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Original Research Article

Phytochemical Composition and Antioxidant Activity of Methanol, Ethanol, and Water Extracts from Pruned Harumanis Mango Leaves

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Abstract: This study investigates the phytochemical composition, antioxidant activity, total phenolic content, and total flavonoid content of methanol, ethanol, and water extracts from pruned Harumanis mango leaves. High-performance liquid chromatography (HPLC) detected mangiferin, with all extracts yielding positive results. The detected phytochemicals include flavonoids, cardiac glycosides, tannins, and steroids. Among the solvents tested, ethanol and methanol were identified as the most effective for extracting phytochemicals from Harumanis mango leaves. Both extracts contained flavonoids, cardiac glycosides, tannins, and steroids. The ethanol extract exhibited the highest antioxidant activity (94.559 ± 0.425%), total phenolic content (0.055 ± 0.002 GAE mg/ml), and total flavonoid content (0.071 ± 0.002 mg/ml). Ethanol was the optimal solvent for extracting bioactive compounds from Harumanis pruned leaves, likely due to its less polar nature, which enhances extraction efficiency. The study concludes that ethanol is the most effective solvent for extracting bioactive compounds, providing the highest antioxidant activity, phenolic, and flavonoid content. This research offers valuable insights into the potential health benefits of Harumanis pruned leaves, especially concerning their antioxidant properties attributed to specific phytochemicals.

Keywords: Phytochemical activities; antioxidant activities; mango leaves, Harumanis mango pruned leaves, extraction

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1. Introduction

Mango (*Mangifera indica L.)*, belonging to the *Anacardiaceae* family within the order Sapindales, is a luscious stone fruit predominantly cultivated in tropical regions (Parvez, 2016). The Mangifera genus comprises 69 species, primarily concentrated in tropical Asia. Malaysia, particularly in the peninsula, hosts about 28 mango species, including notable cultivars like Sala, Maha 65 (MA 165), Masmuda (MA 204), Chok Anan (MA 224), and the renowned Harumanis. Harumanis, revered as the monarch of mangoes, is exclusive to the state of Perlis, Malaysia.

After the harvesting season of Harumanis mangoes, growers engage in a meticulous pruning process to maintain tree health and optimise future fruit production. By systematically thinning out the canopy and selectively removing overcrowded or crossing branches, growers ensure adequate airflow and sunlight penetration, reducing the risk of fungal diseases and promoting uniform fruit ripening. Ultimately, post-harvest pruning sets the stage for healthy regrowth and robust fruit production in the next growing season, ensuring the continued success of Harumanis mango cultivation. However, pruning mango trees results in a significant accumulation of residues, often leading to the burning of leaves and contributing to environmental concerns.

Mango by-products, particularly leaves, contain a high concentration of phenolic compounds, notably mangiferin and quercetin (Barreto *et al*., 2008). The bioactive polyphenol mangiferin found in mango leaves holds promise for various cosmetic, pharmaceutical, and food applications. The phytochemicals in Harumanis mango leaves, such as flavonoids, cardiac glycosides, tannins, and steroids, provide antioxidant, anti-inflammatory, and heart health benefits, making them valuable for potential therapeutic applications. The possible health benefits of mango leaves are that they are anti-inflammatory and provide immune support, have potential anticancer effects, and have antioxidant properties. The antioxidant activity of mango leaves is crucial in protecting the body from oxidative stress, which can contribute to chronic diseases like cancer, cardiovascular diseases, and neurodegenerative conditions. The phenolic and flavonoid content in mango leaves plays a significant role in neutralising harmful free radicals. For instance, mangiferin's antioxidant properties in cosmetics make it valuable for anti-ageing and skin rejuvenation products. In the pharmaceutical sector, mangiferin's diverse health benefits, including anti-inflammatory and antimicrobial properties, are being explored to treat various conditions. In the food industry, mangiferin's antioxidant properties can help extend the shelf life of products, and its potential health benefits may be incorporated into functional foods or dietary supplements.

In summary, the phytochemicals in Harumanis mango leaves have antioxidant properties and contribute to various health benefits, such as improving heart health, reducing inflammation, and protecting against oxidative damage. These findings highlight the potential of mango leaves as a source of natural bioactive compounds with therapeutic uses.

Consequently, recent interest has been in exploring the possibility of locally pruned Harumanis mango leaves. However, information regarding the extraction of these pruned leaves is currently limited. Therefore, this research aims to extract Harumanis mango pruned leaves and investigate the extracts' phytochemical composition and antioxidative properties.

