quality and stability of the final product. Excessive moisture content in raw materials can lead to microbial growth, oxidation, and degradation of active constituents, which can compromise the efficacy and safety of the final product [18]. This analysis is crucial in evaluating the quality of raw materials used in the pharmaceutical and herbal industries. Researchers and manufacturers can produce high-quality products with consistent efficacy and safety by ensuring the raw materials have the appropriate moisture content. A high-quality herbal product should have less than 15% moisture. Overall, the moisture content of the harumanis leaves was revealed to have 8.11±0.41% moisture, indicating that harumanis leaves are acceptable and potentially can be made into high-quality herbal products.

3.2 Total Extraction Yield of Harumanis Leaves

The extraction yield of harumanis leaves extracts with five different solvents, 100% methanol, 50% methanol, 100% ethanol, and 50% ethanol and distilled water was obtained using ultrasonic-assisted extraction (UAE). Based on the results obtained, the highest extraction yield of harumanis leaves extract was obtained with 50% methanol extraction (14.47±0.19 %), followed by 100% methanol (13.73±0.24 %), 50% ethanol (12.61±0.26 %), 100% ethanol (10.35±0.19 %) and finally distilled water, (8.09±0.21 %). It can be seen that the extraction yield of 50% methanol (14.47±0.19 %) is higher than that of 100% methanol (13.73±0.24 %). This shows that the extraction yield increases when the polarity of the solvent is higher. When water and organic solvents are combined, the extraction of soluble compounds may be more efficient. Therefore, this explains extraction with water-based solvents provides higher yields than pure solvents. Other studies also reported that aqueous methanol was the best solvent for plant extraction, like rice bran and some medicinal plants, *Terminalia arjuna* and *Ficus religiosa* [19]. Hence, 50% methanol is the most suitable and effective solvent for obtaining harumanis leaves extract.

3.3 Qualitative Analysis of Harumanis Leaves

Colour tests are commonly used in pharmacognosy as a primary tool for identifying plant constituents or chemical compounds. These tests involve adding a specific reagent to a plant material sample or extract and observing the resulting colour change. The colour change indicates the presence of specific functional groups or chemical compounds in the sample, allowing researchers to determine the likely identity of the constituents. Using the colour test (Table 1), the phytochemical composition of harumanis leaves was analysed using three distinct polyphenols categories. The finding through this approach revealed that the harumanis leaves consist of polyphenols, namely flavonoids, tannins and saponins.

Table 1
Preliminary phytochemical screening of Harumanis mango leaves via colour test

Phytochemicals	Chemicals/solvents	Observation	Results
Flavonoids	Sodium hydroxide, hydrochloric acid	Formation of dark green	Present
Tannins	Ferric (III) chloride	Formation of yellow to colourless (Mild yellow)	Present
Saponins	Distilled water	Formation of a foam layer	Present

3.4 Fourier Transform Infrared Spectrometry (FTIR) Analysis of Harumanis leaves

FTIR analysis (Fourier-transform infrared spectroscopy) is a powerful analytical tool used in pharmacognosy to identify and quantify chemical constituents in plant extracts or herbal products. FTIR analysis works by measuring the interaction of infrared radiation with a sample, producing a spectrum that reflects the unique molecular vibrations of the sample. Confirmation of the polyphenols available in the harumanis leaves was conducted via fingerprinting analysis, namely the FTIR (Figure 1).

