

Phytochemical Profile, Spectroscopic Characterization, and Antimicrobial Properties of *Carica papaya* Seed Extracts Obtained from Aqueous, Ethanol, and Hexane Solvents

Issah Yahaya ^{1,*}, Bright Darko ¹, Bismark Addo Oppong ¹, Isaac Williams ²

1 Department of Chemical Sciences, School of Sciences, University of Energy and Natural Resources, Sunyani, Ghana; issah.yahaya@uenr.edu.gh (I.Y.); darkobright11@gmail.com (B.D.); bismark.oppong@uenr.edu.gh (B.A.O.);

2 Center for Research in Applied Biology, School of Sciences, University of Energy and Natural Resources, Sunyani, Ghana; isaac.williams@uenr.edu.gh (I.W.);

* Correspondence: issah.yahaya@uenr.edu.gh;

Received: 15.09.2025; Accepted: 07.02.2026; Published: 30.09.2026

Abstract: This study investigates the phytochemical composition, phenolic and flavonoid contents, spectroscopic features, and antimicrobial properties of *Carica papaya* seed extracts obtained using water (Aqua), ethanol (EtOH), and hexane (Hex) as solvents. Extract recovery (mean $\pm$ SD with $n = 3$) was highest in Aqua ($4.16 \pm 0.02\%$), followed by EtOH ($3.95 \pm 0.03\%$) and Hex ($3.38 \pm 0.05\%$). Phytochemical screening revealed the presence of saponins in all extracts, with alkaloids additionally detected in EtOH. Total phenolic content (mean $\pm$ SD with $n = 3$) was highest in Aqua (162.03 ± 0.05 ppm), while total flavonoid content (mean $\pm$ SD with $n = 3$) was also highest in Aqua (148.24 ± 0.07 ppm). UV-Vis spectra exhibited solvent-dependent absorption bands, while FT-IR spectra confirmed the presence of functional groups corresponding to phenolics, alkaloids, and saponins. Antimicrobial assays (mean $\pm$ SD with $n = 3$) demonstrated that EtOH extracts exhibited significant activity against *Staphylococcus aureus* (16.0 ± 0.58 mm) and *Klebsiella pneumoniae* (16.0 ± 1.00 mm), comparable to the positive control (tazobactam/piperacillin). Aqua extracts showed moderate activity, while Hex extracts were the least effective. Overall, ethanol extraction yielded the most bioactive extract, suggesting *Carica papaya* seeds can be a source of natural antimicrobial agents.

Keywords: *Carica papaya* seeds; phytochemicals; UV-Vis and FT-IR spectroscopy; antimicrobial activity; natural products.

© 2026 by the authors. This article is an open-access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. The authors retain copyright of their work, and no permission is required from the authors or the publisher to reuse or distribute this article, as long as proper attribution is given to the original source.

1. Introduction

The increasing prevalence of antibiotic-resistant bacteria has become a significant global health concern, necessitating the exploration of alternative antimicrobial agents [1]. *Carica papaya* Linn., which belongs to the *Caricaceae* family, is both a nutritional and medicinal plant that originated from Central America and has spread to different parts of the world, especially West Africa. Although native to the Americas, it is predominantly grown and consumed in the tropics and subtropics for its delightful tastes, aromas, and nutrient content [2]. *Carica papaya* seed has been reported by several researchers due to its versatility [3]. The plant is known to aid quail production performance [3,4].

Additionally, many people use the seed for its phytotoxic effect and as a contraceptive [5-7]. Furthermore, it can also be employed as an environmentally friendly and efficient alternative for dye adsorption [8]. The growth of the plant is affected by several factors [9-11].

Many studies on the plant have shown the profound clinical and pharmacological importance of the proteolytic enzymes found in its latex, which are more abundant in younger plants than in older ones [12]. A study by Ouedraogo et al. [13] reports on aqueous infusions and decoctions of papaya seeds, assessing polyphenolic content and antimicrobial inhibition. It demonstrates that seed extracts show measurable antimicrobial activity. Okeke-Nwolisa and Benedict [14] conducted research that focused on the ethanolic and aqueous extracts of dry papaya seeds tested against MDR clinical isolates. They reported MIC values as low as ~12.5 mg/mL for the ethanolic extract. In an *in vitro* study by Oboh et al. [15], the scientists compared the ethanolic versus hot-water extracts of papaya seeds against *Salmonella spp.* and *Candida albicans*. The ethanolic extract produced inhibition zones from ~10 mm to ~32 mm; the aqueous extract gave much smaller zones (~4-9 mm).

Most investigations on the bioactive properties of pawpaw have focused on the stem and leaves; little is known particularly from the seeds, which constitute 15 to 20% of the net weight of the fruit. Again, there is no or limited comparative data on solvent-based extraction of papaya seeds. This study was therefore designed to investigate the biological properties of pawpaw seed extracts. Hence, the phytochemical investigations and the characterization of three different extracts of *Carica papaya* seed were conducted using FT-IR and UV-visible spectroscopy. Furthermore, this study aims to evaluate the antimicrobial potential of crude extracts from *Carica papaya* seeds against W.H.O. priority pathogens as an alternative drug candidate. Finally, to the best of our knowledge, this study has never been conducted.

2. Materials and Methods

2.1. Collection of plant material.

Five fresh *Carica papaya* fruits were purchased from a Bono City local market. After cutting each *Carica papaya* into four pieces, the seeds were gathered. An experienced taxonomist at Kwame Nkrumah University of Science and Technology in Kumasi's Department of Herbal Medicine laboratory of the Faculty of Pharmacy and Pharmaceutical Sciences correctly identified the seed samples (Voucher number KNUST/2012/CP014). All chemicals used were of analytical grade with the highest purity available (>99.5 %) and procured from Sigma-Aldrich, USA.

The seeds were dried at room temperature in the laboratory for 14 days. For better grinding, the seed materials were then oven-dried for 24 hours at 30 °C. The dried seeds were then ground into a coarse powder in the Department of Chemical Sciences laboratory at the University of Energy and Natural Resources, using a high-capacity grinding machine.

2.2. Extracting phytochemicals from powdered dried *Carica papaya* seeds.

With some modifications, a recently reported protocol [16] was utilized. An accurately measured 50 g of the powdered dried *Carica papaya* seeds was weighed and placed in a 500 mL beaker. To obtain three separate beakers labeled Aqua (aqueous extract), EtOH (ethanolic extract), and Hex (n-hexane extract), the process was repeated three times (n = 3). A measuring cylinder was used to determine how much water, ethanol, and hexane (250 mL) would be needed to dissolve each of the powders in the beakers.

The maceration technique, at 30 °C, was employed for the extraction of the crude extracts. In this case, 50 g of the ground *Carica papaya* dried seeds were weighed into a 500 mL beaker, and 250 mL of the appropriate solvent was added to effect the extraction. The procedure was repeated three times (n = 3) for each solvent to get three different extracts (Aqua, EtOH, and Hex). The beakers were labeled Aqua, EtOH, and Hex. 250 mL of the solvent was employed for all three solvents to ensure that the ground *Carica papaya* dried seeds were submerged in each of the solvents to enhance dissolution of the phytochemicals into the solvents. Each mixture was placed on the laboratory shaker to undergo agitation for 24 hours. After the extraction, each beaker was covered with paraffin paper and aluminum foil to avoid evaporation of the solvent. After another 24 hours, each crude extract was filtered using a filter paper (Whatman No.1) to get the filtrate. A rotary evaporator was used to remove solvents from the filtrates to get almost completely dried extracts, which were weighed and placed in a sterile container and then put in a refrigerator at -4 °C for later use.

2.3. Recovery extracts (extraction yields).

The recovery value of each extract of *Carica papaya* dried seeds was calculated using the formula:

$$\%R = \frac{m_2}{m_1} \times 100 \quad (1)$$

Where: %R = percentage recovery; m_1 = mass of dried leaves (g), m_2 = mass of crude extract (g).

The present study was carried out in triplicate, and the findings were presented as mean±SD.

2.4. Examining the phytochemicals in the powdered dried *Carica papaya* seed extracts.

Utilizing various standard qualitative methods described by some scientists [17-19] with some modifications, phytochemical investigations were carried out on the three extracts (Aqua, EtOH, and Hex) of ground *Carica papaya* dried seeds to determine the presence or absence of certain phytochemicals (alkaloids, flavonoids, phenolics, saponins, and tannins). Figures S1–S5 in the Supplementary material present the comprehensive experimental protocol for the phytochemical screenings, which includes color change.

2.5. Examination of total flavonoid content (TFC) and total phenolic content (TPC).

The Folin-Ciocalteu and aluminum chloride methods were used to determine the total phenolic and total flavonoid contents, respectively. The Supplementary Material contains the concentration and the matching absorbance.