2. Materials and Methods

2.1 Sample Preparation

Harumanis mango pruned leaves sourced from the Batu Pahat, Perlis orchard undergo a sample preparation process. Initially, the leaves were rinsed with tap water and dried overnight in an oven set at 60℃. Following drying, the leaves have been finely powdered and sieved to obtain a particle size of two hundred and fifty micrometres for use in a blender and securely stored in an airtight container, as described by Alshammaa (2016).

2.2 Extraction of Harumanis Leaves

Harumanis mango pruned leaves extracts have been prepared using the water bath extraction method. Separate extracts using methanol, ethanol, and water have been obtained by dissolving ten grams of finely ground dried Harumanis mango pruned leaves powder in one hundred millilitres of each solvent, contained in three hundred millilitre conical flasks as outlined by Ali *et al*. (2020). The mouths of the conical flasks have been sealed with cotton and wrapped with aluminium foil before subjecting the mixture to water bath extraction. Extraction has been conducted at 60ºC for 3 hours, after which the extracts have been filtered using Whatman no. 1 filter paper in a filter funnel, with the filtrates collected in conical flasks. Methanol and ethanol extracts have undergone vacuum drying using a rotary evaporator at 50ºC, while water extract has been dried using a freezer dryer at around -40ºC – 80ºC for about 2 hours (Ling *et al*., 2009).

2.3 Phytochemical Screening of Harumanis Leaves Extract

2.3.1 Detection of steroid (Liebermann-Burchard test)

Anhydrous acetic acid (CH_3COOH) with three millilitres of the solvent was added to the zero point, and one millilitre of Harumanis mango pruned leaves extract was shaken well in a test tube. A few drops of concentrated sulfuric acid (H_2SO_4) were added to the mixture. Changes in colour for the solution from red to violet and blue to green indicated the presence of the steroid in the Harumanis mango pruned leaves extract (Alshammaa, 2016). These steps were repeated for the other two extracts.

Anhydrous acetic acid (CH_3COOH) was added to the extract by mixing three millilitres of the solvent (CH_3COOH) with one millilitre of Harumanis mango pruned leaves extract in a test tube, then shaking well. Subsequently, a few drops of concentrated sulfuric acid (H_2SO_4) were added to the mixture. A colour change in the solution from red to violet and from blue to green indicated the presence of steroids in the Harumanis mango pruned

leaves extract (Alshammaa, 2016). The same procedure was repeated for the other two extracts.

2.3.2 Detection of cardiac glycosides (Keller-Killian's test)

In a test tube, 3 ml of glacial acetic acid containing one drop of 5% Iron (III) chloride ($FeCl_3$) solution was added to 0.1 ml of Harumanis mango pruned leaves extract. Then, 1 ml of concentrated sulfuric acid was added to the mixture by drops, forming two layers. A brown ring appeared at the interface, indicating deoxy sugar characteristic of cardenolides in the Harumanis mango pruned leaves extract (Alshammaa, 2016). These steps were repeated for the other two extracts.

2.3.3 Detection of saponins (Frothing test)

0.5 ml of Harumanis mango pruned leaves extract was diluted with 2.5 ml of distilled water in a test tube and shaken vigorously for 15 seconds. The formation of a stable foam layer indicated saponins in the Harumanis mango pruned leaves extract (Alshammaa, 2016). These steps were repeated for the other two extracts.

2.3.4 Detection of tannins

A few drops of an alcoholic solution of 1% Iron (III) chloride ($FeCl_3$) were added to 0.1 ml of Harumanis mango pruned leaves extract in a test tube. The appearance of a dark blue or greenish-black solution indicated the presence of tannins in the Harumanis mango pruned leaves extract (Ahmad Fuad *et al*., 2020). These steps were repeated for the other two extracts.

2.3.5 Detection of flavonoid

A few drops of 10% sodium hydroxide solution (NaOH) were added to 0.1 ml of Harumanis mango pruned leaves extract in a test tube. A yellow-colored solution was produced, which then turned colourless when 5% dilute hydrochloric acid (HCL) was added, indicating the presence of flavonoids in the Harumanis mango pruned leaves extract (Abdillah *et al*., 2015). These steps were repeated for the other two extracts.

2.3.6 Detection of mangiferin

The detection of mangiferin in the Harumanis mango leaves extract was analysed using High Performance Liquid Chromatography (HPLC). The HPLC system comprised two solvent delivery pumps, a UV-VIS detector, an autosampler, and a symmetry C-18 (4.6 ×250 nm, 5 µm) column. Mangiferin was detected on the symmetry C-18 column with a temperature controller maintained at 25°C. Chromatographic separation was carried out on a C-18 column using methanol and 2.5% acetic acid (28:72 v/v) as the mobile phase at a 1 ml/min flow rate. A volume of 0.02 ml of Harumanis mango leaves extract was injected into

the column and detected at 254 nm. The resulting chromatogram was compared to the standard mangiferin (Salomon *et al*., 2014).