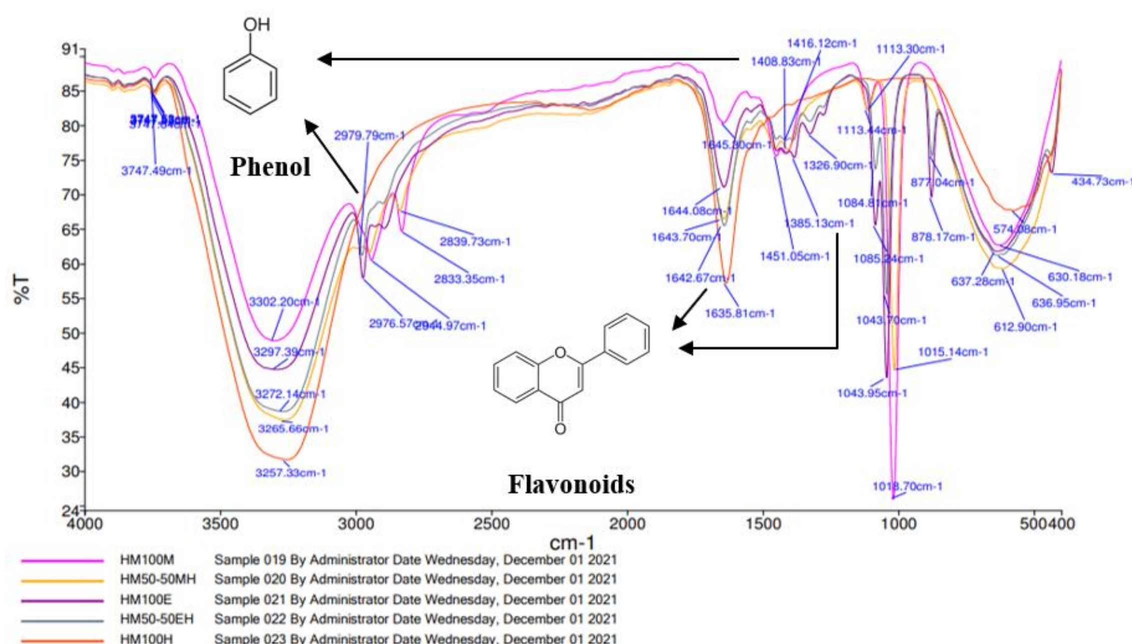

Fig. 1. FTIR spectrum peaks of Harumanis leaves extracts

FTIR analysis is instrumental in identifying and characterizing functional groups in plant constituents such as alcohols, carboxylic acids, amines, and carbonyl groups (Table 2). By analyzing the FTIR spectra of a plant extract, researchers can identify and quantify the constituents present in the extract, allowing for a better understanding of the extract's therapeutic potential. FTIR analysis is also used in quality control testing of herbal products to ensure consistency and purity.

Table 2
Summary of the functional group from FTIR spectra for Harumanis leaves extract

Frequency cm^{-1}	Bond	Functional group
3302.20	N-H stretching	1°, 2° amines and amides group
2944.97	C-H stretching	Alkane
1645.30	C=C stretching	Alkene
1113.44	C-O stretching	Alcohols
1385.13	O-H bonding	Phenol
1326.90	C-N stretching	Amines
636.95	C-Cl stretching	Alkyl halide

From 650 to 4000 cm^{-1}, a solution containing an extract of harumanis leaves was scanned via FIR. Due to their ability to extract various phytochemicals, the solvents utilised in this study may have contributed to the modest variation in peak characteristics. According to Figure 1, 100% ethanol

extracts contain the most peaks, 11 peaks, indicating that the extracts contain a variety of functional groups and have a greater capacity to extract a broader range of phytochemicals. Comparatively, water extracts had the fewest peaks, four, indicating that only a small number of phytochemicals were identified. In addition, a medium peak at 3302.20 cm^{-1} indicates the presence of the 1°, 2° amines and amides group's N-H stretching vibration. The predicted alkane (C-H stretching) and alkene (C=H stretching) peaks for the primary materials are 2944.97 cm^{-1} and 1645.30 cm^{-1}, respectively. In addition, the peak at 1113.44 cm^{-1} indicated the presence of the alcohols group (C-O stretching). At peak 1385.13cm^{-1}, the phenol group (O-H bonding) was detected. The presence of O-H bonds verifies the presence of a phenolic compound in the leaves extract of harumanis. The functional group of alkyl halide (C-Cl stretching) was then detected in the sample at the peak of 636.95 cm^{-1}. For the medicinal properties of the extracts, the presence of alcohols, amines, alkanes, and alkyl halides is crucial. In addition, none of the samples displayed the characteristic cyanide region between 2280 cm^{-1} and 1990 cm^{-1}. Cyanide is a poisonous substance that poses a severe risk to human health and can cause toxicity [20]. Therefore, the harumanis extract sample was harmless for consumption as it contained no cyanide. Overall, FTIR analysis is a versatile and powerful analytical tool used in pharmacognosy for research and quality control purposes. It allows for identifying and quantifying plant constituents, providing valuable insights into the potential therapeutic benefits of herbal products.

3.5 Total Phenolic and Flavonoid Contents of Harumanis Leaves Extracts

Phenolic compounds are a diverse group of secondary metabolites found in plants. They are known to have various beneficial effects on human health due to their antioxidant and anti-inflammatory properties [21]. The total phenolic content (TPC) values for all harumanis leaves extracts were quantified using UV spectroscopy with gallic acid as the test standard. From this analysis, 50:50 methanol extract has recorded the highest phenolic content with a value of 5.76 ± 0.02 mg/g, followed by 50:50 ethanol extract (5.15 ± 0.02 mg/g), 100% distilled water (4.9 ± 0.02 mg/g), 100% ethanol (3.34 ± 0.03 mg/g) and finally 100% methanol (3.08 ± 0.02 mg/g). It was observed that the TPC value was found to be higher in the water-based solvents, 50-50 methanol and 50-50 ethanol, compared to the pure organic solvent, 100% ethanol and 100% methanol. This indicates that the water is favorable for phenolic compound extraction in harumanis leaves. The solubility of antioxidant compounds in a solvent relies on the characteristics of the phytochemicals present in the food [22]. Polyphenols are frequently most soluble in less polar organic solvents than water [23], suggesting that the aqueous solution containing a polar solvent such as methanol is optimal for extracting phenolic compounds from harumanis leaves. Therefore, methanolic extracts are an effective solvent for polyphenol extraction from harumanis leaves extracts.