2.5.1. Examination of the total phenolic content.

A slightly modified version of the procedure described by Johari and Khong [20] was employed for total phenolic content (TPC). The reference standard used in this study was ascorbic acid, which was modified slightly from Singleton et al.'s report [21].

Weighing 10 mg of each extract (Aqua, EtOH, and Hex) and dissolving it in 10 mL of deionized water resulted in a stock solution (1 mg/mL). Each of the extracts (Aqua, EtOH, and Hex) was placed in a different test tube, and each contained 0.5 mL of aqueous solution. After adding 2.5 mL of the Folin-Ciocalteu reagent (1:10 v/v deionized water), 2.5 mL of the 7.5%

(w/v) Na_2CO_3 solution was added. For thirty minutes, each mixture was incubated at 25 °C in the dark. Using a SHIMADZU UV-1800 General Purpose UV-visible spectrophotometer, the absorbance of the solution was measured at 760 nm following the 30-minute incubation period. Using the same procedure, standard solutions of ascorbic acid with concentrations of 100, 120, 140, 160, and 180 ppm were made in place of extracts (Table S1).

Plotting the absorbance (at 760 nm) against concentration led to the establishment of a calibration curve ($y = 0.02116x + 0.0336$, $R^2 = 0.9993$), as shown in Figure S6. Afterward, using the calibration curve, the total phenolic content was calculated and expressed as ascorbic acid equivalents (AAE) in parts per million of dry extract [22]. The findings were presented as mean $\pm$ standard deviation (mean $\pm$ SD) after this analysis was conducted three times ($n = 3$).

2.5.2. Examination of the total flavonoid content.

With only minor adjustments, the method described by Pękal and Pyrzynska [22] was used to determine the total flavonoid content.

In this investigation, a stock solution (1 mg/mL) was prepared by weighing 5 mg of each extract (Aqua, EtOH, and Hex) and adding 5 mL of deionized water. Placed in a test tube, 1.5 mL of each stock solution (1 mg/mL) of each extract was added, followed by 0.50 mL of NaNO_2 (5 %w/v) and 5 minutes of incubation. A 5-minute incubation period was followed by adding 0.50 mL of 2 % w/v AlCl_3 . 2.0 mL of 4% w/v NaOH and 5.0 mL of distilled water were added following the incubation period. A half-hour incubation period was then added to the mixture. Utilizing a SHIMADZU UV-1800 General Purpose UV-VIS spectrophotometer, the absorbance was measured at 410 nm following the incubation period.

The same procedure was used to prepare standard quercetin solutions (100, 120, 140, 160, and 180 ppm), as illustrated in Table S2, for the purpose of creating a calibration curve ($y = 0.02089x + 0.03993$, $R^2 = 0.9989$), as depicted in Figure S7. The same process was carried out using 1.5 mL of distilled water as the blank solution. The outcome was stated in dry extract parts per million. Quercetin equivalent, or QE, was used to express the total flavonoid content in parts per million of dry extract. The results were recorded as mean $\pm$ standard deviation (mean $\pm$ SD) after conducting this analysis three times ($n = 3$).

2.6. Examination of ultraviolet-visible and FT-IR spectroscopic analyses.

2.6.1. Examination of the ultraviolet-visible spectroscopy.

The process described by Bashyam and associates [23] was used in this instance with minimal modifications.

A 250 mL conical flask containing 1 g of dry powdered *Carica papaya* seeds was macerated for 48 hours at room temperature in 50 mL of n-hexane with sporadic shaking. Whatman No. 1 filter paper was used to filter the sample. After centrifuging the extract for ten minutes at 12,000 rpm, it was filtered once more. The sample was scanned at wavelengths between 200 and 500 nm using the SHIMADZU UV-1800 UV-Vis spectrophotometer (1.00 cm path-length quartz cuvettes, resolution/data interval: 0.5–1.0 nm step, and bandwidth (spectral slit): ~ 1 nm) after being diluted 1:10 with hexane. The wavelength scans for the Aqua and EtOH extracts were also acquired, just like for the Hex extract.

2.6.2. Examinations of the FT-IR spectroscopy.

The method previously outlined [23] was followed for this analysis, with some slight adjustments, for FT-IR analysis.

Fourier Transform Infrared (FT-IR) spectroscopy was performed to identify the functional groups present in the aqueous (Aqua), ethanol (EtOH), and hexane (Hex) extracts of *Carica papaya* seeds. Approximately 10 mg of each dried extract was thoroughly mixed with 250 mg of spectroscopic-grade potassium bromide (KBr). The mixtures were compressed into 13 mm diameter transparent pellets using a hydraulic press at a pressure of 75 kN cm⁻² for three minutes. Spectral measurements were carried out using a Shimadzu FTS-8000 FT-IR spectrophotometer (Shimadzu Corporation, Japan). The instrument was calibrated using a standard polystyrene film prior to analysis to ensure wavelength accuracy. The optical path length of the KBr pellet was approximately 1 mm. Each spectrum was recorded over a scanning range of 4000–500 cm⁻¹ at a resolution of 4 cm⁻¹, with background correction performed using a pure KBr pellet under identical conditions.

2.7. Antimicrobial evaluation.

2.7.1. Retrieval of testing organisms.

Bacterial strains for the in vitro analysis were retrieved from the microbiology laboratory unit of the Center for Research in Applied Biology (CeRAB). Following the manufacturer's instructions, isolates were grown on fresh MacConkey agar and Mannitol salt agar. Bacterial isolates were Gram-stained and biochemically tested using API 20E. The score confirmed the bacterial strains obtained as *Staphylococcus aureus*, *Citrobacter freundii*, *Klebsiella pneumoniae*, *Escherichia coli*, and *Enterobacter cloacae*.

2.7.2. In vitro assessment of the crude extract against the bacterial strains.

The disc diffusion method was used to determine the antibacterial activity of *Carica papaya* seeds using previously reported [24] methods with slight modification. With the help of a microbiological loop, the bacterial strains were suspended in 3 mL of normal saline and vortexed. The concentration was adjusted using DensiCHEK to a McFarland standard of 0.50 turbidity.

A sterile swab stick was dipped into the suspension. The swab was rotated against the side of the tube using firm pressure to remove excess fluid. The dried surface of the Mueller-Hinton agar plate was inoculated by streaking the swab over the entire agar surface to ensure an even distribution of the inoculum. In each of these plates, 3 wells with a diameter of 6 mm in diameter were cut using a sterile cork borer, and agar discs were removed. 0.10 mL of each solvent was tested against the bacterial strains as a negative control and compared to the efficacy of the extracts.

Again, the wells were filled with a 0.10 mL sample of each extract (10 mg/mL) using a micro-pipette, kept in a fridge at 2°C to 8°C, and allowed to diffuse for 30 minutes. Tazobactam/piperacillin (10 µg/disc) was used as a positive control. The plates were then incubated upright at 37°C for 24 hours. The zone of inhibition around the central zone was measured according to the guidelines set by the Clinical and Laboratory Standards Institute (CLSI).

2.7.3. Statistical analysis.

Every investigation was performed on three separate occasions ($n = 3$). One-way analysis of variance was used to analyze the data, which are presented as mean $\pm$ standard deviation (SD). The SPSS statistical software (Version 28, IBM, Armonk, NY, USA) was employed for evaluating the data. Using the Tukey post hoc test, significant differences ($p < 0.05$) between the groups were observed.

3. Results and Discussion

3.1. Recovery of the extracts

Table 1 shows the findings of the percentage recovery of the dried seeds of *Carica papaya* extracts. The Aqua extract of *Carica papaya* seeds gave the highest recovery among the three solvents, with a yield of 4.16 ± 0.02 %. This suggests that water was more efficient at solubilizing and extracting water-soluble phytochemicals from the seed matrix compared to ethanol and hexane. The EtOH extract had a percentage recovery of 3.95 ± 0.03 %, slightly lower than the aqueous extract but higher than the hexane extract. Ethanol's polarity allows it to extract a wider range of bioactive compounds, including both polar and moderately nonpolar phytochemicals. The Hex extract had the lowest percentage recovery of 3.38 ± 0.05 %, reflecting hexane's selectivity for nonpolar compounds such as lipids and certain terpenoids, which may be less abundant in papaya seeds compared to polar constituents.

Table 1. This shows the percentage recovery, results of phytochemical tests, TPC, and TFC of the different extracts of *Carica papaya* seeds.