2.4 Antioxidant Activity Assay of Harumanis Leaf Extracts

1,1-diphenyl-2-picrylhydrazyl (DPPH) was used to evaluate the antioxidant activity of the Harumanis mango leaves extract based on the capacity of the antioxidant compounds in the Harumanis mango leaves extract to scavenge the DPPH free radicals in vitro (Ghadage *et al*., 2017).

Different concentrations (0–5 mg/ml) of Harumanis mango pruned leaves extract were prepared. The test sample was prepared by mixing 0.1 ml of the Harumanis mango pruned leaves extract with 2.9 ml of 0.1mM DPPH methanolic solution, and the mixture was allowed to react and incubate in the dark for 30 minutes. A small amount of the solution was transferred to a cuvette. The absorbance of the reaction mixture was measured at 517 nm using a spectrophotometer (Sprada *et al*., 2014). The control was prepared by adding 2.9 ml of 0.1 mM DPPH methanol solution with 0.1 ml of methanol. The optical density obtained was converted into the percentage of DPPH free radical scavenging activity (% inhibition) using Equation 1 (Irawan *et al*., 2017). These steps were repeated for another two extracts. Three replicate absorbance readings were taken for each extract.

$$\% \text{ inhibition} = \frac{(Ac - As)}{Ac} \times 100\% \quad (1)$$

where,
Ac = Absorbance of the control
As = Absorbance of the tested sample

The free radical scavenging activity was expressed as IC_{50}, representing the amount or concentration of antioxidant compounds (extract) needed to inhibit free radical scavenging activity by 50%. The IC_{50} of a compound was inversely proportional to its antioxidant activity. It was determined from linear regression of plots where the x-axis represented the various concentrations (0–5 mg/ml) of the Harumanis mango pruned leaves extract. In contrast, the y-axis represented the percentage of free radical scavenging activities. High antioxidant activity of a sample indicated a lower IC_{50} (Do *et al*., 2014). These steps were repeated for the other two extracts.

2.5 Determination of Total Phenolic Content (TPC)

The total phenolic content of Harumanis mango pruned leaves extract was determined using the Folin-Ciocalteu colourimetric method. Folin-Ciocalteu reagent was utilised to assess the total phenolic compounds in the Harumanis mango pruned leaves extracts.

To prepare the test sample, 0.1 ml of Harumanis mango pruned leaves extract was pipetted and transferred to a test tube, with the addition of 2 ml of 15% sodium carbonate

solution, 0.5 ml of Folin-Ciocalteu reagent, and then made up to a volume of 10 ml by adding distilled water. The solution was left for 15 minutes in the dark before the absorbance was read. A small amount of the solution was transferred to the cuvette. Gallic acid standard solutions in the 0.01–0.08 mg/ml range were prepared from a 1 mg/ml stock solution of gallic acid in ethanol. The absorbance of the sample extract and the gallic acid standard solutions was determined at a wavelength of 718 nm against a blank solution using a spectrophotometer (Jadhav *et al*., 2012). These steps were repeated for the other two extracts. Three replicate absorbance readings were taken for each extract. The obtained absorbance value for the test sample was compared to the standard gallic acid calibration curve, and the total phenolic content was determined.

2.6 Determination of Total Flavonoid Content (TFC)

The total flavonoid content of Harumanis mango pruned leaves extracts was quantified using the aluminium chloride colourimetric method with a spectrophotometric technique.

To prepare the test sample, 0.05 ml of Harumanis mango pruned leaves extract was pipetted and transferred to a test tube. 0.3 ml of 5% sodium nitrate solution and 4 ml of distilled water were added to the test tube. After five minutes, 0.3 ml of 10% aluminium chloride was added to the test tube. At the sixth minute, 2 ml of 1M sodium hydroxide was added to the test tube, and the volume was made up to 10 ml by adding distilled water. The solution was left dark for 15 minutes before the absorbance was read. An orange-yellowish colour appeared. Quercetin standard solutions in the 0.01-0.10 mg/ml range were prepared from a 1 mg/ml stock of quercetin in ethanol. The absorbance of the sample extract and the standard solutions was determined at a wavelength of 415 nm against the blank solution using a spectrophotometer (Kamtekar *et al*., 2014). These steps were repeated for the other two extracts. Three replicate absorbance readings were taken for each extract. The obtained absorbance value for the test sample was compared to the standard quercetin calibration curve, and the total content of flavonoid compounds in the Harumanis mango pruned leaves extract was determined.

