



Research Article

Phytochemical Extraction, Evaluation of Antioxidant and Antimicrobial Activities of *Carica Papaya* Leaf Extract

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DOI: <https://doi.org/10.5281/zenodo.23341676>

Article Info:-

Received: 25 July 2026
Received: 15 August 2026
Accepted: 04 September 2026

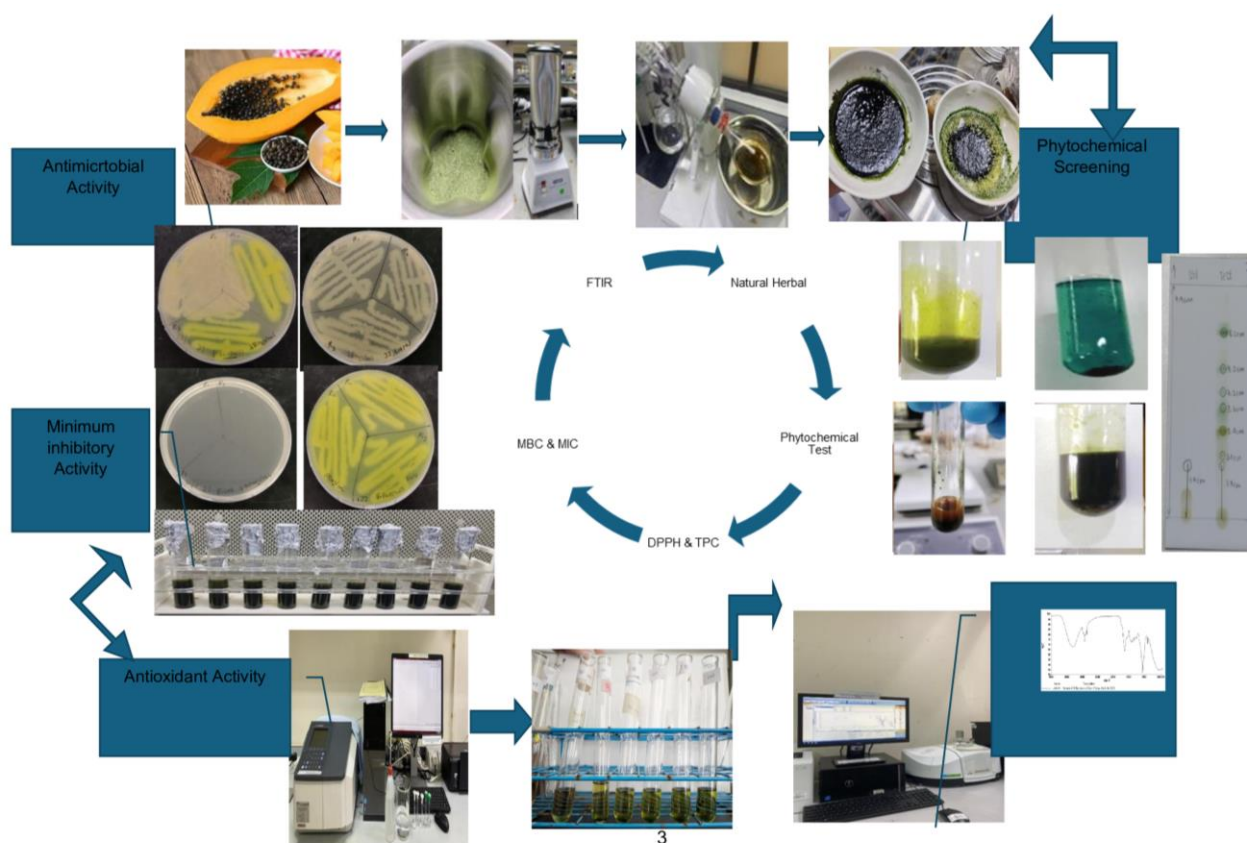
How to cite this Article:

Nabila Perveen, Naeem Hasan Khan, Geethaa Sahgal, Hii Jia Jia (2026) Phytochemical Extraction, Evaluation of Antioxidant and antimicrobial Activities of *Carica Papaya* Leaf Extract *International Journal of Advanced Pharmaceutical and Healthcare*, 1(2), 01-16.

ABSTRACT

Introduction: *Carica papaya* L. leaves are known for their antioxidant, antimicrobial, anti-inflammatory, and anti-dengue properties. Extraction efficiency and phytochemical recovery are influenced by the extraction solvent used. **Objective:** This study evaluated the extraction yield, phytochemical constituents, antioxidant activity, and antimicrobial potential of *Carica papaya* leaf extract obtained through cold maceration using a mixed solvent system of 95% ethanol and n-hexane (4:1, v/v). **Methodology:** Dried *Carica papaya* leaf powder was extracted using cold maceration, followed by concentration of the filtrate to obtain crude extract. Phytochemical screening, thin-layer chromatography (TLC), and Fourier Transform Infrared (FTIR) spectroscopy were performed to characterize the extract. Antioxidant activity was assessed using DPPH radical scavenging and total phenolic content (TPC) assays, while antimicrobial activity was evaluated using minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) methods. **Results:** The extraction yielded 104 g of crude extract from 160 g of dried leaf powder (65%). Phytochemical screening revealed the presence of saponins, alkaloids, reducing sugars, flavonoids, steroids and triterpenoids, tannins, phenolic compounds, and lipids. TLC confirmed the presence of flavonoids, while FTIR identified characteristic hydroxyl, carbonyl, alkane, alkene, and aromatic functional groups. The extract exhibited moderate antioxidant activity with an IC₅₀ value of 157.09 µg/mL in the DPPH assay and a TPC value of 105.02 mg GAE/g extract. Antimicrobial assays demonstrated inhibitory and bactericidal activities against selected Gram-positive and Gram-negative bacteria, with MIC and MBC values of 400 mg/mL. **Conclusion:** Cold maceration using a mixed solvent system of 95% ethanol and n-hexane effectively produced a high extraction yield and recovered a wide range of bioactive phytochemicals from *Carica papaya* leaves. The extract demonstrated moderate antioxidant activity and broad-spectrum antimicrobial potential, supporting its potential application as a natural source of antioxidant and antimicrobial agents for pharmaceutical and nutraceutical development.

KEYWORDS: *Carica papaya*; papaya leaf extract; cold maceration; phytochemicals; TLC; FTIR; antioxidant activity; DPPH; total phenolic content; antimicrobial activity; MIC; MBC.