Extract	%Recovery (mean $\pm$ SD)	Phytochemicals					TPC in ppm (mean $\pm$ SD)	TFC in ppm (mean $\pm$ SD)
		Alkaloids	Flavonoids	Phenols	Saponins	Tannins		
Aqua	4.21 $\pm$ 0.03	–	–	–	+	–	162.03 $\pm$ 0.05	148.24 $\pm$ 0.07
EtOH	4.13 $\pm$ 0.15	+	–	–	+	+	148.55 $\pm$ 0.07	133.94 $\pm$ 0.10
Hex	4.07 $\pm$ 0.03	–	–	–	+	–	90.42 $\pm$ 0.28	99.64 $\pm$ 0.08

Presence of phytochemical = +; Absence of phytochemical = –; TPC = Total phenolic content; TFC = Total flavonoid content.

3.2. Tests of the phytochemicals from the dried *Carica papaya* seed powder extracts.

An overview of the findings from the phytochemical screening of the dried papaya seed extracts is provided in Table 1. The Supplementary material (Figures S1-S5) also illustrates the screening results.

The screening revealed saponins, while alkaloids, flavonoids, phenols, and tannins were absent. This indicates that the aqueous solvent primarily extracted glycosidic compounds with surfactant properties, which are typical of saponins. The EtOH extract tested positive for alkaloids and saponins, while flavonoids, phenols, and tannins were not detected. The presence of alkaloids in the EtOH extract is noteworthy since these compounds often contribute to antimicrobial and pharmacological effects. Only saponins were detected in the Hex extract, while alkaloids, flavonoids, phenols, and tannins were absent. This suggests that hexane solubilized fewer classes of secondary metabolites.

3.3. Total phenolic content (TPC) and total flavonoid content (TFC).

3.3.1. TPC.

Utilizing the calibration curve regression equation ($y = 0.0224x + 0.03079$; $R^2 = 0.9992$), the TPC of the compounds (ppm) in each extract was calculated. As shown in Table 1 and the Supplementary Material (Figures S6 and S7), the results suggested that there was a significant variation in the TPC of the extracts (Aqua, EtOH, and Hex) made using different extraction solvents. As expected, the Aqua extract had a higher TPC (162.03 ± 0.05 ppm) than the EtOH extract (148.55 ± 0.07 ppm), and the Hex had the lowest amount of TPC (90.42 ± 0.28 ppm).

3.3.2. TFC.

The calibration curve's regression equation, $y = 0.02165x + 0.01584$ ($R^2 = 0.9998$), was utilized in calculating the TFC (ppm) in each extract. The findings demonstrated that there are notable differences in TFC between the various extracts (Figure S7 in the Supplementary material, Table 1). It also revealed that the Hex extract had the lowest TFC (99.64 ± 0.08 ppm), while the Aqua extract had a higher TFC (148.24 ± 0.07 ppm) than the EtOH extracts (133.94 ± 0.10 ppm).

In a nutshell, both TPC (162.03 ± 0.05 ppm) and TFC (148.24 ± 0.07 ppm) values were the highest in the Aqua extract, highlighting water's effectiveness in concentrating phenolic and flavonoid-related compounds, even though screening tests did not detect phenols/flavonoids qualitatively. This may reflect sensitivity differences between qualitative and quantitative methods. The EtOH extract values (TPC = 148.55 ± 0.07 ppm and TFC = 133.94 ± 0.10 ppm) were slightly lower than those in the Aqua extract but still substantial, indicating ethanol's efficiency in extracting polyphenolic compounds. However, the TPC (90.42 ± 0.28 ppm) and TFC (99.64 ± 0.08 ppm) values were lowest in the Hex extract, consistent with its inability to efficiently extract phenolic- or flavonoid-rich compounds.

3.4. Examinations of the ultraviolet-visible and FT-IR spectroscopy

Following the phytochemical screenings, UV-Vis and FT-IR spectroscopy studies were conducted in order to corroborate the phytochemical test results.

3.4.1. Ultraviolet-visible spectroscopy.

At wavelengths ranging from 200 to 500 nm, *Carica papaya* seed extracts were scanned to detect compounds containing aromatic rings, lone pairs of electrons, σ -bonds, and chromophores.

The Aqua extract displayed absorption maxima at 204 nm, 233 nm, 281 nm, 458 nm, and 494 nm. Peaks in the 200–280 nm region suggest π – π^* transitions in unsaturated bonds and aromatic systems, while the visible region absorptions (458–494 nm) indicate the presence of conjugated compounds or pigments, as depicted in Figure 1 [25].

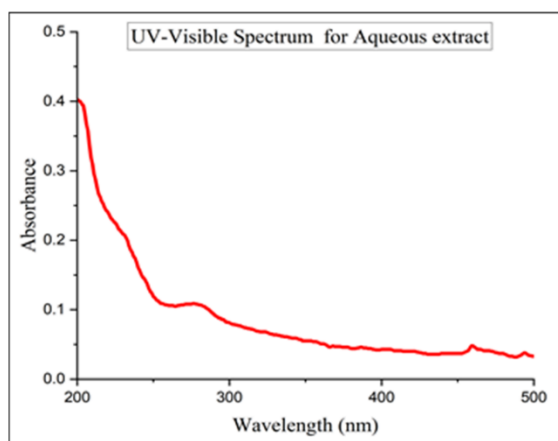


Figure 1. UV-Visible spectrum of Aqua extract of *Carica papaya* seeds, revealing absorptions and wavelengths of absorption that point to the presence of certain bonds.

The EtOH extract showed absorption bands at 205 nm, 229 nm, 272 nm, 460 nm, and 493 nm. The sharp absorptions below 300 nm confirm the presence of aromatic/phenolic systems, while visible region bands suggest conjugated molecules similar to those seen in the Aqua extract [25].

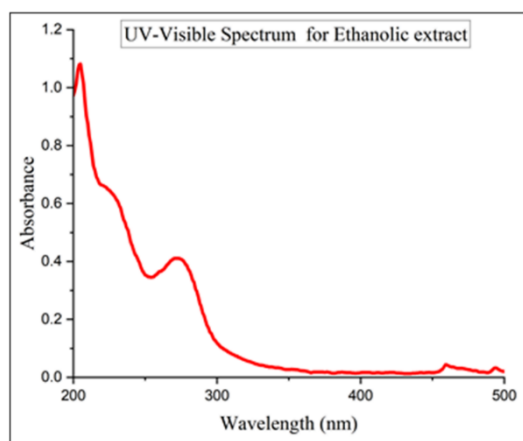


Figure 2. UV-Visible spectrum of EtOH extract of *Carica papaya* seeds, revealing absorptions and wavelengths of absorption that point to the presence of certain bonds.

The Hex extract displayed absorptions at 202 nm, 234 nm, 261 nm, 400 nm, and 465 nm. The UV region bands indicate unsaturated systems, while the visible absorptions point toward nonpolar pigments or terpenoid-type compounds [25].

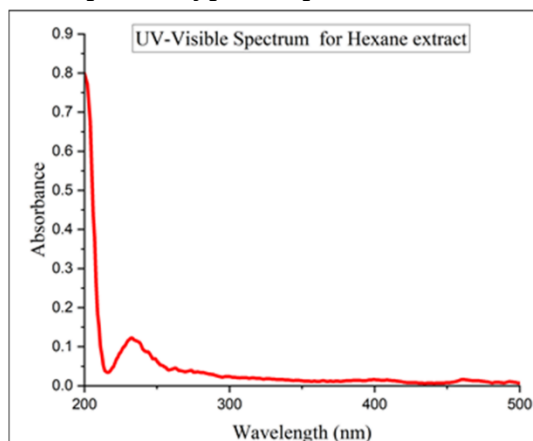


Figure 3. UV-Visible spectrum of the Hex extract of *Carica papaya* seeds, revealing absorptions and wavelengths of absorption that point to the presence of certain bonds.

3.4.2. FT-IR spectroscopy.

Table 2 displays the findings from the FT-IR Spectroscopic analysis.

Table 2. Peak designation and FT-IR absorption frequency.

Aqua extract		EtOH extract		Hex extract	
Frequency (cm ⁻¹)	Inferred group	Frequency (cm ⁻¹)	Inferred group	Frequency (cm ⁻¹)	Inferred group
3309.53(st)	-OH	3311.19(st)	-OH	2953.30(st)	sp ³ C-H
2346.91(st)	C≡N	2923.35(st)	sp ³ C-H	2848.46(st)	sp ³ C-H
1709.41(st)	C=O	2852.80(st)	sp ³ C-H	2320.17(st)	C≡N
1596.75(st)	Ar-C=C	2348.06(st)	C≡N	2201.29(st)	C≡N
1382.53(b)	-CH ₃	1728.76(st)	C=O	1735.01(st)	C=O
1303.07(b)	-CH ₃	1517.80(st)	Ar-C=C	1462.71(st)	Ar-C=C
1262.35(b)	C-O	1203.66(b)	C-O	1388.89(b)	-CH ₃
1114.23(b)	C-N	1029.88(b)	C-O	1365.53(b)	-CH ₃
				1229.11(st)	O=C-O-C
				951.34(st)	C=C-H
				923.12(st)	C=C-H
				728.85(oop)	C-Br
				719.41(oop)	C-Br

st = stretching; b = bending; oop = out of the plane bending.