2.7 Statistical Analysis

The results obtained in the determination of antioxidant activity, total phenolic content, and total flavonoid content of the Harumanis mango pruned leaves extracts were represented as the values of three individual replicates as mean ± standard error (SE) using the One-way ANOVA with Tukey's Test which the test was used to establish the differences between means of various groups at the significance level fixed at $p<0.05$. Software programs SigmaPlot 12.0 and Minitab 17 were utilised for data analysis.

3. Results and Discussions

3.1 Extraction of the Harumanis Mango Pruned Leaves

Liquid ethanol and methanol extracts exhibited a greener colour, while water appeared brown. Subsequently, methanol and ethanol were subjected to the rotary evaporator after drying, while the water extract underwent freeze-drying. The crude extracts were utilised for DPPH free radical scavenging activity testing.

3.2 Phytochemical Screening of Harumanis Mango Pruned Leaves Extract

The qualitative outcomes of the phytochemical screening conducted on Harumanis mango pruned leaves extracts are displayed in Table 1.

Table 1. Phytochemical screening of Harumanis mango pruned leaves extract.

Phytochemicals	Sample		
	Ethanol Extract	Methanol Extract	Water Extract
Flavonoid	+	+	+
Cardiac Glycocides	+	+	+
Tannins	+	+	+
Steroid	+	+	-
Saponin	-	-	-

Key: + indicates presence, - indicates absence

The analysis revealed the presence of flavonoids, cardiac glycosides, tannins, and steroids in Harumanis mango pruned leaves extracts. Flavonoids, cardiac glycosides, and tannins were found in all the Harumanis mango pruned leaves extracts. This finding aligns with the results of Divyalashmi and Sharmili (2017) and De and Pal (2014), who also noted the presence of flavonoids, cardiac glycosides, and tannins in ethanol, methanol, and water extracts of Mangifera indica leaves. In the present investigation, steroids were detected in all the leaf extracts except the water extract. This result is supported by the findings of Tarrsini *et al*. (2019) and Sharif *et al*. (2021), who reported the presence of steroids in ethanol and methanol extracts of Mangifera indica leaves, respectively.

Additionally, Willberforce and Olivia (2017) confirmed the absence of steroids in the water extract. All solvent extracts of the leaves showed the lack of saponins, consistent with the results of Divyalashmi and Sharmili (2017) and De and Pal (2014). While the absence of saponins in Harumanis mango leaves extract might reduce its utility in certain medicinal or functional areas (e.g., antimicrobial or emulsifying roles), its high antioxidant activity ensures that it remains valuable in other critical aspects, such as disease prevention, skin protection, and food preservation. The extract can still be marketed and applied effectively,

especially for its antioxidant and anti-ageing benefits, which might overshadow the missing saponins in many applications.

The ethanol and methanol extracts obtained from Harumanis mango pruned leaves exhibited the highest concentration of bioactive compounds, whereas the water extract showed the lowest detection of such compounds. Variations in the yield of phytochemicals in plant extracts can be significantly influenced by the solvent or extraction method. Therefore, it is reasonable to infer that ethanol and methanol solvents are inherently more effective than water in the extraction process. This is likely due to the increased solubility of bioactive compounds in the leaves in ethanol and methanol solvents, resulting in a more efficient extraction of bioactive metabolites than water, as Bhandary *et al*. (2012) reported. The findings of Bhandary *et al*. (2012) are corroborated by Willberforce and Olivia (2017), who suggested that the solubility of bioactive compounds in different solvents varies, with certain types of phytochemicals exhibiting greater solubility in specific solvents. Consequently, in the present investigation, methanol and ethanol emerge as the optimal solvents for extracting phytochemicals from Harumanis mango pruned leaves.

3.3 Detection of Mangiferin

The presence of mangiferin in the Harumanis mango pruned leaves extracts was assessed using High Performance Liquid Chromatography (HPLC) with a detection wavelength of 254 nm. It was observed that mangiferin was detected in all the Harumanis mango pruned leaves extracts. The mangiferin analysis of the methanol, ethanol, and water leaf extracts is outlined in Table 2.

Table 2. Mangiferin analysis in Harumanis mango pruned leaves extracts.

Sample	Retention time (min)	Area under the peak	Height of the peak
Standard Mangiferin	3.851	2510929	119496
Methanol Extract	3.400	4318496	832087
Ethanol Extract	3.761	1777533	608453
Water Extract	3.868	2070809	728180

During the investigation, it was qualitatively determined that mangiferin was present in all the extracts of Harumanis mango pruned leaves, with a retention time close to that of the standard mangiferin at 3.851 minutes. The retention time obtained for mangiferin in the standard sample was notably shorter than that reported by Medina Ramírez *et al*. (2016) at 7.75 minutes. This discrepancy could be attributed to using a different column type during analysis or a different solvent to dissolve the standard mangiferin.

