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Antimicrobial activity and potential of aqueous and ethanolic extracts of unripe *Carica papaya* linn. (Papaya) fruit

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Abstract
Papaya (*Carica papaya Linn.*) is valued in tropical and subtropical regions for its nutraceutical and medicinal properties. This study evaluated the antibacterial activity of aqueous and ethanolic extracts of unripe papaya fruit against microbial isolates. Extracts were obtained via maceration, and their antimicrobial activities were assessed using agar dilution and agar well diffusion techniques. Gas chromatography-mass spectrometry (GC-MS) was employed to identify bioactive compounds in the extracts. The extracts were tested against *Salmonella typhi*, *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Candida albicans*. Both extracts demonstrated significant antimicrobial activity, with the aqueous extract showing the highest efficacy. However, *S. aureus* was resistant to the ethanol extract, while *S. typhi* was resistant to the aqueous extract. The optimal concentration for inhibition was 200 mg/mL for both extracts. The aqueous extract exhibited minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of 12.5 mg/mL against *S. aureus*, *B. subtilis*, *K. pneumoniae*, and *C. albicans*. The ethanol extract had MICs of 50 mg/mL for *B. subtilis*, 25 mg/mL for *S. typhi*, and 12.5 mg/mL for *K. pneumoniae* and *C. albicans*. GC-MS analysis identified key bioactive compounds, including hexamethylcyclotrisiloxane, dodecamethylcyclohexasiloxane, and dihydroxyphenylglycol, contributing to the extracts' antimicrobial properties. These findings support the traditional use of *C. papaya* as a natural antimicrobial agent and highlight its potential as a complementary treatment for antimicrobial-resistant infections. Further studies on its pharmacodynamics are recommended.

Keywords: Antimicrobial, gas chromatography-mass spectrometry, bioactive, agar dilution, *Carica papaya*

Introduction

The papaya (*Carica papaya* Linn.) is a widely grown and significant fruit tree in tropical and subtropical regions. Across the globe, different parts of the plant are used to treat various animal and human ailments. In Cuba, its latex is used to treat conditions such as ringworm, malignant growths, and psoriasis. Numerous pharmacological properties of *C. papaya* include its hepatoprotective, hypoglycemic, anthelmintic, antifungal, anti-inflammatory, anticancer, and antioxi-dant capabilities [1]. The discovery of new drugs is urgently needed to treat and manage antimicrobial resistance (AMR) infections, as AMR is growing at a faster rate than the development of traditional antibiotics. Traditional medical practitioners have long been aware of the therapeutic properties of plants [2]. Plants are capable of producing an endless variety of secondary metabolites, the majority of which are aromatic compounds such as alkaloids, glycosides, terpe-noids, saponins, steroids, flavonoids, tannins, quinones, and coumarins [3].
Clinical isolates are affected by these metabolites; for example, coumarins are active against *Staphylococcus aureus* but inactive against gram-negative bacteria [4]. Worldwide, and particularly in emerging nations, AMR is considered a threat to public health systems. With microbial infections emerging, especially those resistant to frequently used antibiotics, the future of medical care concerning the use of known antibiotics has become unpredictable [5]. Herbal medicines, derived from plants or plant extracts containing therapeutic substances, offer a promising alternative [6].
While papaya has been traditionally used for its antimicrobial potential, scientific studies are essential to confirm its potency, particularly against clinically relevant pathogens. The antimicrobial activity of various papaya extracts, including aqueous and ethanol extracts, remains underexplored, particularly concerning their effectiveness against antibiotic-resistant strains. This study aims to address this gap by evaluating the antibacterial proper-ties of unripe papaya fruit extracts against select microbial isolates, potentially validating its role as a natural antimicrobial agent in clinical settings.

Materials and Method

Plant collection and identification: The *Cassia. papaya L*inn. fruit was collected and sent to the herbarium for identification and authentication. The fruit was verified at the Department of Pharmacognosy's Medicinal Plant Herbarium, Faculty of Pharmacy, Dr. Bhimrao Ambedkar, Agra, with voucher number FPI2474. It was then washed, sliced with its peel, dried, and ground into a powdery form.

Extraction of active ingredients: The dried fruit was ground into a fine powder using a mechanical grinder. A 150 g quantity was macerated in 500 mL of ethanol, and 313 g of the dried fruit powder was macerated in 4,280 mL of water for 48 hours in conical flasks, respectively. The mouths of the conical flasks were covered with aluminum corks to prevent solvent evaporation. After 48 hours, the mixtures were properly filtered using What man filter paper (Cat. No. 1001 155) to separate the marc from the extract,

which was collected in a beaker. The beakers were then transferred to a water bath and heated at 40°C until all the solvent evaporated, leaving behind the extracts [7]. The ethanol and aqueous extracts weighed 15 and 22 g, respectively, giving percentage yields of 10% and 7%.