The FT-IR of the dried *Carica papaya* seed aqueous extract is displayed in Figure 4. The presence of the -OH group, indicated by the broad absorption at 3271.52 cm⁻¹, was shown in the FT-IR analysis of the aqueous extract of dried *Carica papaya* seeds. There is evidence of aliphatic C-H stretching at wavenumbers 2921.97 and 2851.75 cm⁻¹. Possible nitrile (-C≡N) or cumulative bonds have been shown by stretching frequencies of 2191.85 and 2162.23 cm⁻¹. The characteristic C=C or aromatic ring stretching can be found at 1619.53 cm⁻¹. The absorptions at 1559.88 and 1405.11 cm⁻¹ further indicate the presence of N-H bending or aromatic C=C stretching. Absorption at 1071.51 cm⁻¹ indicates the vibration of C-O stretching (alcohols or ethers). In summary, this spectrum (Figure 4) suggests a mixture of aliphatic hydrocarbons, aromatic systems, and oxygenated functional groups [25].

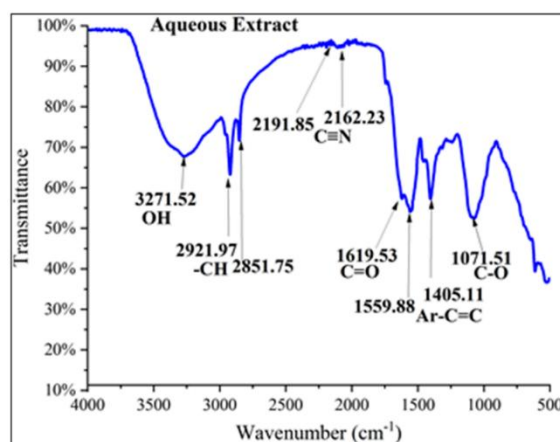


Figure 4. FT-IR spectrum of the aqueous extract of *Carica papaya* seeds, indicating absorption and the corresponding functional groups.

As shown in Figure 5, the O-H/N-H stretching (alcohols, phenols, amines) is present at 3271.52 cm⁻¹ in the FT-IR spectrum of the EtOH extract of dried *Carica papaya* seeds. The wavenumbers at 2921.97 and 2851.75 cm⁻¹ also suggest the presence of aliphatic C-H stretching. Nitriles (C≡N) or cumulative bonds are observed at 2191.17 cm⁻¹. The presence of C=O stretching (carbonyl, ester, or ketone) is indicated at 1711.74 cm⁻¹. Absorption at 1463.41 cm⁻¹ indicates the presence of an aromatic C=C or CH₃. Absorption at 1043.14 cm⁻¹ suggests

the presence of C–O stretching. The out-of-the-field absorption in the fingerprint region at 717.87 cm^{-1} indicates the presence of aromatic C–H bending. Thus, it can be deduced that the EtOH extract exhibited a broader diversity of functional groups compared to Aqua and Hex, including strong carbonyl features [25].

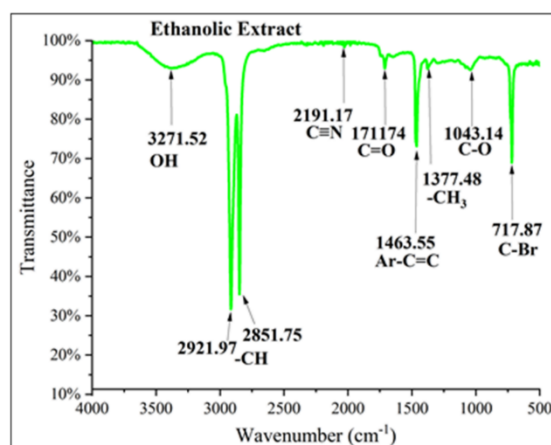


Figure 5. FT-IR spectrum of EtOH extract of *Carica papaya* seeds indicating absorption and the corresponding functional groups.

The FT-IR spectrum of the dried *Carica papaya* seeds' Hex extract is displayed in Figure 6. The aliphatic C–H stretching with sharp edges was observed at 2851.19 cm^{-1} and 2920.10 cm^{-1} . The presence of a nitrile or triple bond is indicated by absorption at 2263.67 cm^{-1} . The absorption at 1744.47 cm^{-1} indicates the presence of a strong carbonyl absorption (esters, carbonyl, or ketone), which is one of the common characteristics of compounds containing flavonoid groups. The absorptions at 1462.32 cm^{-1} and 1376.99 cm^{-1} suggest that an asymmetric $-\text{CH}_3$ group is present. The wavenumbers at 1236.41 cm^{-1} and 1161.06 cm^{-1} show that C–O stretching (esters, ethers) is indicated. Finally, aromatic/alkene bending is observed at 964.98 cm^{-1} and 720.24 cm^{-1} [25].

In summary, the spectrum points to fatty acid esters, lipids, and terpenoid-like compounds as the dominant constituents.

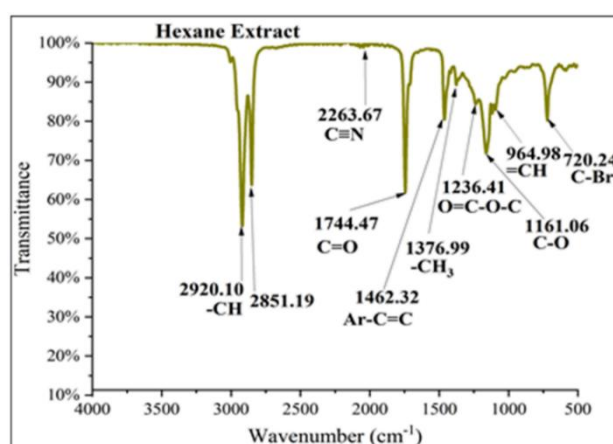


Figure 6. FT-IR spectrum of the Hex extract of *Carica papaya* seeds, indicating absorption and the corresponding functional groups.

3.5. Antimicrobial evaluation.

The search for alternative medicine to combat infections caused by drug-resistant bacteria has gained significant momentum [26]. Plant extracts have emerged as potential

sources of new antimicrobial agents due to their bioactive compounds [27]. Our study explores the antimicrobial potential of crude extracts of *Carica papaya* seed against clinically relevant bacteria. The results indicate that the EtOH extract of *C. papaya* seed exhibited the highest inhibitory activity, followed by the Aqua extract, with the Hex extract showing the least activity.

The estimated mean inhibition (mm) effect is based on the organisms and the crude extracts. The test was conducted in triplicate. Our data suggest that Tazobactam/piperacillin (10.00 µg/disc), the positive control, was generally the most potent extract, followed by the EtOH and Aqua extracts. The Hex extract had the least effect across all organisms. Interestingly, the EtOH extract demonstrated a higher efficacy of 16.0 ± 1.00 mm against *Klebsiella pneumoniae* than the Tazobactam/piperacillin, as shown in Table 3.

Table 3. The mean inhibitory effect of crude extracts of *Carica papaya* seed against relevant bacterial strains.

Bacterial strains	Zone of inhibition (mm)			Tazobactam/piperacillin (10.00 µg/disc)
	Aqua extract (0.10 mL)	EtOH extract (0.10 mL)	Hex extract (0.10 mL)	
<i>Citrobacter freundii</i>	12.0±1.00	15.0±1.00	7.5±2.10	15.5±1.00
<i>Klebsiella pneumoniae</i>	8.5±1.50	16.0±1.00	6.5±1.50	16.0±0.20
<i>Enterobacter cloacae</i>	8.0±1.00	13.0±1.50	6.5±1.00	14.0±0.00
<i>Escherichia coli</i>	8.5±1.30	13.5±0.60	6.5±3.10	13.5±1.00
<i>Staphylococcus aureus</i>	13.5±1.70	16.±0.58	9.5±2.60	17.5±0.00

Staphylococcus aureus is a gram-positive bacterium recognized for its ability to cause infections, ranging from minor skin infections to severe systemic diseases such as pneumonia, sepsis, and endocarditis. In this study, all crude extracts of *C. papaya* seed showed substantial inhibition against *S. aureus*. The EtOH extract had the highest inhibitory zone of 16.0 ± 0.58 mm, followed by the Aqua extract (13.5 ± 1.70 mm) and the Hex extract (9.5 ± 2.60 mm). These findings are supported by previous studies [29] in which Al-Rajhi et al. reported an EtOH extract of *Thevetia peruviana* with a zone of inhibition of 15.2 ± 1.30 mm against *S. aureus*. Similarly, Singh et al. reported a zone of inhibition of 14.5 ± 0.80 mm for the EtOH extract, further confirming the efficacy of this extract.