In the methanol, ethanol, and water extracts, mangiferin was detected with retention times varying. Specifically, the methanol extract exhibited the shortest retention time of 3.400 minutes, followed by the ethanol extract, while the water extract showed the longest

retention time of 3.878 minutes. The deviations in retention time observed in different Harumanis mango pruned leaf extracts compared to the standard mangiferin's retention time could be attributed to potential column contamination (Yi *et al*., 2020). In Harumanis mango leaves extract, the key bioactive compounds like mangiferin typically have well-established retention times under standard HPLC conditions. Mangiferin often elutes at shorter retention times due to its higher polarity. The retention time of compounds in Harumanis mango leaves extract during HPLC analysis is determined by their polarity, molecular size, interaction with the column, and the chromatographic conditions used, making it a crucial parameter for identifying and quantifying specific bioactive compounds.

The methanol extract exhibited the most significant area under the peak for mangiferin, approximately 43,184,955, followed by the water extract, while the ethanol extract showed the smallest area under the peak for mangiferin, measuring approximately 17,775,333. All the areas under the peak for mangiferin in the extracts were notably larger than the area under the mangiferin peak in the standard sample, which was 2,510,929, as detailed in Table 2. The concentration of mangiferin in the sample was directly proportional to the area under the peak for mangiferin. The standard mangiferin was prepared at a concentration of 0.001 M, while the solvent extracts used in the analysis were prepared in liquid form without specifying their molarity. Consequently, it is plausible that the concentration of the standard mangiferin prepared was lower than that of the sample extracts, leading to the smaller area under the peak for mangiferin in the standard sample compared to all the extracts. Therefore, the results indicate that mangiferin was detected in all the Harumanis mango pruned leaf extracts.

The peak area of mangiferin from the methanol extract was the highest (approximately 43,184,955), followed by the water extract, and the smallest was from the ethanol extract (approximately 17,775,333). The mangiferin peaks obtained in the solvent extracts were significantly larger than the peak area of the mangiferin standard (2,510,929), as shown in Table 2.

At the same HPLC conditions, the same injection volume, detector setting and other instrument parameters, the peak area is directly correlated to the concentration of the analyte. Peak area was therefore used as a reliable measure of the relative mangiferin content and extraction efficiency by using different solvents.

Although an external standard was analyzed to confirm peak identity, absolute quantification was not the primary objective of this study. Instead, the comparative evaluation based on peak area sufficiently demonstrates that mangiferin was successfully extracted from Harumanis mango pruned leaves using all solvents, with methanol showing the highest extraction efficiency.

Studies on Harumanis mango leaves often highlight mangiferin, a potent antioxidant, as the primary compound, alongside flavonoids and phenolic acids. Other varieties (e.g., Alphonso, Tommy Atkins) similarly report mangiferin but may show differences in

flavonoid profiles or concentrations of polyphenols. For example, some varieties have higher levels of quercetin or kaempferol, influencing the overall antioxidant and anti-inflammatory activities.

3.4 Antioxidant Activity Assay of Harumanis Mango Pruned Leaves Extract

The antioxidant activity of methanol, ethanol, and aqueous extracts of Harum Manis leaves in scavenging free radicals was assessed using the 1-Diphenyl-2-picryllhydrazyl (DPPH) assay. This method relies on transforming the deep violet colour of stable DPPH free radical solution to light yellow as it undergoes reduction by antioxidant compounds (Krishnaiah *et al*., 2011).

Figure 1 illustrates the percentage of DPPH scavenging activity exhibited by various extracts of Harumanis mango pruned leaves, suggesting that they possess antioxidant properties capable of scavenging DPPH free radicals. The evaluation of the potential of Harumanis mango pruned leaf extracts to scavenge DPPH free radicals was determined by the reduction in absorbance value of the solution, measured at a wavelength of 517 nm, as well as the degree of discolouration of the DPPH free radical solution (Azlim Almey *et al*., 2017). The DPPH scavenging properties observed in the Harumanis mango pruned leaf extracts are likely attributable to flavonoids and other polyphenols within the extracts, as indicated by the current research findings. This observation is supported by Das *et al*. (2014), who similarly found that the DPPH radical scavenging activity in Crescentia cujute leaves could be attributed to the presence of free radical scavenger compounds such as flavonoids (e.g., quercetin), tannins, and steroids.