Total flavonoid content (TFC) is the total amount of flavonoid compounds present in a sample. Flavonoids are a class of plant secondary metabolites widely distributed in the plant kingdom and have diverse biological activities, including antioxidant, anti-inflammatory, and anti-cancer properties. This study determined the total flavonoid content of harumanis leaves extract using an Aluminium Chloride Colorimetric test calibrated with quercetin as the primary standard. The quantified value unravelled that the 50:50 ethanol harumanis leaves extract has the highest flavonoid content with a value of 4.38 ± 0.04 mg/g followed by 50:50 methanol (3.49 ± 0.03 mg/g), distilled water (3.31 ± 0.04 mg/g), 100% ethanol (2.44 ± 0.03 mg/g) and finally 100% methanol (2.40 ± 0.03 mg/g). This indicates that the polarity of the solvent influences the display of TFC values and that the optimal TFC values were obtained with a 50% ethanol solvent. Compared to other solvents, ethanol was demonstrated to be the most effective for flavonoid extraction. This is because ethanol is

superior to water and methanol regarding its traceability. According to Masturi *et al.*, [24], the optimal total flavonoid content of harumanis seeds was obtained using a 96% ethanol solvent. Thus, it can be concluded that organic solvents derived from water, such as 50% ethanol and 50% methanol, were notably effective at extracting the flavonoid content of harumanis leaves.

3.6 Antioxidant Assay of Harumanis Leaves Extracts

The DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging assay is a widely used method to measure the antioxidant activity of a sample. When a sample with antioxidant activity is added to the DPPH solution, the free radical is reduced, resulting in a loss of color. The degree of color change can be measured spectrophotometrically to indicate the sample's ability to scavenge free radicals. The DPPH scavenging assay is a simple and relatively fast method to measure the antioxidant activity of a sample. It can evaluate the antioxidant activity of a wide range of samples, including plant extracts, foods, and dietary supplements.

Additionally, the IC_{50} is commonly used in DPPH scavenging studies to represent the sample concentration required to scavenge 50% of the DPPH free radicals. It is a measure of the antioxidant potency of a sample and is commonly used to compare the antioxidant activity of different samples. In this study, the potential of harumanis leaves extracts was tested on the DPPH for antioxidant potential (Table 3) and was recorded as an IC_{50} value.

Table 3
DPPH scavenging activity of Harumanis leaves extracts

Extracts	DPPH scavenging activity value (IC_{50})
100% methanol	365.2 ± 0.25 µg/ml
50% methanol	75.2 ± 0.22 µg/ml
100% ethanol	329.2 ± 0.43 µg/ml
50% ethanol	266.6 ± 0.27 µg/ml
Distilled water	1528.5 ± 0.38 µg/ml
Ascorbic acid (standard)	62.6 ± 0.31 µg/ml

The DPPH scavenging activity of harumanis leaves extracts dissolved in 100% methanol, 50% methanol, 100% ethanol, 50% ethanol, and distilled water. According to the findings, 50% methanol harumanis leaves extracts exhibited a significant level of antioxidant activity, as indicated by the lowest IC_{50} value of 75.2 ± 0.22 µg/ml. The high content of phenolic and flavonoids from available methanolic extracts may contribute to the high antioxidant properties as shown in the 50% methanol harumanis leaves extracts since these metabolites are well known for inhibiting free radicals [25].