Contrarily, *Enterobacter cloacae* showed the least inhibitory zone against all crude extracts. The EtOH extract had an inhibitory zone of 13.0 ± 1.50 mm, while the Hex extract had the smallest zone of 6.5 ± 1.00 mm. These findings are comparable to those reported by some researchers [28], who reported that an EtOH extract of *C. papaya* seed had a zone of inhibition of 12.0 ± 1.00 mm against *E. cloacae*. The lower efficacy of the Hex extract against *E. cloacae* is also supported by Khan et al. [29], who reported a zone of inhibition of 7.0 ± 0.50 mm.

Escherichia coli is a Gram-negative bacterium that is a normal inhabitant of the human intestinal tract. While many strains of *E. coli* are harmless, some pathogenic strains can cause a range of infections, including meningitis in newborns, urinary tract infections (UTIs), sepsis, and gastroenteritis. In the current study, the EtOH extract had a zone of inhibition of 13.5 ± 0.60 mm against *E. coli*, which is consistent with the findings of Alam et al. [30], who reported an inhibitory zone of 13.0 ± 0.80 mm. The Aqua extract had a zone of inhibition of 8.5 ± 1.30 mm.

Furthermore, *Klebsiella pneumoniae* is a gram-negative bacterium commonly found in the human intestinal tract and soil. It is known for its ability to cause severe infections, particularly in immunocompromised individuals and hospital settings. *Citrobacter freundii* is another gram-negative bacterium that can cause infections, especially in immunocompromised

individuals. In our study, both *K. pneumoniae* and *C. freundii* were inhibited by the EtOH extracts with zones of 16.0 ± 1.00 mm and 15.0 ± 1.00 mm, respectively. These results are consistent with the findings of Zhang et al., who stated a zone of inhibition of 15.5 ± 0.80 mm [31].

3.5.1. Interpretation of Table 3 through the lens of membrane-disruption by alkaloids.

The EtOH extract shows the highest zone (among the three extracts) across all strains (e.g., 16.00 mm vs 8.50 mm for *Klebsiella*). This suggests that the EtOH extract likely has higher amounts (or more bioavailable) alkaloid (or other lipophilic) compounds that effectively disrupt membranes. The Hex extract shows the lowest zones across all strains (6.50-9.50 mm). Possibly the Hex extract yields very hydrophobic compounds that may not diffuse well in the agar/disc system, or the compounds are less bioactive (or the concentration is lower). For Gram-negative strains (*Citrobacter*, *Klebsiella*, *Enterobacter*, *E. coli*), the zones tend to be lower than for *Staphylococcus aureus* (a Gram-positive).

For example, *S. aureus* shows 13.50 mm (Aqua) vs *K. pneumoniae* 8.50 mm (Aqua). This is consistent with the fact that Gram-negatives have an outer membrane barrier, which makes membrane disruption more challenging — but if alkaloids act via outer membrane destabilization, this could explain why some extracts have relatively lower effect. Because membrane disruption is one mechanism (among others like DNA/protein inhibition), the zone of inhibition reflects a composite of mechanisms. But the relative differences between extracts can be interpreted: EtOH extract is likely better at permeabilizing membranes → higher zones. The control antibiotic (tazobactam/piperacillin) gives higher zones for most strains (14.00-17.50 mm) than the extracts, which is expected since it's a prepared antibiotic at a known effective dose. The fact that the EtOH extract comes close (16.00 mm for *K. pneumoniae*) suggests the extract's potency is reasonably good, and perhaps the alkaloid-mediated membrane disruption is contributing significantly.

3.5.2. Mechanism of membrane-disruption by alkaloids using Table 3.

The extracts likely exert their antibacterial activity at least in part through alkaloid-mediated membrane disruption. Plant alkaloids can associate with bacterial membranes, destabilize lipid bilayers or outer membranes (in Gram-negatives), increase permeability, induce leakage of intracellular ions and metabolites, disrupt the proton motive force, and ultimately cause bacterial death or growth inhibition [32,33]. In our disc-diffusion assay, the EtOH extract showed the largest zones of inhibition across all strains (e.g., 16.00 ± 1.00 mm for *K. pneumoniae*), which may reflect greater extraction of membrane-active alkaloids. The comparatively lower zones of the Hex extract (e.g., 6.50 ± 1.50 mm for *K. pneumoniae*) may reflect either lower extractable active alkaloid concentration or poorer agar diffusion of the hydrophobic constituents. Moreover, the Gram-positive strain *S. aureus* showed slightly higher susceptibility (16.00 ± 0.60 mm with EtOH extract) than most Gram-negatives, consistent with the absence of an outer membrane barrier and increased vulnerability to membrane-disrupting compounds. In a nutshell, these data are consistent with membrane disruption being a major contributor to the antibacterial effect of the extracts, although other mechanisms (e.g., DNA/protein synthesis inhibition) cannot be excluded.

In summary, the Aqua extract showed moderate inhibition against both Gram-positive and Gram-negative bacteria. Activity was notable against *S. aureus* and *C. freundii* but was

overall weaker than the EtOH extract and the standard drug. The EtOH extract showed the strongest antimicrobial effects among the tested extracts, nearly comparable to the standard control. The results suggest ethanol is the most effective solvent for extracting antimicrobial phytochemicals, consistent with the presence of alkaloids. The Hex extract exhibited the weakest antimicrobial effects, with inhibition zones significantly smaller than EtOH and the positive control. This suggests hexane-soluble compounds in papaya seeds may have limited antibacterial potential.

4. Conclusions

This study shows that the bioactivity and phytochemical yield of *Carica papaya* seed extracts are influenced by solvent choice. Aqueous extraction provided higher phenolic and flavonoid contents, whereas EtOH extracts showed the broadest antimicrobial efficacy, particularly against *Staphylococcus aureus* and *Klebsiella pneumoniae*, with activity close to that of the standard drug. The Hex extracts yielded the least phytochemicals and the weakest antimicrobial performance. Spectroscopic analyses further confirmed the presence of diverse functional groups, supporting the observed bioactivity. These findings highlight the EtOH extract of *Carica papaya* seeds as the most active amongst the three extracts, but it requires further isolation, characterization of bioactive constituents, and cytotoxic evaluation.

Author Contributions

Conceptualization, I.Y.; methodology, B.D., B.A.O., and I.W.; software, I.Y., B.A.O., and I.W.; validation, I.Y.; formal analysis, B.D., B.A.O., and I.W.; investigation, B.D., B.A.O., and I.W.; resources, I.Y.; data curation, I.Y., B.D., B.A.O., and I.W.; writing—original draft preparation, I.Y. and I.W.; writing—review and editing, I.Y.; visualization, I.Y.; supervision, I.Y.; project administration, I.Y. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

Funding

This research received no external funding.