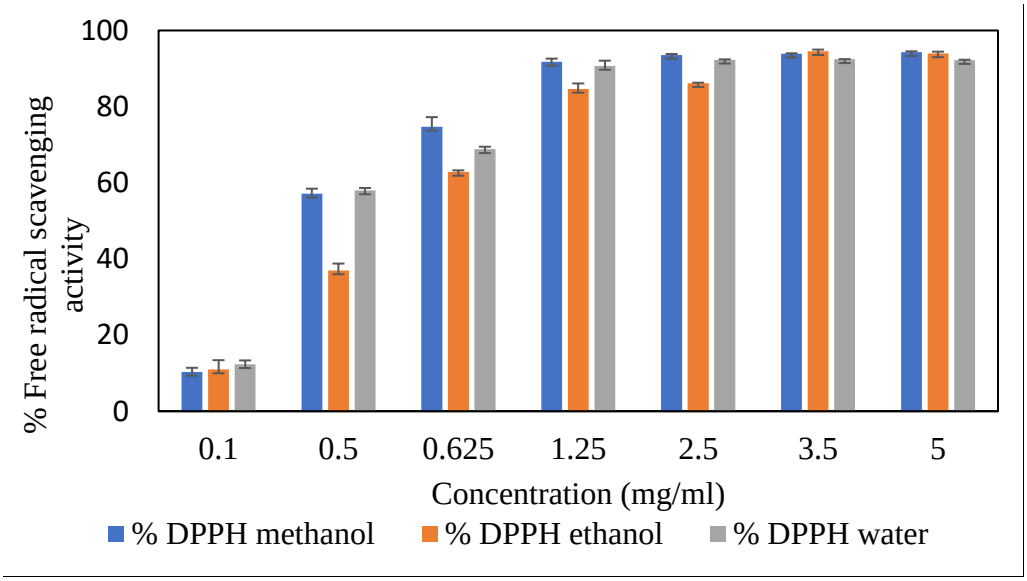

Figure 1. DPPH scavenging activity of the different Harumanis leaf extracts. Data are expressed as Mean ± SE, n=3, p<0.05.

Figure 1 illustrates the concentration-dependent increase in DPPH free radical scavenging activity exhibited by all the methanol, ethanol, and aqueous extracts of

Harumanis mango pruned leaves. ANOVA analysis revealed significant differences between the extracts' ability to scavenge DPPH free radicals. Specifically, ethanol extract exhibited significantly lower activity in scavenging free radicals than methanol and water extracts at lower concentrations. However, all the Harumanis mango pruned leaf extracts demonstrated DPPH free radical scavenging activities ranging from 92% to 94% at concentrations of 3.5 mg/mL and 5.0 mg/mL, suggesting their high potential as effective free radical scavengers. Ethanol exhibited the highest antioxidant activity, reaching its maximum DPPH scavenging activity of 94.559 ± 0.425% at a concentration of 3.5 mg/mL, followed by the methanol extract. In contrast, the aqueous extract displayed the lowest antioxidant activity, with its maximum DPPH scavenging activity of 92.433 ± o.046% at a concentration of 3.5 mg/mL. This finding is consistent with the report by Chewchinda *et al*. (2020), who observed that extracts' DPPH radical scavenging activities are influenced by the extraction solvent used and the plant species.

The maximum DPPH scavenging activity value of the water extract was comparable to the previously reported value of 91.48 by Irawan *et al*. (2017) for the aqueous extract of mango leaves. However, there is a lack of studies comparing the DPPH free radical scavenging activity among ethanol, methanol, and water extracts of Harumanis mango pruned leaves. Therefore, the results of the current study were compared to those of other plant leaf extracts. The findings of this study are consistent with research conducted by Sahu *et al*. (2013), who observed that the ethanol extract of leafy vegetable Olax psittacorum exhibited the highest DPPH scavenging activity (97.81%), followed by its methanol leaf extract (91.14%). Additionally, Do *et al*. (2014) demonstrated that the ethanol extract of Limnophila aromatica plant showed the highest DPPH scavenging activity, followed by the methanol and water extracts. These findings further support the current study's conclusion that the ethanol extract of Harumanis mango pruned leaves possesses the highest antioxidant activity. The antioxidant activity of Harumanis mango leaves extract is significant because antioxidants play a crucial role in neutralising free radicals, which can damage cells and lead to various diseases, including cancer, cardiovascular diseases, and ageing-related disorders. The bioactive compounds present in Harumanis mango leaves, such as flavonoids, phenolic acids, and mangiferin, contribute to its strong antioxidant potential

3.5 Determination of Total Phenolic Content (TPC)

The total phenolic content (TPC) of the extracts from Harumanis mango pruned leaves was determined using the calorimetric Folin-Ciocalteu method. This method relies on the interaction between phenolic compounds in the plant extract and the Folin-Ciocalteu reagent under alkaline conditions, resulting in the formation of blue-colored complexes, which are then quantified spectrophotometrically at a wavelength of 718 nm (Blainski *et al*., 2013). The quantification was performed by referencing a gallic acid standard curve with the equation y=79.19x + 0.0295, as illustrated in Figure 2, where R^2 = 0.9894, and the results were expressed as mg GAE/ml. Table 3 displays the total phenolic content of the extracts from Harumanis mango pruned leaves.