INTRODUCTION

In the modern era, antibiotic resistance is one of the major global health issues due to the abuse, misuse, overuse, and poor control of antibiotics. The capacity of bacteria to resist the effects of antibiotics that were previously effective against them is known as antibiotic resistance (Ahmed et al., 2024). This has caused bacterial infections to become more difficult to treat by antibiotics and has given limited treatment options. One of the effective approaches for developing antibacterial treatments is the investigation of herbal medicines (Alhodieb et al., 2025). The herbal medicines are widely used in wound healing, tissue repair, and regeneration. The population is shifting towards herbal medication treatment from modern medication. The preference for herbal medication use is increasing as the population considers the products to be safe, useful, high-quality, cheaper, and with fewer adverse effects compared to modern and branded medication treatments. Papaya leaf has been found and detected by previous studies to contain various bioactive constituents such as glycosides, alkaloids, tannins, phenolics, saponins, steroids and flavonoids. These phytochemical substances give different pharmacological effects such as antioxidant, antimicrobial, anti-dengue, anticancer, anti-diabetes and antimalarial (Riaz et al., 2023). Papaya leaf shows antimicrobial properties against some Gram-positive and Gram-negative bacterial species. They have been used as a therapeutic alternative because of their antimicrobial effects (Mohd Danial et al., 2020). The properties are effective in the treatment of infectious diseases and are

also the reason to use them as a traditional remedy to cure ailments and infections. The bioactive constituents such as phenolic compounds and organic acids. The use of natural antimicrobial compounds in herbal plants is more generally safer and reduces the risk of drug-drug interactions, adverse side effects, and the development of antibiotic resistance. For possible health benefits and reduced toxicity, scientific research focuses more on naturally occurring plant parts that are enriched with antioxidants. The phytochemicals present in the papaya leaf are known as natural antioxidants as an alternative. Antioxidants are important to maintain human health by inhibiting oxidative damage. Oxidative damage is formed because of exposure to ionizing radiation such as X-ray that causes cell damage and interferes with normal cellular metabolism. The imbalance between production and accumulation of ROS in cells (Masyita et al., 2022) and the ability of the metabolism system to detoxify the reactive products may lead to the development of free radicals. Free radicals in the human body indicate the presence of unpaired electrons. Because of their possible antioxidant, antibacterial, anti-inflammatory, and anti-dengue qualities, *Carica Papaya L.* leaves are widely known as therapeutic herbs.

Besides, the choice of extraction solvent is important in determining the extraction yield and biological efficacy of bioactive constituents. However, none of these investigations have conducted phytochemical screening tests, antioxidant evaluation and antimicrobial assays using the same papaya leaf extract. Furthermore, there

are also limited studies that using a mixture of polar and non-polar solvents as extracting solvent to maximize the extraction yield and bioactive constituents of papaya leaf extract. There is limited evidence to determine the effectiveness of extracting solvent.

METHODS AND METHODOLOGY

Collection & Preparation of papaya extract

Fresh, healthy, mature *Carica papaya* leaves free of disease were plucked at Bao Shu Yuan, an organic farm in Bedong, Kedah, Malaysia. The 600g papaya leaf was cleaned with running tap water to remove impurities. They were air-dried at room temperature for five days.

The air-dried papaya leaf was milled into powder form using a commercial electric blender that available in MDL 4 lab, AIMST University. The 160 grams of dried papaya leaf were obtained. The cold maceration method was used for extraction. The powder was soaked in two 500 mL conical flasks with a mixture of 250 mL extracting solvent (80% of 95% ethanol solvent and 20% n-hexane solvent). To achieve pure extract solution, the mixture was mixed, left to stand for 5 days, and then filtered through a woven mesh filter cloth. After that, the extract was evaporated by a rotary evaporator at 130 rpm at 50°C for 2 hours and 30 minutes to obtain concentrated extract.

Following that, the concentrated extract was placed in an evaporating dish, sealed with aluminium foil, and boiled in a water bath at 50°C until a concentrate paste of extract was formed. To prevent damage and contamination, store the extract paste in a refrigerator at 4°C. The stages were repeated for the second maceration technique, which used the same solid residue to maximize extraction yield.



Figure 1: The evaporation process of extracting solvents by using hot plate and the final product of *Carica papaya* leaf extract.

Phytochemical analysis of papaya extract

A little amount of extract was collected and dissolved in DMSO solvent. When the extract had been completely dissolved, distilled water was added to get the mixture up to 15mL.

All the test for saponin glycosides, alkaloids, reducing sugars, flavonoids, steroids and triterpenoid, tannins and phenolic compounds & lipid were performed.

DPPH RADICAL SCAVENGING ASSAY

The DPPH test was used to assess the antioxidant activity of *Carica papaya* leaf extract. The 0.3mM DPPH reagent was created by dissolving 11.83mg of DPPH powder in 100mL of ethanol.

The 1mg/mL ethanolic extract used as a stock solution was made by dissolving 100mg of extract in 100mL ethanol. The first trial involved preparing extract solutions at various concentrations (10, 20, 40, 60, 80, 100 µg/mL). However, it yielded a bad outcome because the purple tint of the mixture did not turn yellow. Consequently, greater quantities of *Carica papaya* leaf extract were utilized. The second study included extracts with concentrations of 120, 140, 160, 180, and 200 µg/mL. The colour shift was noticed, and the absorbance was measured.

Each solution made up to 10mL of solution with different volume of ethanol that mentioned in Table 1: Formulae for different dilution of extract. The 0.3mM of DPPH reagent was prepared and added 1mL of DPPH reagent into all test tubes. The test was performed in triplicate for each concentration of extract. Besides, the control solution was prepared by ethanol and 1mL of 0.3mM of ethanolic solution of DPPH. The blank solution was prepared only with 3mL of 95% of ethanol. Similar steps were repeated by replacing the plant extract to standard ascorbic acid solution.

All test tubes containing mixture were covered with aluminium foil as DPPH is light photosensitivity reagent and they were allowed to incubate for 30 minutes in dark condition, and the absorbance was measured at 518nm by using UV-Vis spectrophotometer.

The absorbance values of each different concentrations of standard ascorbic acid, plant extracts, blank and control were recorded. The standard curve was obtained using ascorbic acid. The absorbances of each concentration were recorded.

The DPPH radical scavenging activity (% DPPH_{SC}) was calculated using the formula below:

$$\%DPPH_{SC} = \frac{(\text{Absorbance of control at 517nm} - \text{Absorbance of sample extract at 517nm})}{\text{Absorbance of control at 517nm}} \times 100\%$$

Lastly, the graph was presented the relationship between the concentration in µg/mL and the percentage of inhibition. The concentration used was within the range of 120 µg/mL to 200 µg/mL. The equation of the curve used to calculate the IC₅₀ corresponding to the sample concentration that reduced the initial DPPH absorbance of 50%.

Table 1: Formulae for different dilution of extract.

Trial	Concentration of extract solution (µg/mL)	Volume of stock solution (mL)	Volume of ethanol (mL)
1	10	0.1	9.9
	20	0.2	9.8
	40	0.4	9.6
	60	0.6	9.4
	80	0.8	9.2
	100	1.0	9.0
2	120	1.2	8.8
	140	1.4	8.6
	160	1.6	8.4
	180	1.8	8.2
	200	2.0	8.0

DETERMINATION OF TOTAL PHENOLIC CONTENT (TPC) ASSAY

The total phenolic content (TPC) of *Carica papaya* leaf extract was calculated using the Folin-Ciocalteu technique. It is an antioxidant assay based on electron transfer that determines the reductive capacity of the tested substance. The standard curve was created using Gallic acid and represented as mg of Gallic acid equivalent (GAE) per gram of papaya leaf extract.

Preparation of *Carica papaya* leaf extract

Table 2: Formulae for preparation of different concentration of Gallic *acid solution* showed the preparation of various Gallic acid standard concentrations for TPC by serial dilution.