3.7 Antimicrobial Test

Harumanis leaves extract antibacterial activity was determined using the disc diffusion method. This method assessed the antibacterial properties of harumanis extracts against three distinct bacteria: *E. coli, B. subtilis*, and face pathogen. Following incubation, an inhibitory zone will form around the filter paper disc if the plant extracts or isolated compounds are microbiologically active. The diameter of the inhibition zone accurately represents the antibacterial activity of plant extracts or particular components [26]. This experiment uses methanol as a negative control because it can dissolve polieugenol and lacks antibacterial properties [27]. The purpose of using negative controls is to ensure that the employed solvent has no impact on the antibacterial test results. The positive control in this antimicrobial activity test was the broad-spectrum antibiotic ciprofloxacin, which is

effective against the activity of gram-positive and the majority of gram-negative bacteria. Based on Table 4, it can be seen that the leaves extracts of various solvents of harumanis possess antimicrobial activity against each of the three microorganisms tested. Methanolic extracts, particularly 100% methanol, consistently exhibit a large diameter of inhibition against *E. coli* (13 mm), *B. subtilis* (22 mm), and other microorganisms (14 mm). According to Handayani *et al.*, [28], antibacterial activity can be categorized based on the diameter zone displayed, with less than 5 mm indicating weak, 5-9 mm indicating medium, 10-19 mm indicating strong, and more than 20 mm meaning exceptionally strong. This suggests that the antibacterial properties of 100 % methanol against *B. subtilis* are highly potent, while those against E. coli and the face pathogen are moderate. This indicates that 100% methanol extracts were the most antimicrobial. The flavonoids content is also an effective antibacterial by forming complex compounds against extracellular proteins that disrupt the integrity of bacterial cell membranes, microorganism cell function, and inhibits the microbial cell cycle. At high concentrations, phenol content penetrates, disrupts the bacterial cell wall, and precipitates proteins in bacterial cells. In lower concentrations, phenols activate important enzyme systems in bacterial cells [29]. For that reason, the antibacterial properties of harumanis leaves were probably due to the presence of polyphenols, namely the flavonoids. Overall, the results indicate that methanolic extracts of harumanis leaves extracts, including 100% methanol and 50% methanol, possessed significant antimicrobial properties that supported using harumanis leaves extract as a potential medicinal herbal product.

Table 4
Summary of antimicrobial tests for Harumanis mango leaves extract

Test organism	Sample	Inhibition zone diameter (mm)
E. coli	Positive control (Ciprofloxacin)	32
	Negative control (Methanol)	6
	100% methanol	13
	50% methanol	16
	100% ethanol	9
	50% ethanol	24
	Distilled water	13
B. Subtilis	Positive control (Ciprofloxacin)	35
	Negative control (Methanol)	6
	100% methanol	22
	50% methanol	13
	100% ethanol	10
	50% ethanol	6
	Distilled water	22
Face microbe	Positive control (Ciprofloxacin)	36
	Negative control (Methanol)	13
	100% methanol	14
	50% methanol	12
	100% ethanol	9
	50% ethanol	10
	Distilled water	11

4. Conclusions

Natural products have been used for centuries as a source of medicine. Pharmacognosy provides a scientific basis for the use of these products. Researchers can identify the active ingredients in natural products and study their pharmacological effects. This information can mainly contribute to the development of a more effective and safer drugs. Additionally, pharmacognosy plays a crucial

role in the quality control of herbal medicines. Identifying active compounds in herbal extracts is vital to ensure the efficacy and safety of herbal medicines. Hence, this study conducted several investigations and observations to determine the therapeutic properties of harumanis mango leaves via a pharmacognostic approach. The research is comprised of quality control, chemical profiling and therapeutical analysis. The analysis shows that harumanis leaves contain various phytochemicals, including flavonoids, tannins, and saponins, typically found in medicinal plants. Determining foreign matter, total ash content, acid-insoluble ash content, and total moisture content are included in the gravimetric evaluation of the quality of harumanis leaves as raw material. Through this analysis, it has been determined that harumanis leaves have a high level of quality control, as all test values fall within an acceptable range. As a result, the quality control of harumanis leaves as a raw material is adequately ensured to maintain the quality of botanical products. The fingerprinting analysis via Fourier-transform infrared spectroscopy (FTIR) evaluates the availability of functional groups in harumanis leaves extracts. Through this analysis, FTIR identified numerous phytochemicals, including phenols, and flavonoids, in the extract of harumanis leaves in various solvents. These phytochemicals are the critical component that can contribute to developing herbal products, as their high antioxidant capacity can provide health benefits when ingested. On five distinct extracts of harumanis leaves, antioxidant and antimicrobial tests were also conducted. Due to the presence of phytochemicals such as flavonoids and phenolic, which are antioxidant-rich compounds, the antioxidant assay revealed that all the extracts possessed exceptional antioxidant properties. For the microbial test, a disc diffusion test was conducted to assess the zone inhibition of the extracts. The antimicrobial test has succeeded since all of the extracts displayed visible zone inhibition, particularly in the *B. subtilis* test. Therefore, it can be concluded that the harumanis leaves possess good antioxidant and antimicrobial properties that can be used to manufacture a commercial herbal product.

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