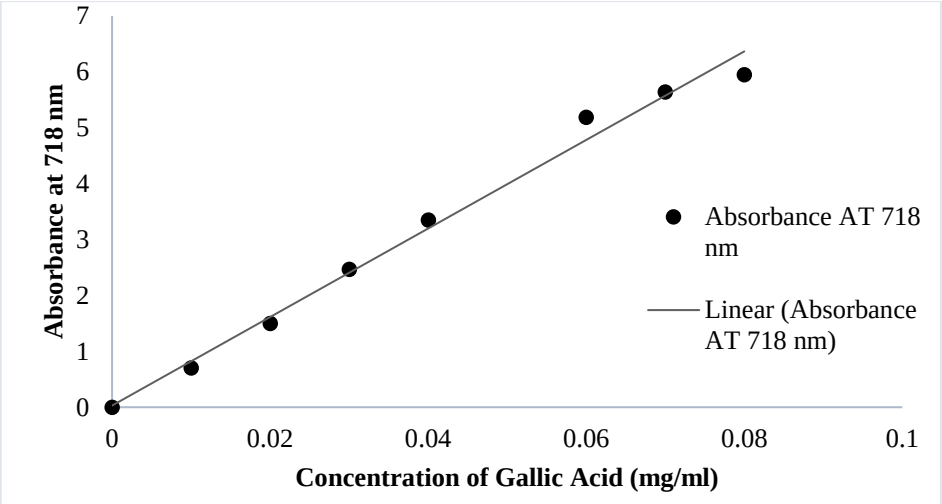

Figure 2. Standard curve of Gallic Acid.

Table 3. Total phenolic content (mg GAE/ml) in Harumanis mango pruned leaves extracts.

Sample	TPC (mg/ml)
Methanol Extract	0.050 ± 0.003^{a}
Ethanol Extract	0.055 ± 0.002^{a}
Water Extract	0.042 ± 0.001^{b}

Dates given are mean ± standard error for triplicate values of each sample, $p<0.05$ by using one-way ANOVA with Tukey test

The total phenolic content (TPC) findings were consistent with the current study on DPPH free radical scavenging activity. The ethanol extract exhibited the highest phenolic compound content at 0.055 ± 0.002 mg GAE/ml, followed by the methanol extract. Conversely, the water solvent yielded the lowest extraction of total phenolic compounds, with a concentration of 0.042 ± 0.001 mg GAE/ml. Statistically, the methanol and ethanol extracts showed significantly higher TPC than the water extract.

These findings align with prior research by Ling *et al*. (2009), who observed that the ethanolic extract of mango leaves exhibited a higher total phenolic volume (TPV) at 590 ± 48 mg GAE/g, compared to the aqueous extract with a TPC of 189 ± 109 mg GAE/g. Similarly, another study conducted by Kawpoomhae *et al*. (2010) indicated that the methanolic extract of mango leaves possessed a higher TPC at 420 ± 4.30 mg GAE/g, in contrast to the aqueous extract of fresh mango leaves, which had a TPC of 187 ± 3.20 mg GAE/g. Upon comparison of these two studies, it becomes evident that the ethanolic extract of mango leaves consistently displays the highest total phenolic content, followed by the methanolic extract, and then the aqueous extract. These findings corroborate the study on Harumanis mango pruned leaves, supporting the notion that the ethanolic extract exhibits greater antioxidant capacity than the methanolic and aqueous extracts.

3.6 Determination of Total Flavonoid Content (TFC)

The Aluminium trichloride colourimetric method assessed the total flavonoid content (TFC) in the extracts from Harumanis mango pruned leaves. TFC values were determined by referencing a Quercetin standard curve, represented by the equation y= 16.459x + 0.0144, with an R^2 value of 0.9993, and were expressed in mg/ml. Absorbance measurements of the samples were conducted at a wavelength of 415 nm. The results of TFC in the extracts from Harumanis mango pruned leaves are summarised in Table 4.