Acknowledgments

Declared none.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Serwecińska, L. Antimicrobials and Antibiotic-Resistant Bacteria: A Risk to the Environment and to Public Health. *Water* **2020**, *12*, 3313, <https://doi.org/10.3390/w12123313>.
2. Jeon, Y.A.; Chung, S.W.; Kim, S.C.; Lee, Y.J. Comprehensive Assessment of Antioxidant and Anti-Inflammatory Properties of Papaya Extracts. *Foods* **2022**, *11*, 3211, <https://doi.org/10.3390/foods11203211>.
3. Bayode, A.A.; Anthony, E.T.; Ore, O.T.; Alfred, M.O.; Koko, D.T.; Unuabonah, E.I.; Helmreich, B.; Omorogie, M.O. A review on the versatility of *Carica papaya* seed: an agro-genic waste for the removal of organic, inorganic and microbial contaminants in water. *J. Chem. Technol. Biotechnol.* **2023**, *98*, 2095–2109, <https://doi.org/10.1002/jctb.7415>.
4. Rahmasari, R.; Hertamawati, R.T.; Rafli, A.K.; Nugraha, B.A. Addition of papaya (*Carica papaya* L.) seed meal on quail production performance. *IOP Conf. Ser.: Earth Environ. Sci.* **2021**, *672*, 012040, <https://doi.org/10.1088/1755-1315/672/1/012040>.
5. Memudu, A.E.; Oluwole, T.J. The contraceptive potential of *Carica papaya* seed on oestrus cycle, progesterone, and histomorphology of the Utero-ovarian tissue of adult wistar rats. *JBRA Assist. Reprod.* **2021**, *25*, 34–43.
6. Cadena-Álava, A.P.; Cevallos-Cedeño, R.E.; García-Muentes, S.A. Evaluation of the coagulant property of *Carica papaya* seeds in surface water treatment. *Rev. Mex. Ing. Quim.* **2024**, *23*, 1A24301, <https://doi.org/10.24275/rmiq/1A24301>.
7. Iwuagwu, C.C.; Eze, P.A. Phytotoxic effect of *Carica papaya* extract on seedborne fungus of African Yam Bean (*Sphenostylis stenocarpa* Hochst ex. A. Rich. Harms) seeds. *J. Agric. Sci. Pract.* **2024**, *9*, 73–80, <https://doi.org/10.31248/JASP2024.466>.
8. Cano, F.J.; Sánchez-Albores, R.; Ashok, A.; Escorcia-García, J.; Cruz-Salomón, A.; Reyes-Vallejo, O.; Sebastian, P.J.; Velumani, S. *Carica papaya* seed-derived functionalized biochar: an environmentally friendly and efficient alternative for dye adsorption. *J. Mater. Sci. Mater. Electron.* **2025**, *36*, 663, <https://doi.org/10.1007/s10854-025-14725-y>.
9. Pandey, A.; Bahadur, V. Effects of Different Plant Growth Regulators on Seed Germination, Seedling Growth and Establishment of Papaya (*Carica papaya*) Cv. Pusa Nanha. *J. Adv. Biol. Biotechnol.* **2024**, *27*, 717–724, <https://doi.org/10.9734/jabb/2024/v27i6932>.
10. Kumawat, M.; Topno, S.E. Effect of Different Growing Media on Seed Germination, Seedling Growth and Establishment of Papaya (*Carica papaya* L.) cv. Red Lady. *J. Adv. Biol. Biotechnol.* **2024**, *27*, 962–967, <https://doi.org/10.9734/jabb/2024/v27i5858>.
11. Aboryia, M.S.; Al-Saoud, A.M.A.; El-Boraie, E.-S.A.; El-Gawad, H.G.A.; Ismail, S.; Alkafafy, M.; Aljuaid, B.S.; Abu-Ziada, L.M. Seed germination, seedling growth performance, genetic stability and biochemical responses of papaya (*Carica papaya* L.) upon pre-sowing seed treatments. *Folia Hortic.* **2025**, *36*, 533–558, <https://doi.org/10.2478/fhort-2024-0036>.
12. Raju, S.K.; Savitha, H.S.; Kumar, A.; Yadav, S.; Rai, M.; Yadav, A.K.; Mukherjee, R.K.; Mahapatra, S. Phytochemical And Pharmacological Assesment Of *Carica papaya* Seed Extract Revealed That It Shows Neuroprotection Reveses Oxidative Damage And Neutralized Free Radical. *Rev. Electron. Vet.* **2024**, *25*, 1461–1468.
13. Ouedraogo, A.; Nikiema, P.A.; Nikiema, M.E.M.; Yameogo, J.G.; Sourabie, B.P.; Bassave, B.R.H.; Nikiema, O.; Goumbri, F.W.B.; Barro, N. Phytochemicals properties and antimicrobial activities of *Carica papaya* and *Balanites aegyptiaca* seeds aqueous infusion and decoction extracts. *J. Drug Deliv. Ther.* **2024**, *14*, 46–52, <https://doi.org/10.22270/jddt.v14i2.6393>.
14. Okeke-Nwolisa, B.C.; Benedict, J.C. Assessment of antimicrobial potentials and minimum inhibitory concentration (MIC) of extracts of dry *Carica papaya* seeds on multidrug resistant (MDR) clinical microbial isolates. *J. Biomed. Invest.* **2024**, *12*, 113–139.
15. Oboh, E.; Ashefo, D.; Oleghe, P.; Ojei, O. Investigating the Astonishing Antimicrobial Potential of Papaya seeds against *Salmonella* spp and *Candida albicans*. *Int. J. Tradit. Complement. Med. Res.* **2023**, *4*, 26–30, <https://doi.org/10.53811/ijtcmr.1249215>.

16. Yahaya, I.; Gyasi, S.F.; Hamadu, A. Phytochemical screening of bioactive compounds and antimicrobial activity of different extracts of *Syzygium samarangense* leaves. *Pharmacol. Res. - Nat. Prod.* **2024**, *4*, 100059, <https://doi.org/10.1016/j.prenap.2024.100059>.
17. Goriainov, S.; Orlova, S.; Nikitina, E.; Vandshev, V.; Ivlev, V.; Esparza, C.; Vasil'ev, V.; Platonov, E.; Sheremeta, A.; Kalabin, G. Study of the Chemical Composition of *Carica papaya* L. Seed Oils of Various Geographic Origins. *Horticulturae* **2023**, *9*, 1227, <https://doi.org/10.3390/horticulturae9111227>.
18. Konmy, B.; Bossikponsi, M.G.; Dansou, C.C.; Arigbo, K.B.; Houmenou, G.T.; Adoho, C.A.; Azando, E.V.B.; Doko Allou, S.-y.; Olounladé, P.A. Evaluation of the effect of *Carica papaya* seed on the growth performance of fattening rabbits. *Trop. Anim. Health Prod.* **2024**, *56*, 134, <https://doi.org/10.1007/s11250-024-03969-1>.
19. Johari, M.A.; Khong, H.Y. Total Phenolic Content and Antioxidant and Antibacterial Activities of *Pereskia bleo*. *Adv. Pharmacol. Pharm. Sci.* **2019**, *2019*, 7428593, <https://doi.org/10.1155/2019/7428593>.
20. Singleton, V.L.; Orthofer, R.; Lamuela-Raventós, R.M. [14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. In *Methods in Enzymology*; Academic Press: **1999**; Volume 299, pp. 152-178, [https://doi.org/10.1016/S0076-6879\(99\)99017-1](https://doi.org/10.1016/S0076-6879(99)99017-1).
21. Pękal, A.; Pyrzyńska, K. Evaluation of Aluminium Complexation Reaction for Flavonoid Content Assay. *Food Anal. Methods* **2014**, *7*, 1776–1782, <https://doi.org/10.1007/s12161-014-9814-X>.
22. Bashyam, R.; Thekkumalai, M.; Sivanandham, V. Evaluation of Phytoconstituents of *Bryonopsis laciniosa* fruit by UV-Visible Spectroscopy and FTIR analysis. *Pharmacogn. J.* **2015**, *7*, 165–170.
23. Satish, A.; Farha, S.S.; Urooj, A. Quantification of flavonoids by UPLC-MS and its antibacterial activity from *Brassica oleracea* var. *Capitata* L. *GSC Biol. Pharm. Sci.* **2018**, *5*, 109-114, <https://doi.org/10.30574/gscbps.2018.5.1.0105>.
24. Pavia, D.L.; Lampman, G.M.; Kriz, G.S.; Vyvyan, J.R. Chapter 2 Infrared Spectroscopy. In *Introduction to Spectroscopy*, 5th Edition; Pavia, D.L., Lampman, G.M., Kriz, G.S., Vyvyan, J.R., Eds.; Cengage Learning: Stamford, CT, USA, **2015**; pp. 14-106.
25. Dhama, K.; Tiwari, R.; Chakraborty, S.; Saminathan, M.; Kumar, A.; Karthik, K.; Wani, M.Y.; Amarpal; Singh, S.V.; Rahal, A. Evidence Based Antibacterial Potentials of Medicinal Plants and Herbs Countering Bacterial Pathogens Especially in the Era of Emerging Drug Resistance: An Integrated Update. *Int. J. Pharmacol.* **2014**, *10*, 1-43, <https://doi.org/10.3923/ijp.2014.1.43>.
26. Taylor, P.W. Alternative natural sources for a new generation of antibacterial agents. *Int. J. Antimicrob. Agents* **2013**, *42*, 195–201, <https://doi.org/10.1016/j.ijantimicag.2013.05.004>.
27. Al-Rajhi, A.M.H.; Yahya, R.; Abdelghany, T.M.; Fareid, M.A.; Mohamed, A.M.; Amin, B.H.; Masrahi, A.S. Anticancer, Anticoagulant, Antioxidant and Antimicrobial Activities of *Thevetia peruviana* Latex with Molecular Docking of Antimicrobial and Anticancer Activities. *Molecules* **2022**, *27*, 3165, <https://doi.org/10.3390/molecules27103165>.
28. Barnabas, F.O.; Wuyep, P.A.; Obeta, U.M.; Mantu, E.C.; Ukpa, S.P.; Nduke, S.J.; Ashien, U.U. Phytochemical analysis and antimicrobial effects of *Carica papaya* leaf extracts on urinary tract infections. *J. Bio. Innov.* **2022**, *11*, 350–374.
29. Khan, M.; Khan, T.; Wahab, S.; Aasim, M.; Sherazi, T.A.; Zahoor, M.; Yun, S.-I. Solvent based fractional biosynthesis, phytochemical analysis, and biological activity of silver nanoparticles obtained from the extract of *Salvia moorcroftiana*. *PLOS ONE* **2023**, *18*, e0287080, <https://doi.org/10.1371/journal.pone.0287080>.
30. Alam, F.; Khan, S.H.A.; Asad, M.H.H.B. Phytochemical, antimicrobial, antioxidant and enzyme inhibitory potential of medicinal plant *Dryopteris ramosa* (Hope) C. Chr. *BMC Complement. Med. Ther.* **2021**, *21*, 197, <https://doi.org/10.1186/s12906-021-03370-7>.
31. Zhang, S.; Li, P.; Wei, Z.; Cheng, Y.; Liu, J.; Yang, Y.; Wang, Y.; Mu, Z. *Cyperus* (*Cyperus esculentus* L.): A Review of Its Compositions, Medical Efficacy, Antibacterial Activity and Allelopathic Potentials. *Plants* **2022**, *11*, 1127, <https://doi.org/10.3390/plants11091127>.
32. Yan, Y.; Hou, P.; Duan, F.; Niu, L.; Dai, T.; Wang, K.; Zhao, M.; Li, S.; Zhou, W. Improving Photosynthesis to Increase Grain Yield Potential: An Analysis of Maize Hybrids Released in Different Years in China. *Photosynth. Res.* **2021**, *150*, 295–311. <https://doi.org/10.1007/s11120-021-00847-x>.
33. Othman, L.; Sleiman, A.; Abdel-Massih, R. M. Antimicrobial Activity of Polyphenols and Alkaloids in Middle Eastern Plants. *Front. Microbiol.* **2019**, *10*, 911. <https://doi.org/10.3389/fmicb.2019.00911>.