Preparation of the serial dilution: A serial dilution of the two extracts was prepared using a 2-fold dilution method. Two grams of the aqueous and ethanol extracts were weighed into beakers and dissolved in 10 mL of dimethyl-sulfoxide (DMSO) each, resulting in a concentration of 200 mg/mL. This served as the stock solution. After mild agitation to ensure proper mixing, dilutions were made from the stock into six test tubes. Ciprofloxacin (5 µg) and fluconazole (10 µg) were used as positive controls for bacterial and fungal isolates, respec-tively. DMSO was used as the negative control.

Test isolates confirmation and standardization: The isolates obtained from the Pharmaceutical Microbiology Lab of Dr. Bhimrao Ambedkar University, yamuna river, Agra were subcultured from preserved agar slants onto various selective media, such as Sabouraud dextrose agar (L: S-BIOTECH, San Diego, CA), *Salmonella–Shigella* agar, mannitol salt agar (FLUMEDIA, Berkshire, UK), eosin methylene blue agar (TM MEDIA, Titan Biotech, Rajasthan, India), and nutrient agar (L: S-BIOTECH, San Diego, CA), 24 hours before use for the former and 72 hours before use for the latter. To verify the isolates taken from the laboratory, biochemical tests were also performed. Biochemical tests included gram staining, lactophenol cotton blue staining for fungus, coagulase test, indole test, oxidase test, and catalase test. Using a spectrophotometer set to 600 nm, the turbidity of the bacterial culture was adjusted to meet the 0.5 McFarland standard (0.08–0.1 OD) [7].

Antimicrobial susceptibility testing: The agar well diffusion technique was used to test the susceptibility of the test isolates to the extracts. An agar diffusion test, using the punch-hole method, was carried out to determine the susceptibility of the bacterial isolates to the plant extract. A 2-fold serial dilution of the extracts was performed using six test tubes to produce six different concentrations of the extract. Sixteen sterile Mueller–Hinton agar plates were prepared using the pour plate method eight plates for each extract (ethanol and aqueous extracts). The various bacteria were swabbed onto four plates each using a sterile swab stick.

For the susceptibility testing of *C. albicans*, four sterile Mueller–Hinton supplementary agar plates were prepared and poured into Petri dishes—two plates for each extract. The Mueller–Hinton supplement was prepared by adding 0.5 mg/L of methylene blue and 2% glucose to the Mueller–Hinton agar. The organism was swabbed onto the four plates using a sterile swab stick.

Using a sterile standard cork borer of 7 and 8 mm for the aqueous and ethanol extracts, respectively, six wells were made on the agar plates at equal distances using the punch-hole method. A 50 µL volume of each extract concentration was aseptically introduced into each well. The inoculated plates were left for the extract to diffuse for about 30 minutes before incubation. The inhibition zone diameter (IZD) was measured for all wells using a well-calibrated ruler after 24 hours of incubation [7].

Minimum inhibitory concentration: The minimum inhibitory concentration (MIC) of the aqueous and ethanol extracts on the microbial isolates was determined using the agar dilution method, which was plated out and observed for visibility after 24 hours. For this process, 12 bijou bottles containing 19 mL of nutrient agar were autoclaved. Before pouring into the Petri dishes, 1 mL of each extract concentration was added to the corresponding bijou bottles to make up 20 mL volumes. The mixture was shaken slightly for proper mixing before being poured into the Petri dishes and allowed to solidify. The dishes were divided into four equal quadrants, where each quadrant was swabbed with the corresponding bacterial organism. For the fungus *C. albicans*, 12 agar plates were prepared to check the MIC using both the ethanol and aqueous extracts. Following the same method used to determine the MIC for bacteria, six bijou bottles containing 19 mL of Mueller–Hinton supplementary agar were autoclaved. Afterward, 1 mL of each ethanol extract concentration was added to the corresponding bottle to make up 20 mL volumes. The bottles were slightly shaken to ensure proper mixing before being poured into the Petri dishes and left to stand. The fungal organism was then properly swabbed onto the Petri dishes and observed for 24 hours to check for growth.

Minimum bactericidal and fungicidal concentrations: The minimum bactericidal and fungicidal concentrations (MBC and MFC, respectively) of the aqueous and ethanol extracts on the microbial isolates were determined by re-incubating the MIC plates for another 24 hours.

GC-MS methodology: The following settings were used to link the gas chromatograph with the mass spectrometer system for the GC-MS analysis, which was performed using the GC-MS-QP 2010 Plus Shimadzu system: Elite fused silica capillary column composed of 100% dimethyl polysiloxane (30 m × 0.25 mm, 1 D × µL). A 70-eV electron ionization energy device was used. With an injection volume of 2 µL and a flow rate of 1 mL/min, 99.99% helium gas was used as the carrier gas. The injection and ion source tempera-tures were set at 280°C. The oven was set to operate at 110°C. Data from the analysis were compared with the National Institute of Standards and Technology library to determine the proportional percentage *quantity* of each component [8].