Total phenolic content test

A 2.5% sodium carbonate solution was prepared by dissolving 2.5g of sodium carbonate in 100mL of distilled water.

To prepare the plant extract, 1mL of extract was added into a test tube by using a micropipette and added with 1mL of Folin-Ciocalteu reagent and 2mL of 2.5% sodium carbonate solution.

Besides, the standard gallic acid with different concentrations were prepared by using the same procedure by replacing the extract. Each test tube containing different volume of gallic acid were added with 1mL of Folin-Ciocalteu reagent and 2mL of 2.5% of sodium carbonate solution. The steps above were repeated in triplicate for each concentration of extract.

The 1mg/mL methanolic extract used as stock solution was prepared by dissolving 10mg of extract in 10mL of methanol. Only one concentration of *Carica papaya* leaf extract was prepared and used for the test.

Preparation of different concentrations of Gallic Acid standard

Gallic acid was used as the standard for this test. The six different concentrations of standard solutions were prepared in 10, 20, 40, 60, 80, 100µg/mL.

Other than that, the control solution was prepared by replacing the extract with 1mL of methanol and 1mL of Folin-Ciocalteu reagent and 2mL of sodium carbonate solution while the blank solution was prepared by adding 3mL of methanol. All test tubes containing mixture were allowed to incubate for 2 hours in dark condition and then subjected to UV visible analysis. Approximately 2mL content of each test tube was transferred into the quartz cuvette, and the absorbance was measured at 760nm by using UV-Vis spectrophotometer. The absorbance values of each different concentration of standard gallic acid, plant extracts, blank and control were recorded. The standard curve was obtained using gallic acid and showed the line graph of absorbance against concentration of gallic acid. The total phenolic content was expressed in mg of Gallic acid equivalents per g dry extract (mg GAE/g).

The formula below was used to calculate the total concentration of phenolic content:

$$\text{Total phenolic content (mg GAE/g dry extract)} = \frac{c \times v}{m}$$

Table 2: Formulae for preparation of different concentration of Gallic acid solution.

Concentration of extract solution (µg/mL)	Volume of stock solution (mL)	Volume of methanol (mL)
10	0.1	9.9
20	0.2	9.8
40	0.4	9.6
60	0.6	9.4
80	0.8	9.2
100	1.0	9.0

IDENTIFICATION OF FLAVONOIDS USING TLC SCANNING ANALYSIS

Chromatogram preparation

A small TLC chamber was required. The 20ml solvent (mobile phase) was produced with 13ml of ethyl acetate, 1.5ml of glacial acetic acid, 1.5ml of formic acid, and 4ml of water. To saturate the chamber with vapor, a filter paper was cut and inserted within its side wall. After the solvent has been poured into the chamber, the lid is greased to seal it. The papaya leaf extract paste was placed in a beaker and dissolved in 95% ethanol. A small spot of extract solution and a standard solution containing 1% quercetin were put on the baseline of the TLC plate using a capillary tube. To allow for capillary action, the TLC plate was allowed to dry before being carefully placed in the TLC chamber using forceps. The chamber lid was closed, and the spotted samples were moved to the solvent fronts. Once at the top, the TLC plate was allowed to be removed from the chamber. The plate was dried using a dryer. The visible spots were identified, and a UV light was employed. All Rf values were calculated using the formula below and meticulously noted.

$$\text{Rf value} = \frac{\text{Distance travelled by analyte (mm)}}{\text{Distance travelled by solvent (mm)}}$$

FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR) ASSAY

The small amount of *Carica papaya* leaf extract was analysed by Fourier transform infrared spectroscopy (ATR-FTIR) to determine the functional groups available in the extract. Firstly, the instrument start-up and settings such as resolution 4cm⁻¹, 16 scans (background) + 16 scans (sample), range 4000 to 400 cm⁻¹, and absorbance mode. After that, the ATR crystal was cleaned with isopropyl alcohol and acquire background spectrum. Then, the dried papaya leaf extract was placed on the crystal and apply pressure arm more than 90% to get better match value and click the 'Preview Mode' on PC which allows the real-time monitored the quality of spectrum. The spectrum obtained was recorded and documented.

Determination of Antimicrobial Activity

The antimicrobial activity of *Carica papaya* leaf extract was determined by the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).

Preparation of nutrient medium

The nutrient mediums were prepared according to the directions given by the manufacturer, HIMEDIA. The 28g of nutrient agar powder was suspended in 1000mL of distilled water while 13g of nutrient broth powder was suspended in 1000mL of distilled water. Besides, 38g of Muller-Hinton agar powder was suspended in 1000mL of distilled water while 21g of Mueller-Hinton broth

powder was suspended in 1000mL of distilled water. The suspensions were prepared in the conical flask respectively and covered with cotton wool and aluminium foil. They were sterilized in an autoclave at 121°C at 15 lbs pressure for 15 minutes. For agar medium, the suspension was cooled and poured into sterile petri dishes to solidify and stored in fridge for future use while for broth medium, the suspension was cooled and stored in room temperature.

Preparation of bacteria strains

The two species of bacteria, one gram-positive *Enterococcus faecalis* (ATCC 29212) and one gram-negative *Escherichia coli* (ATCC 8739) were obtained from UG Lab AIMST UNIVERSITY was used for testing.

Preparation bacteria cultures and inoculation

Pure cultures of *Enterococcus Faecalis* and *Escherichia coli* obtained from Central Laboratory Management Unit of AIMST University. The bacteria were inoculated into nutrient broth and incubated in inoculator shaker at 100 rpm at 37°C for 24 hours. To obtain single colony of respective bacteria, the quadrant streak plate method was performed on the nutrient agar plate and incubate in oven incubator for 18 hours at 37°C. After that, the single colony can store in chiller for further use.

Preparation of (0.5) McFarland standard

The 20mL of (0.5) McFarland standard was prepared by mixing 0.1mL of 1% barium chloride with 19.9mL of 1% sulphuric acid in a screw-cap universal bottle and stored in room temperature. It should be shake before each use.

Preparation of bacterial suspension

The bacterial suspension was freshly prepared. The bacterial culture was taken from the 100mL of Mueller-Hinton broth in 250mL of conical flask after 24 hours shaking incubation at 37°C by using sterile 200µL micropipette. The culture was added drop by drop into 15mL of autoclaved sterile distilled water in screw-cap universal bottle. The turbidity of bacterial suspension was adjusted and compared visually with the (0.5) McFarland standard (1.5×10⁸ CFU/ml).

Test for the positive control and negative control

A positive control was performed by combining standard antibiotics (Amoxicillin) with 1mL of *E. coli* bacterial suspension in 10mL of nutrient broth, whereas a negative control was performed by adding 1mL of *E. coli* bacterial suspension to 10mL of nutrient broth. The procedure was repeated using *E. faecalis*. The universal bottles were left to stand in the oven incubator for 24 hours. The results have been recorded.