Publisher's Note & Disclaimer

The statements, opinions, and data presented in this publication are solely those of the individual author(s) and contributor(s) and do not necessarily reflect the views of the publisher and/or the editor(s). The publisher and/or the editor(s) disclaim any responsibility for the accuracy, completeness, or reliability of the content. Neither the publisher nor the editor(s) assume any legal liability for any errors, omissions, or consequences arising from the use of the information presented in this publication. Furthermore, the publisher and/or the editor(s) disclaim any liability for any injury, damage, or loss to persons or property that may result from the use of any ideas, methods, instructions, or products mentioned in the content. Readers are encouraged to independently verify any information before relying on it, and the publisher assumes no responsibility for any consequences arising from the use of materials contained in this publication.

Supplementary materials

Materials and Methods

Phytochemical Screenings

Phytochemical screening of the dried seeds of *Carica papaya* was done in water, ethanol, and hexane and their extracts were named aqueous, ethanolic, and hexane extracts, respectively. The phytoconstituents investigated were the alkaloids, flavonoids, phenols, saponins, and tannins were found in *Carica papaya* dried seeds.

Screening for alkaloids

2 mL of each extract was placed in three different test tubes. 3 mL of Wagner's reagent was added to each test tube and the results were recorded.

Screening for flavonoids

2 mL of each extract was placed in three different test tubes. In each of the test tubes, 2 to 3 drops of 2% NaOH solution were added. Later, a few drops of dilute HCl were also added to the solution containing the extract and the 2% NaOH and the extract.

Screening for phenols

2 mL of each extract was placed in three different test tubes. 5 % Ferric chloride solution was added and the results were recorded.

Screening for saponins

2 mL of each extract was placed in three different test tubes. 3 mL of distilled water was added in each test tube followed by a 5-10 minute shake. The results of the test were recorded.

Screening for tannins

2 mL of each extract was placed in three different test tubes. 5 % Ferric chloride solution was added and the results were recorded.

Results of the phytochemical test

Results for the screening for Alkaloids

Wagner's reagent was added to the beige original solution in the extract's Aqua extract test, indicating the absence of alkaloids as the solution turned orange. Wagner's reagent was added to the EtOH extract, causing the original solution's lemon colour to turn reddish, signifying the presence of alkaloids. But after adding Wagner's reagent, the Hex extract's original gold colour changed to wine-red, indicating that there were no alkaloids present, as seen in **Figure S1** of the Supplementary data.

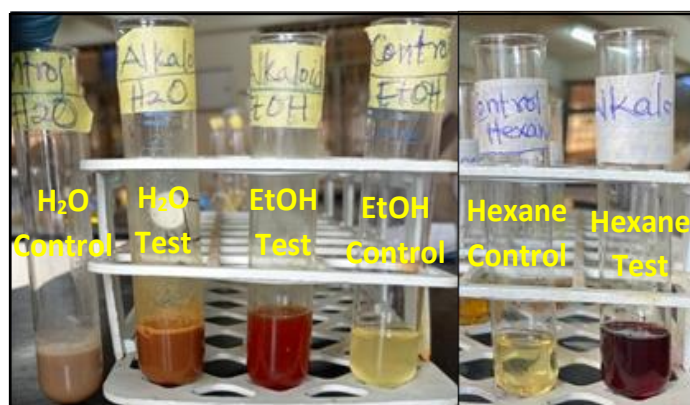


Figure S1. Results for the Test for Alkaloids.

Results for the screening for Flavonoids

The beige colour of the original solution turned brown upon adding 10% NaOH to the mixture containing the Aqua extract. The brown colour of the mixture containing the 10% NaOH and the Aqua extract changed to a light brown colour after diluted HCl was added. This suggested that flavonoids were not present. 10% NaOH was added to the EtOH extract solution, but the solution's original lemon colour persisted. Nevertheless, after adding diluted HCl to the mixture of 10% NaOH and the EtOH extract, the lemon colour remained intact. This demonstrated the absence of flavonoids. After adding 10% NaOH, the original Hex extract solution's gold colour turned to lemon. The mixture of 10% NaOH, Hex extract, and lemon colour turned white with the lemon colour suspended on top when diluted HCl was added. This verified that flavonoids were not present. **Figure S2** of the Supplementary data shows the outcomes.

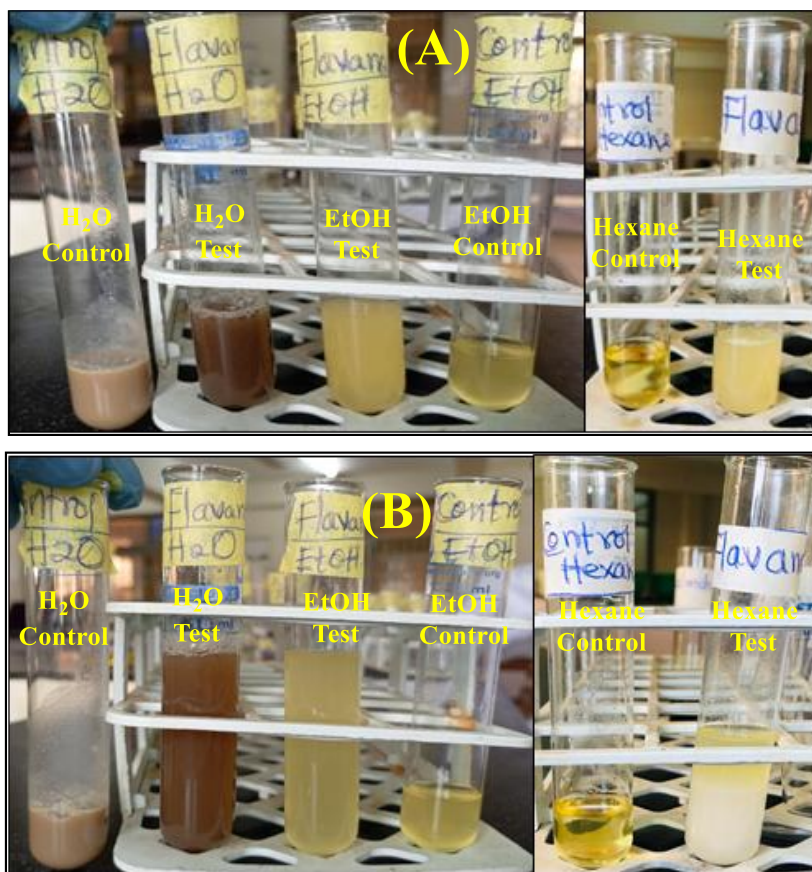


Figure S2. Results of screening for Flavonoids: (A) after adding NaOH; and (B) after adding HCl.