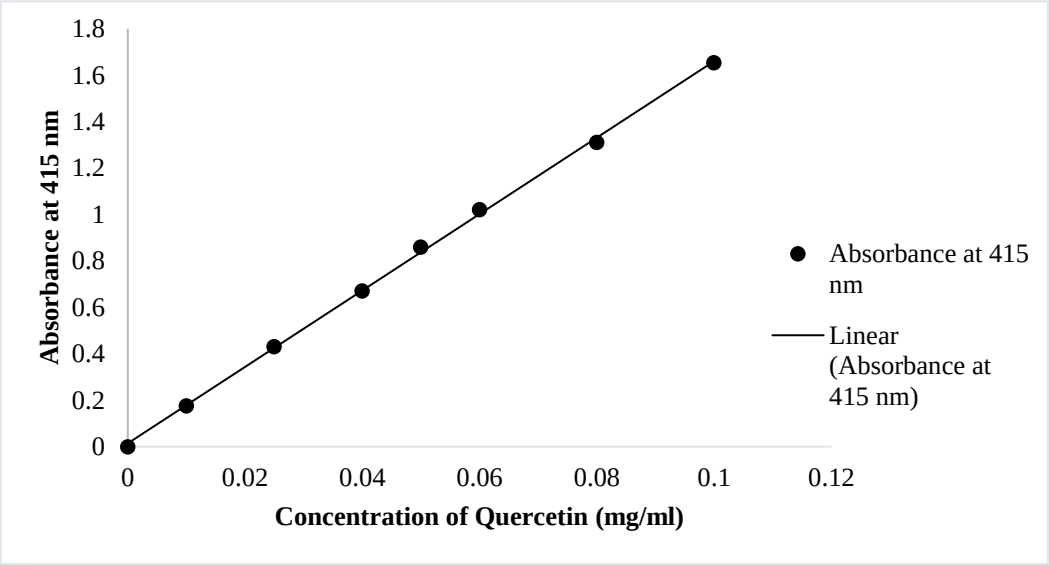

Figure 3. Standard curve of Quercetin.

Table 4. Total flavonoid content (mg/ml) in Harumanis mango pruned leaves extracts.

Sample	TFC (mg/ml)
Methanol Extract	0.047 ± 0.002^{c}
Ethanol Extract	0.071 ± 0.002^{a}
Water Extract	0.055 ± 0.000^{b}

The dates given are mean ± standard error for triplicate values of each sample, $p<0.05$ by using One Way ANOVA with Tukey test

The findings reveal that the ethanol extract exhibited the highest TFC at 0.071 ± 0.002 mg/ml, followed by the water extract. Conversely, the methanol extract showed the lowest content of total flavonoid compounds, measuring 0.0047 ± 0.002 mg/ml. According to ANOVA statistical analysis, there were significant variations in the flavonoid content among all the Harumanis mango pruned leaves extracts. Specifically, the analysis indicated that the ethanol extract contained significantly more flavonoid compounds than the methanol and water extracts.

Since there is a lack of studies comparing the total flavonoid content among ethanol, methanol, and water extracts of mango leaves, the present results are compared with findings

from studies on other plant leaf extracts. The current findings align with research conducted by Das *et al*. (2014), who observed that the ethanol extract of *C. cujete* leaves exhibited a higher TFC at 139.57 ± 3.75 mg/g compared to the aqueous leaf extract, which measured 16.04 ± 3.23 mg/g. Similarly, the present study corresponds to earlier research by Do *et al*. (2014) on the investigation of TFC in methanol, ethanol, and water extracts of the L. aromatica plant, wherein the ethanol extract demonstrated the highest TFC. Based on the current TFC results, the ethanol extract exhibited the highest total flavonoid compounds among the extracts.

4. Conclusions

Ethanol and methanol emerged as the most effective solvents for extracting phytochemicals from Harumanis mango pruned leaves. The polarity of the solvent used will determine the kind of compounds that can be extracted. Ethanol and methanol have been suitable solvents for polyphenol extraction and are safe for humans. Most of the time, polar solvents used to extract bioactive compounds from extracts have excellent yields compared to other solvents. Mangiferin was detected in all extracts of Harumanis mango pruned leaves using HPLC.

Additionally, all extracts exhibited significant potential as free radical scavengers, with DPPH free radical scavenging activities ranging from 92% to 94% at concentrations of 3.5 mg/ml and 5.0 mg/ml, as per the DPPH assay. However, the ethanol extract of Harumanis mango pruned leaves demonstrated the highest antioxidant activity, reaching 94.559 ± 0.425 % at a 3.5 mg/ml concentration. Furthermore, the ethanol extract boasted the highest phenolic compound content at 0.055 ± 0.02 mg/ml among all extracts and also showed the highest concentration of total flavonoid compounds at 0.071 ± 0.002 mg/ml. Hence, ethanol is the optimal solvent for extracting bioactive compounds from Harumanis mango pruned leaves, as it exhibited superior efficacy compared to methanol and water solvents in phytochemical extraction. Moreover, the ethanol extract has the highest antioxidant properties based on the DPPH assay, TPC, and TFC testing results. From the findings, a part of the quantitative value of the qualitative outcomes from mango leaves extract includes potent antioxidant, anti-inflammatory, antimicrobial, and cytoprotective properties, along with potential benefits for skin health, disease prevention, and natural product formulations.

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