Test for the dissolving solvent and extracting solvent

The 1mL of extracting solvent (80% of 95% ethanol and 20% n-hexane) was added into 10mL of nutrient broth

with 1mL of *E. coli* bacterial suspension. Besides, the 1mL of extracting solvents (1% of DMSO, 1.5% of DMSO and 2% of DMSO) were added into three different universal bottles that containing 10mL of nutrient broth respectively with the addition of 1mL of bacterial suspension. The steps were repeated for *E. faecalis*. The results were recorded.

Preparation of papaya leaf extract solution

The 5000 mg of *Carica papaya* leaf extract in paste form was weighed. The extract was dissolved in 10 mL of 1% DMSO, hence, the 500mg/mL of plant extract was prepared.

Minimum inhibitory concentration test

The broth microdilution method was applied to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) for the samples. The range of samples from 25 mg/mL to

400mg/mL were diluted using sterile Mueller Hilton broth (MHB). The MHB was poured into the test tubes and autoclaved before used. The fresh bacteria culture was prepared and used within 1 hour. The bacteria culture was adjusted to desired concentration by using (0.5) McFarland standards with naked eye.

Then 1mL of adjusted bacterial culture is added into the sample and suspends it. The broth contains bacteria culture without any sample is used as growth control. The broth with sample (25 mg/mL) without bacterial culture is served as negative control. The 5 test tubes with different plant extract concentrations were prepared based on Table After adding, the mouth of the test tubes was closed with cotton wool and aluminium foil. The test tubes were incubated at 37 °C for 24 hours. The different of plant extract concentrations were performed triplicate. The tests were performed by using *E. coli* and *E. faecalis* into different concentration of plant extracts.

Table 3: The formulae for the minimum inhibitory concentration preparation.

Concentration of plant extracts (mg/mL)	Volume of stock solution (mL)	Volume of bacterial suspension (mL)	Volume of MHB (mL)
25	0.05	1	8.95
50	0.10	1	8.90
100	0.20	1	8.80
200	0.40	1	8.60
400	0.80	1	8.20

Minimum Bactericidal Concentration (MBC) test

After 24 hours of bacterial incubation, the MIC of plant extracts was determined. The samples from each concentration were transferred to sterile Mueller-Hilton agar using the streak plate method to detect the presence of bacteria that remained alive after incubation. The samples with identical concentrations were equally distributed on the MHA, with each concentration performed in triplicate and incubated at 37 °C for 24 hours. The MBC results were recorded.

RESULTS AND DISCUSSIONS

Percentage yield of *Carica papaya* leaf extract

The total weight of *Carica papaya* leaf extract was 104g and the percentage yield is 65%.

$$\text{Percentage yield} = \frac{\text{Actual yield}}{\text{Theoretical yield}} \times 100\%$$

$$\text{Percentage yield} = \frac{104g}{160g} \times 100\% = 65$$

Extraction yield and extraction solvent

The extraction yield of *Carica papaya* leaf extract obtained is 65%. The mixture of extracting solvent system consisted 80% of 95% of ethanol, which is a polar solvent that efficiently extracted polar and semi-polar phytochemicals such as flavonoids, phenolics, tannins and other polar bioactive constituents while the n-hexane extracted non-polar components such as lipophilic and aromatic constituents. The combination of polar and non-polar solvents extracted phytochemical

constituents from *Carica papaya* leaf improved the extraction yield efficiency after the cold maceration method and remaining solvent removed using rotary evaporator. It broadened the extraction spectrum efficiency. However, some inactive plant components such as chlorophyll and reducing sugars may be extracted as it didn't reflect the quality of bioactive compounds. It didn't determine that the extract was concentrated with the desired bioactive constituents. Hence, it can be improved by comparison between different extracting solvents such as methanol and acetone and also relevant findings. (Tafonao et al., 2024)

The cold maceration method was performed in this study to minimize thermal degradation of heat-sensitive phytochemicals and allow prolonged solvent penetration into plant tissues, to give an effective extraction yield. Furthermore, the extraction yield may be influenced by the environmental factors, climate conditions and geographical location. These factors can affect the phytochemical compounds in medicinal plants. The second maceration was performed to ensure the maximum extraction yield. (Hariono, 2021) The high extraction yield was obtained from the *Carica papaya* leaf collected in Bedong, Kedah.

Phytochemical tests result discussion

The quantitative analysis was conducted to determine the phytochemical compounds present in *Carica papaya* leaf extract. The phytochemical screening of *Carica papaya* leaf extract was shown the presence of saponin,

alkaloids, reducing sugars, flavonoids, steroids and triterpenoids, tannins and phenolic compounds and lipid (Tafonao et al., 2024).

Table 4: Quantitative analysis of Carica papaya leaf extract by using phytochemical screening tests. Key: ++++: High Present, +++ = moderate present, ++ = low present, - = absence.

Tests	Inferences
Saponin test	++++
Alkaloids	
Mayer's test	++++
Dragendorff's test	++++
Reducing sugars	
Fehling's test	++++
Benedict's test	++++
Flavonoids	
Lead acetate test	++++
Steroids, triterpenoid	
Salkowski reaction	++++
Tannin and phenolic compounds	
5% ferric chloride solution	++++
Lipid	
Sudan test	++++

Phytochemical screening and discussion

The phytochemical tests were performed successfully, and the observations were recorded in Table 4. The various tests showed positive results and confirmed the presence of important secondary metabolites such as saponin, alkaloids, reducing sugar, flavonoids, steroid and triterpenoid, tannins, phenolic compounds and lipids. These compounds are involved in biological and therapeutic activities. (Barnabas et al., 2023)

Firstly, saponin gives the bitter taste of papaya leaf. It also provides antioxidant and anticancer properties. Besides, the alkaloids are used as a therapeutic plant substance as they have analgesic, antimalarial and antimicrobial properties. It is most important to fight against pathogens. Then, the presence of reducing sugar in papaya leaf extract indicates the hypoglycaemic activity which can reduce glucose levels. Moreover, flavonoids inhibit the enzyme activity by reversibly binding to the active sites of the enzyme to disrupt the bacteria's cell wall integrity. Other than that, the phenols can donate hydrogen atoms to neutralize free radicals and reduce oxidative stress. Both compounds have