Results for the screening for Phenols

After adding 5% ferric chloride solution to the Aqua extracts, the original solution's beige colour changed to brown, indicating the absence of phenols. But after adding 5% ferric chloride, the EtOH extract solution's lemon colour turned to orange, suggesting that phenols were not present in the mixture. After adding 5% ferric chloride solution to the Hex extract solution, the solution's gold colour turned yellow (**Figure S3**). This demonstrated the absence of phenols.

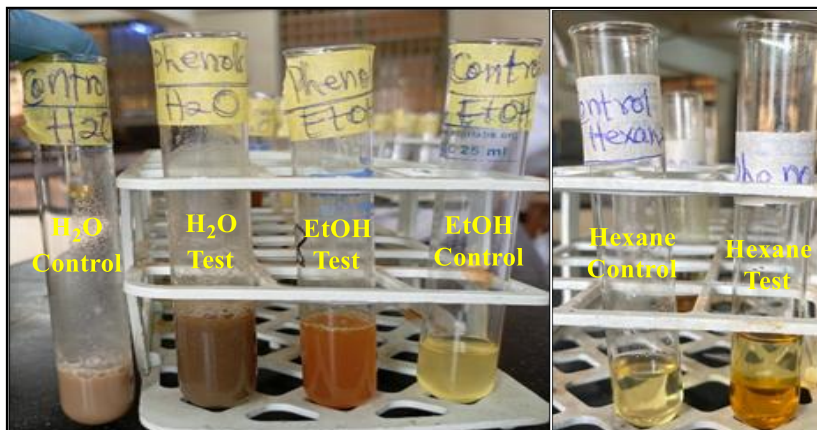


Figure S3. Results for the Test for Phenols.

Results for the screening for Saponins

The saponin test is a "foam" test, which suggests that the extract's original solution would remain colourless. The appearance of the "foam" signifies the test's confirmation. A foam formed in all three extract solutions (Aqua, EtOH, and Hex) following the addition of deionized water and five minutes of agitation (**Figure S4**). The foam persisted in all three extracts for roughly fifteen minutes, indicating the presence of saponins in each (Aqua, EtOH, and Hex).

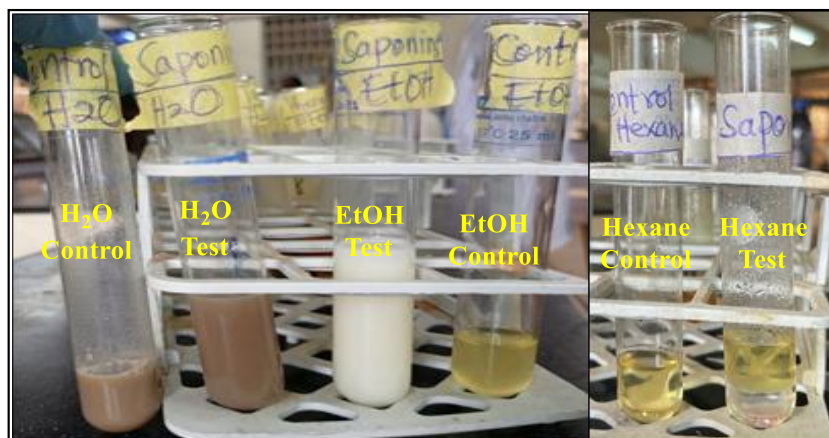


Figure S4. Results for the Test for Saponins.

Results for the screening for Tannins

When the 5% ferric chloride solution was used to test for tannins in the Aqua extract, the orange colour of the original solution changed to a brownish colour, indicating that there were no tannins present. With the EtOH extract solution, the brownish colour of the original solution turned green upon the addition of 5% ferric chloride solution, indicating the presence

of tannins. When 5% ferric chloride solution was added to the original Hex extract, the honey colour of the extract changed to a yellowish colour, indicating the absence of tannins (**Figure S5**).

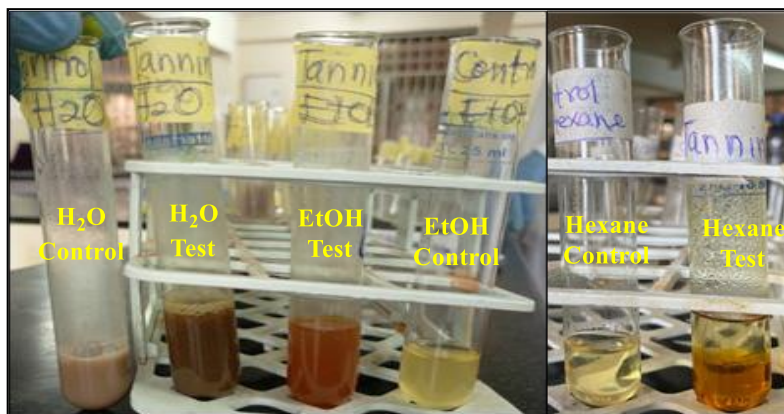


Figure S5. Results for the Test for Tannins.

Results of the total phenolic content (tpc)

Table S1. Plotted Values for TPC Determination.

Concentration (ppm)	Absorbance (at 760 nm)
0	0
100	2.301
120	2.741
140	3.212
160	3.611
180	3.998

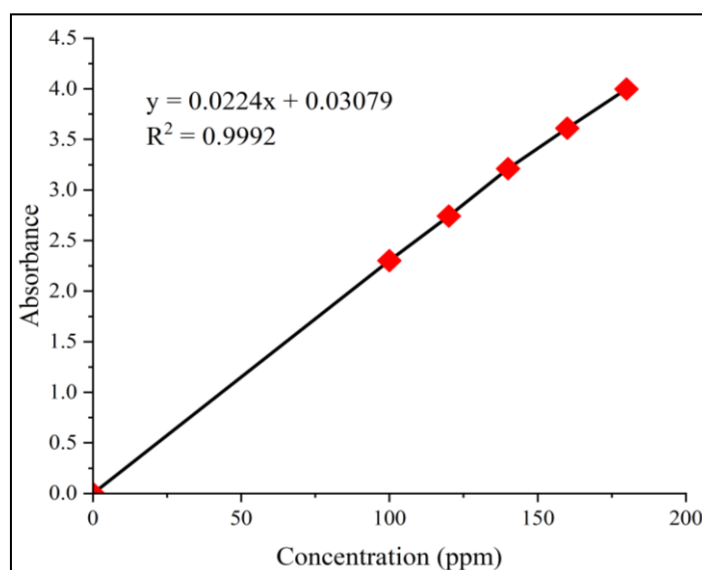


Figure S6. Total Phenolic Content determination calibration curve using ascorbic acid as the standard.

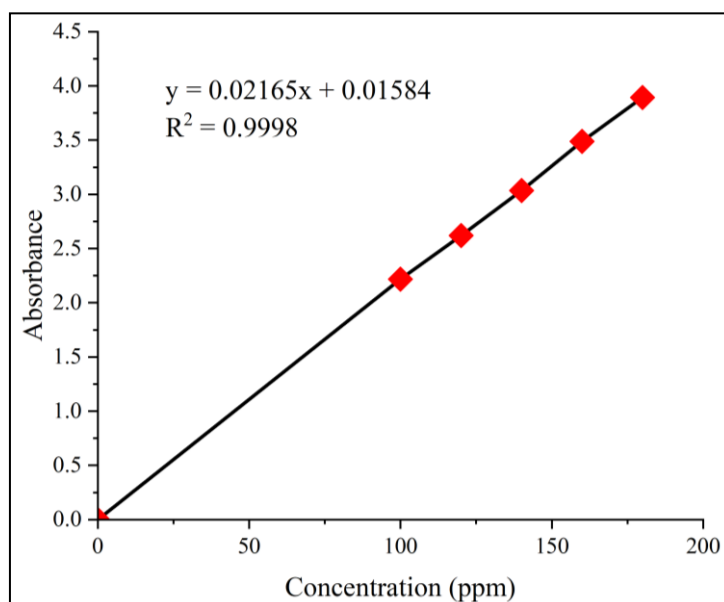


Figure S7. Total Flavonoid Content determination calibration curve using quercetin as the standard.

Results of the total flavonoid content (tfc)

Table S2. Plotted Values for TFC Determination.

Concentration (ppm)	Absorbance (at 410 nm)
0	0
100	2.217
120	2.619
140	3.036
160	3.489
180	3.892