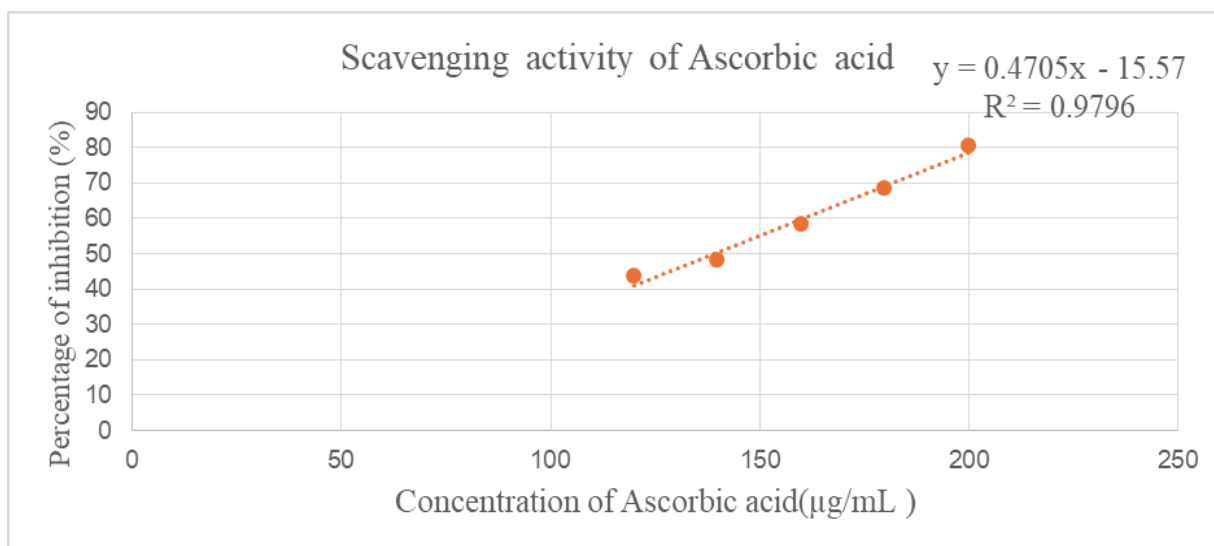
antioxidant activity. Furthermore, the presence of steroids and triterpenoids with anti-inflammatory and antimicrobial properties. Then, tannin is the subclass of phenolic compounds that coagulates the proteins, and leads to denature of the bacterial cell wall, which shows antimicrobial activities. Lastly, the positive result of lipids is due to the presence of waxy cuticles on the leaf surface which protects leaf from dehydration. The presence of bioactive compounds may explain the antioxidant and antimicrobial properties of this study. However, the phytochemical tests only showed the qualitative tests and did not determine the concentration of bioactive compounds. Hence, it is recommended to perform HPLC analysis for individual compounds. The presence of bioactive constituents in this study was matched to previous relevant studies which proved that the papaya leaf was a natural source of antioxidant and antimicrobial agents. (Kong et al., 2021)

Antioxidant activity

DPPH radical scavenging activity was performed, and results were shown as below Table 5.

Table 5: The absorbance value at 518nm of different concentration of ascorbic acid and control.

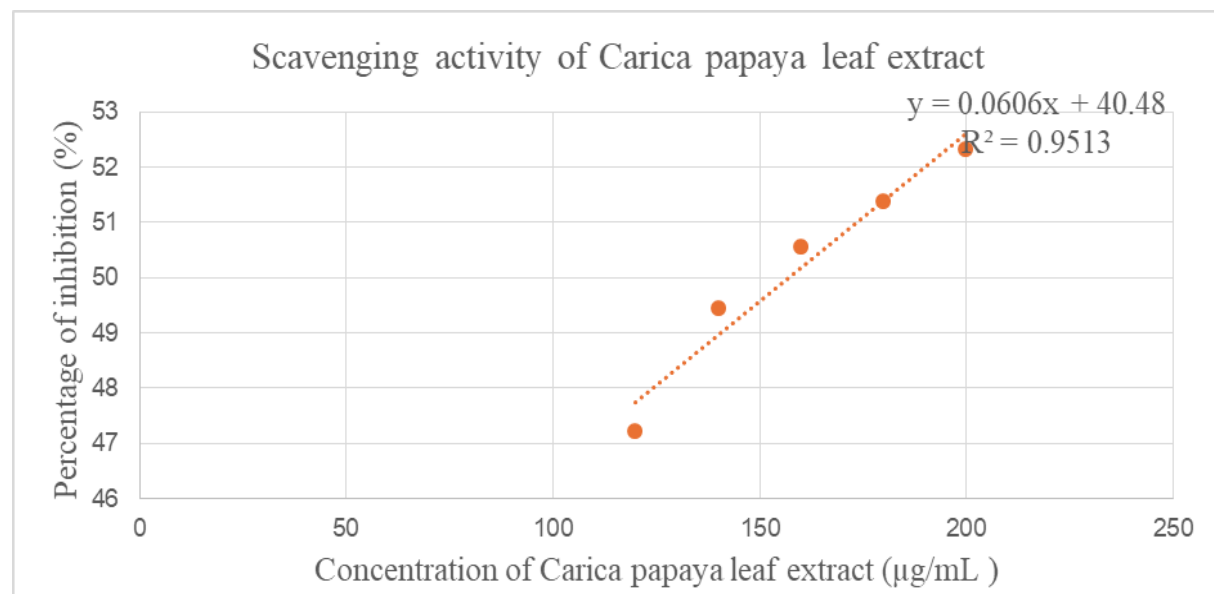
Concentration of ascorbic acid (µg/mL)	Absorbance at 518nm				Standard ± SE	Scavenging activity (%)	IC ₅₀ value µg/mL
	Duplicate 1	Duplicate 2	Duplicate 3	Average			
120	0.352	0.358	0.352	0.354	0.354 ± 0.002	43.54	139.36
140	0.326	0.328	0.324	0.326	0.326 ± 0.001	48.00	
160	0.268	0.262	0.256	0.262	0.262 ± 0.003	58.21	
180	0.201	0.195	0.198	0.198	0.198 ± 0.002	68.42	
200	0.122	0.118	0.128	0.123	0.123 ± 0.003	80.38	
Control	0.624	0.631	0.626	0.627	0.627 ± 0.002		



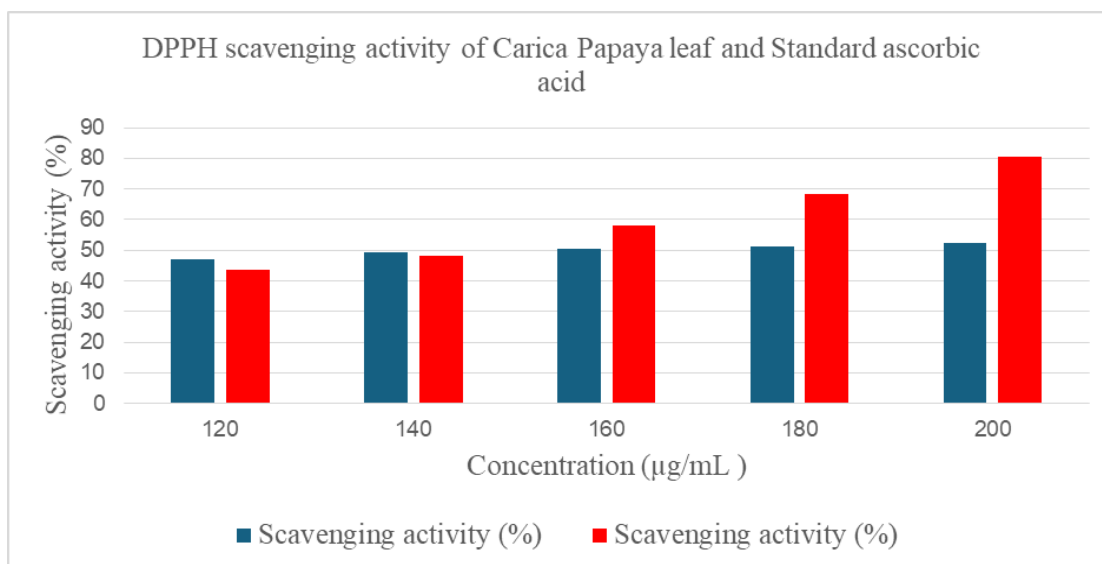
Graph 1: The scavenging activity of standard ascorbic acid with different concentrations.

Table 6: The absorbance value at 518nm of different concentration of Carica papaya leaf extract.

Concentration of papaya leaf extract ($\mu\text{g/mL}$)	Absorbance at 518nm				Standard $\pm$ SE	Scavenging activity (%)	IC ₅₀ value $\mu\text{g/mL}$
	Duplicate 1	Duplicate 2	Duplicate 3	Average			
120	0.334	0.329	0.33	0.331	0.331 ± 0.001	47.21	157.09
140	0.321	0.317	0.313	0.317	0.317 ± 0.002	49.44	
160	0.308	0.312	0.310	0.310	0.310 ± 0.001	50.56	
180	0.305	0.309	0.301	0.305	0.305 ± 0.002	51.36	
200	0.295	0.303	0.299	0.299	0.229 ± 0.002	52.31	
Control	0.624	0.631	0.626	0.627	0.627 ± 0.002		



Graph 2: The scavenging activity of Carica papaya leaf extract.



Graph 3: The scavenging activity between Standard Ascorbic acid and Carica papaya leaf extract samples.



Figure 2: The DPPH of papaya leaf extract. The colour changes from light purple to yellow colour.

The Carica papaya leaf had shown concentration-dependent DPPH scavenging activity by conducting the DPPH assay to confirm their antioxidant activity. The IC_{50} value of papaya leaf extract is 157.09 µg/mL showed in Table 6 while the IC_{50} value of ascorbic acid is 139.36 µg/mL showed in Table 5. Besides, the 200µg/mL of papaya leaf extract achieved 52.31% of radical scavenging activity while at 200µg/mL of standard ascorbic acid achieved 80.38% of radical scavenging activity. The 120µg/mL of papaya leaf extract exhibited stronger antioxidant activity compared to ascorbic acid.

Based on the result in Graph 2, the DPPH inhibition activity increased progressively when the extract concentration increased. The IC_{50} value of papaya leaf indicated moderate antioxidant activity as the lower IC_{50} value indicated stronger antioxidant activity. Although the extract was less potent compared to ascorbic acid, it can be classified as having moderate antioxidant activity. (Khor et al., 2021b) The activity is more likely due to the

presence of flavonoids, phenolic compounds and tannins. They can donate hydrogen protons or electrons to neutralize DPPH free radicals and reduce oxidative damage.

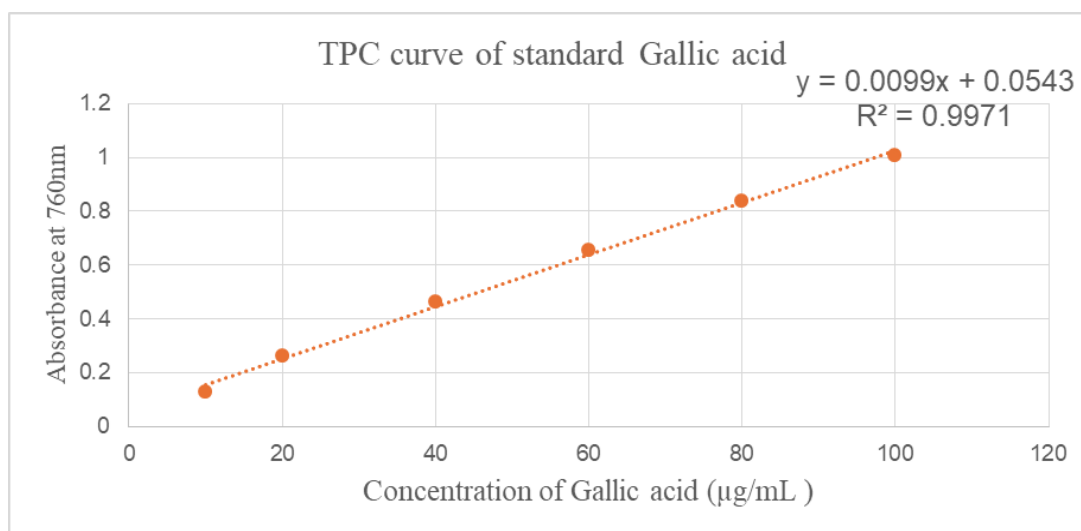
The colour changes and colour intensity from purple to yellow is another important way to evaluate its antioxidant activity. DPPH solution is in a deep violet colour. When it is mixed with a solution of a substance capable of donating a hydrogen atom, the violet colour disappears and a reduced form of DPPH is formed, and colour change to a pale yellow colour. (Gulcin & Alwasel, 2023). There were some possible reasons that affect the inhibition activity of papaya leaf extract such as the extraction solvent, geographical design, and leaf maturity. The presence of bioactive and inactive compounds in the natural medicinal plants can lead to a higher IC_{50} value. This is because the extract didn't undergo purification and isolation processes.

Total phenolic content (TPC) testTotal Phenolic Content ($\mu\text{g GAE/g DW}$)**Table 7: The total phenolic absorbance value of standard gallic acid at different concentration at 760nm wavelength.**

Concentration of gallic acid ($\mu\text{g/mL}$)	Absorbance at 760nm			
	Trial 1	Trial 2	Trial 3	Average
10	0.125	0.128	0.131	0.128 ± 0.008
20	0.261	0.264	0.261	0.262 ± 0.003
40	0.463	0.462	0.465	0.463 ± 0.002
60	0.652	0.654	0.653	0.653 ± 0.001
80	0.872	0.782	0.854	0.836 ± 0.05
100	0.994	0.998	1.023	1.005 ± 0.016
Control	0.035	0.031	0.039	0.035 ± 0.021

Table 8: The absorbance value of Carica papaya leaf extract at 760nm wavelength

Extract ($\mu\text{g/mL}$)	Absorbance of standard at 760nm				
	Trial 1	Trial 2	Trial 3	Average value	Mean $\pm$ SD
Carica papaya leaf extract	1.044	1.126	1.113	1.094	1.094 ± 0.044

**Graph 4: The calibration curve of TPC of Gallic acid.****Table 9: The Total Phenolic Content (TPC) of Carica papaya leaf expressed in Gallic Acid Equivalents (GAE).**

Extract (1mg/mL)	Absorbance of standard at 760nm		
	Average value	Mean $\pm$ SD	Equivalent Gallic acid (mg GAE/g)
Carica papaya leaf extract	1.094	1.094 ± 0.044	105.05

Total phenolic content (TPC) assay

The total phenolic content of Carica papaya leaf extract was 105.05 mg GAE/g in Table 9, it indicated high concentration of phenolic compounds was present in the extract. The phenolic compounds are important bioactive compounds that contribute to antioxidant activity due to their ability to donate a hydrogen proton or an electron to stabilize free radicals. (Chaijan et al., 2024b). Besides, the Gallic acid was used as a standard reference in the TPC assay. The standard calibration curve was determined by using absorbance value at 760nm against various concentrations of Gallic acid as shown in Graph 4. The regression equation of the calibration curve of reference gallic acid was $y = 0.0099x + 0.0543$, $R^2 = 0.9971$.

The Folin-Ciocalteu assay was used for the quantification of total polyphenols in the extract. The redox reaction occurred in this assay in which the antioxidant product containing a phenolic compound acted as the electron donor and the F-C reagent acted as the oxidant. Then, the electrons transfer from phenolic compounds to phosphotungstic acid complex in alkaline solution created a colour shift from yellow to blue colour that detected by UV-Vis spectroscopical at 760nm. (Robert Nastasi, 2025). Some findings showed that the phenolics were higher in a polar solvent such as ethanol as most of the polyphenols were polar compounds. The higher phenolic content in the extract was crucial for bioactivity. Hence, the phytochemical screening, DPPH assay and TPC assay were used to determine the antioxidant properties in Carica papaya leaf extract.

Thin Layer Chromatography

The presence of flavonoids by Thin Layer Chromatography

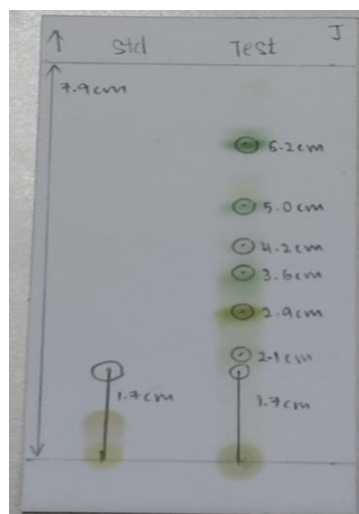


Figure 3: The TLC result for Carica Papaya Leaf extract.

Table 10: The compounds present in Carica papaya leaf extract by determined the Rf values.

Spot	Spot distance (mm)	Rf value	Colour under visible light	Compounds present in Carica Papaya leaf
Standard (Quercetin)	17	0.22	Yellow	Quercetin
Spot 1	17	0.22	Yellow	Quercetin
Spot 2	21	0.27	Greenish yellow	Kaempferol-3-O-glucoside
Spot 3	29	0.37	Greenish	Kaempferol
Spot 4	36	0.46	Pale green	Luteolin
Spot 5	42	0.53	Light green	Luteolin glycoside
Spot 6	50	0.63	Dark green	Apigenin (Flavone)
Spot 7	62	0.79	Dark green	Chlorophyll / non-flavonoid

Thin Layer Chromatography (TLC) analysis

TLC was a simple, fast and effective method for separating mixtures of chemical compounds. The appearance of distinct bands on the TLC plate indicates that multiple phytochemical constituents were successfully extracted from the papaya leaf extract. The TLC profile suggests the presence of flavonoid-related compounds, which are important bioactive compounds to give antioxidant, antimicrobial and anti-inflammatory properties. The mobile phase used was ethyl acetate: glacial acetic acid: formic acid: water (13: 1.5: 1.5: 4, v/v/v/v). They provided suitable polarity for the separation of flavonoids present in papaya leaf extract. Ethyl acetate acts as the primary organic solvent; acetic acid and formic acid improve peak resolution and maintain an acidic environment. Lastly, water increased the polarity of the system and facilitated the migration of polar flavonoid compounds. This solvent system can separate and identify the flavonoid family effectively on the TLC plate. The result of extract showed in Figure 3. The TLC analysis confirmed the presence of quercetin,

kaempferol, kaempferol-3-O-glucoside, luteolin, luteolin glycoside and apigenin in Table 10. The quercetin compound was confirmed in Carica papaya leaf as there was a yellow spot travelled exact same distance as a known standard quercetin spot which the Rf value was 0.22. The polarity of a compound was determined by how fast it travelled up the TLC plate. The less polar compounds travelled faster and had higher Rf values while the more polar compounds interacted strongly with the stationary phase and travelled slower and showed lower Rf values. The solvent was carried up the plate by capillary action at different rates and used for compound separation and identification. The most potent natural antioxidant is quercetin due to its ability to stabilize free radicals through resonance structures. (Nicolas et al., 2021) However, the TLC only showed the visual colour interpretation and did not directly confirm the exact compounds in the extract. Hence, LC-MS/MS confirmation can be used to improve the outcome.

Fourier Transform Infrared Spectroscopy (FTIR) spectrum

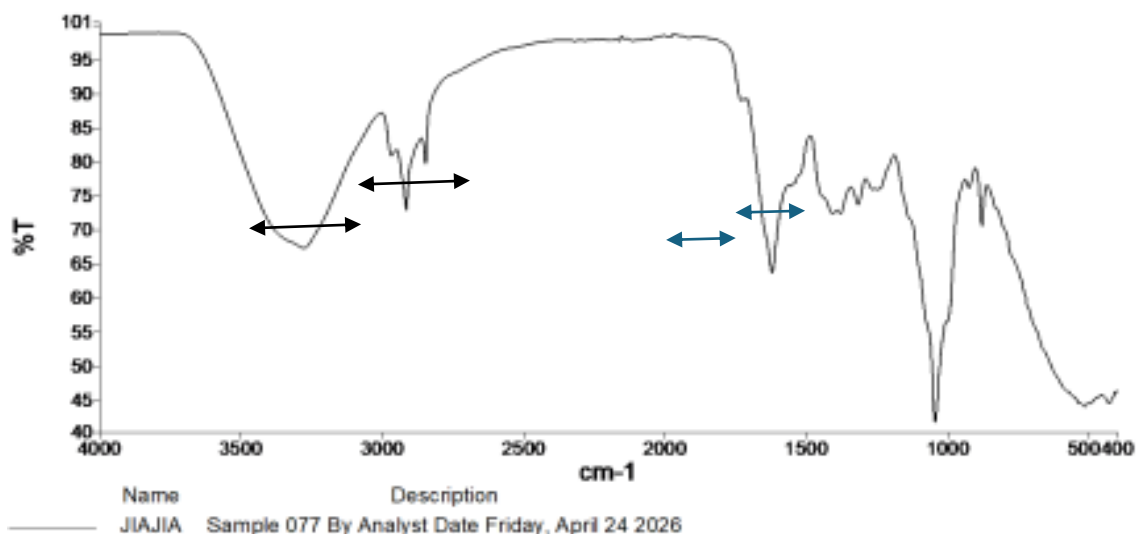


Figure 4: The FTIR spectrum for *Carica papaya* leaf extract.

Table 11: The compounds in *Carica papaya* leaf extract in FTIR spectrum.

Peak position (cm ⁻¹)	Peak shape	Functional Groups	Compounds
3281cm ⁻¹	A strong and broad peak	OH- group	Phenols, flavonoids, tannins, and alcohol compounds
2917cm ⁻¹ and 2849cm ⁻¹	Two small peaks	C-H stretching vibrations	Fatty acids, and terpenoids
1622 cm ⁻¹	A strong peak	C=C aromatic group stretching	Phenolic compound
1408cm ⁻¹	A small peak	C-H bending	Phenols and carboxylic acid
1317cm ⁻¹	A small peak	OH- group bending	Alkaloids and polyphenols compounds
1245cm ⁻¹	A medium peak	C-O group stretching	Flavonoids and glycosides
1044cm ⁻¹	A strong and sharp peak	C-O stretching	Glycosides and saponins compounds

Fourier transform infrared spectroscopy (FTIR) assay

FTIR assay is a powerful, non-destructive analytical technique used to identify chemical structures and functional groups of chemical constituents of *Carica papaya* leaf extract. The FTIR spectrum is used to identify a specific organic functional group in an unknown molecule by changes in vibrational and rotational energy levels when absorbing the infrared ray. Their vibrations and intensity (% transmission) were plotted against the frequency of light (cm⁻¹) to which the sample was exposed to produce an FTIR spectrum. The FTIR spectrum reflected the position and intensity of a vibrational band and the conformation of atoms involved in the chemical bond. Based on the FTIR spectrum results, some functional groups had been detected such as phenolics, flavonoids, alcohol, terpenoids, alkanes and aromatic compounds. The FTIR confirms the functional groups present in *Carica papaya* leaf extract, supporting the medicinal properties such as antioxidant, antimicrobial and anti-inflammatory. The FTIR spectrum showed in Figure 4.

The compounds present in extract were showed in Table 11 where broad absorption band in the 3200–3600 cm⁻¹ region showed the OH group stretching vibrations of phenolic compounds and alcohol. The hydroxyl group contributes to antioxidant properties as it can donate hydrogen protons to neutralize free radicals. The peaks at 3281cm⁻¹, 2917 and 2849cm⁻¹, 1622cm⁻¹, 1245cm⁻¹, 1044cm⁻¹, and 878cm⁻¹ were assigned in the thesis to O–H, C–H, aromatic C=C, C–O, glycosidic/saponin-related C–O, and aromatic ring-associated vibrations, respectively. (R. M. PradikalisyroRizqiravendra Al-Hakiim, 2025).

The phytochemical screening, FTIR spectrum and TLC chromatogram can provide a comprehensive profile of *Carica papaya* leaf extract. The screening indicated broad compound classes, the FTIR spectrum supported the expected functional groups within the compound classes, and the TLC assay provided the presence of the flavonoid family.

Antimicrobial test

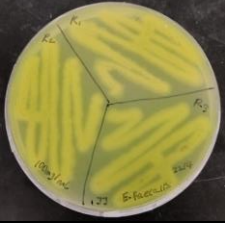
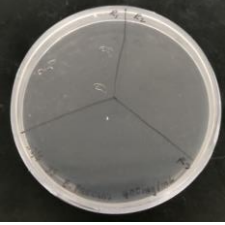
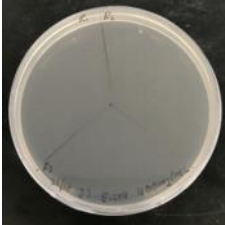
Concentration of Carica papaya leaf extracts (mg/mL)	Observation of minimum bactericidal concentration (MBC) for <i>E. Faecalis</i>	Observation of minimum bactericidal concentration (MBC) for <i>E. Coli</i>
25		
50		
100		
200		
400		

Figure 6: The figure showed the observation of Minimum Bactericidal Concentration (MBC) of Carica papaya leaf extract for gram-positive bacteria & gram-negative bacteria (*E. faecalis* & *E. coli* respectively).

Antimicrobial activity of Carica papaya leaf extract

The Carica papaya leaf extract inhibited both *E. Faecalis* and *E. coli* with MIC and MBC values of 400mg/mL respectively. This investigated the papaya leaf extract which possessed antimicrobial activity against both Gram-positive and Gram-negative bacteria, but only at a relatively high concentration. The antimicrobial assay demonstrated that the extract exhibited inhibitory and bactericidal effects against both microorganisms. The extract has bactericidal activity at the inhibitory concentration. The antimicrobial activity may be proved by the presence of phenolics, flavonoids, saponins, terpenoids, and steroids that were identified in the phytochemical screening. These compounds can inhibit the growth of bacteria through cell membrane disruption

and nucleic acid inhibition. The Carica papaya leaf suggested to have moderate antimicrobial potency compared to antibiotics such as amoxicillin. The high concentration value needed to kill the bacteria may be due to some reasons such as solvent selection, extraction method, geographic variation and bacterial strain differences. Other than that, the active compounds are mixed with inactive constituents. Hence, purification and isolation of active phytochemicals may improve antimicrobial effectiveness.

The results of MBC for *E. coli* and *E. Faecalis* in Figure 6 showed the broad-spectrum antimicrobial potential of the extract and supported the natural medicinal use of Carica papaya leaf in treating bacterial infections.

The 200mg/mL of plant extract cannot be considered as MIC due to the visible turbidity in the broth at 200mg/mL and the bacterial colonies on the MBC agar plates, indicated the bacterial growth still occurred at this concentration. The concentration of plant extract required to kill the bacteria in this study is higher compared to previous studies. The possible reasons are the crude extracts are less potent compared to pure antibiotics. The papaya leaf extract contains bioactive and inactive compounds, waxes and chlorophyll. Each compound contributes differently to antimicrobial activity. At the concentrations between 25 to 200mg/mL, the amount of bioactive compounds may still below the minimum concentration that required to damage the bacterial cells. At 400mg/mL extract concentration, high enough to disrupt bacterial membranes and interfere with essential metabolic pathways. The bacterial defence system becomes overwhelmed and resulting in complete bacterial inhibition.

The limitation of this antimicrobial study is the concentration series used doubles at each step between the range of 25mg/mL to 400mg/mL. The exact MIC lies within the 200mg/mL to 400mg/mL interval. A finer dilution series required to identify the more precise MIC and determine the transition is gradual or truly abrupt.

CONCLUSION AND FUTURE PERSPECTIVES

Carica papaya leaf is well known medicinal plants for therapeutic benefits and traditional use for treatment of inflammation, infection, antioxidant and antimicrobial properties. They are rich in phytochemicals and bioactive compounds that support antimicrobial and antioxidant activity. The phytochemical tests, TLC assay and FTIR assay can support the presence of bioactive compounds in papaya leaf extract.

The recent study reports that the 65% of percentage yield of *Carica papaya* leaf by using 80 % of 95% ethanol and 20 % of n-hexane. The polar and semi polar compounds can be extracted by ethanol while the non-polar compounds can be extracted by n-hexane. The plant extract showed moderate antioxidant activity as the DPPH scavenging activity of plant extract (161.66 µg/ml) was higher than the standard ascorbic acid (136.57 µg/ml). The polar solvent such as ethanol showed higher efficiency in extracting the phenolic compounds of *Carica papaya* leaf which gives 105.05 mg GAE/g. It is proven that the potential of natural antioxidants which have free radical scavenging activity and useful for reduction oxidative stress, hepatoprotection and radioprotection.

The *Carica papaya* leaf also has been proved the antibacterial efficacy against both Gram-positive and Gram-negative microorganisms. This indicates the plant is a potential source for drug production with a broad spectrum of activity.

ACKNOWLEDGEMENT

We are thankful to Ms. Amalina for providing us the original microbial strain.

DISCLOSURE STATEMENT:

No potential conflict of interest was reported by the author(s)

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