Data analysis: The experiments were carried out in duplicates, and the results were expressed as the mean and standard error of the mean $\bar{x}$ with a statistical significance of ($p \leq 0.05$). The statistical package used was the Graph Pad Prism 5 software.

Results and Discussion

Morphological and biochemical identification of the microbial isolates: The isolates obtained and used were confirmed by performing biochemical tests, as reported in Table-1. The gram-staining test identified *S. aureus* as gram-positive cocci and *Bacillus* sp. as gram-positive rods. It also identified *Salmonella* sp. and *Klebsiella* sp. as gram-negative rods. The results showed that *Bacillus* sp., *Klebsiella* sp., and *Salmonella* sp. tested positive in the catalase test. In the coagulase test, *S. aureus* gave a positive result, while the others gave a negative result. For the indole and oxidase tests, all isolates gave negative results.

Antimicrobial studies of *Carica papaya* Linn. extract on the microbial isolates: Tables-2 and 3 show the zones of inhibition observed at different concentrations of the ethanol and aqueous extracts of unripe *C. papaya* on *S. aureus*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Salmonella typhi*, and *Candida albicans.*

These results represent the mean zones of inhibition calculated from duplicate experi-ments. With $p = 0.1122$ and $p = 0.9374$ for aqueous and ethanol extracts, respectively, there is no significant difference in the analysis conducted horizontally between the columns when comparing them using one-way ANOVA

Table 1: Morphological and biochemical results of the test isolates

Microbial isolates	Cultural characteristics	Gram staining	Catalase	Coagulase	Indole	Oxidase	LPCB staining	Organisms
Staphylococcus aureus	On mannitol salt agar, colonies are 2–3 mm in diameter, with a smooth, shiny surface, opaque appearance, and often pigmented golden yellow.	+ve cocci	+ve	+ve	−ve	−ve	ND	*Staphylococcus aureus*
Salmonella typhi	On *Salmonella–Shigella* agar, colonies are round, smooth, and opaque with black-centered colonies.	−ve rod	+ve	−ve	−ve	−ve	ND	*Salmonella* sp.
Klebsiella pneumoniae	Large, mucoid, and purplish pink colonies with no green metallic sheen.	−ve rod	+ve	−ve	−ve	−ve	ND	*Klebsiella* sp.
Bacillus subtilis	Colonies on nutrient agar have jagged edges and seem rough, opaque, fuzzy white, or slightly yellow.	+ve rod	+ve	+ve	−ve	−ve	ND	*Bacillus* sp.
Candida albicans	On Sabouraud dextrose agar, *Candida albicans* show a white-colored, smooth, and yeast-like appearance.	ND	ND	ND	ND	ND	Circular bluish colonies appearing in clusters	*Candida albicans*

Table 2: Mean zone of inhibition of aqueous extract of *Carica papaya* Linn. on the microbial isolates

Isolates	Concentration ($x \pm$ SEM)								
	200 mg/mL	100 mg/mL	50 mg/mL	25 mg/mL	12.5 mg/mL	6.25 mg/mL	Ciprofloxacin	Fluconazole	DMSO
Salmonella typhi (mm)	0	0	0	0	0	0	17.50 ± 0.5	ND	0
Klebsiella pneumoniae (mm)	7.50 ± 0.5	3.00 ± 1.0	4.50 ± 0.5	5.00 ± 0.00	6.50 ± 0.5	5.00 ± 1.0	15.00 ± 1.0	ND	0
Bacillus subtilis (mm)	9.50 ± 0.5	4.00 ± 1.0	2.00 ± 1.0	4.00 ± 2.0	4.00 ± 0.0	6.00 ± 1.0	18.00 ± 4.0	ND	0
Staphylococcus aureus (mm)	9.00 ± 1.0	4.00 ± 0.0	8.00 ± 3.0	3.00 ± 3.0	6.50 ± 1.5	6.50 ± 0.5	26.00 ± 0.5	ND	0
Candida albicans (mm)	6.50 ± 0.5	4.50 ± 1.5	4.50 ± 0.00	3.50 ± 1.5	1.50 ± 1.5	1.50 ± 0.5	ND	18.00 ± 0.0	0

Table 3: Mean zone of inhibition of ethanol extract of *Carica papaya Linn* on the microbial isolates

Isolates	Concentration ($x \pm$ SEM)								
	200 mg/mL	100 mg/mL	50 mg/mL	25 mg/mL	12.5 mg/mL	6.25 mg/mL	Ciprofloxacin	Fluconazole	DMSO
Salmonella typhi (mm)	4.50 ± 4.5	3.50 ± 3.2	0	0	0	0	17.50 ± 0.5	ND	0
Klebsiella pneumoniae (mm)	7.50 ± 0.5	5.50 ± 0.5	5.50 ± 0.5	4.50 ± 0.5	4.00 ± 0.0	3.50 ± 0.5	15.0 ± 1	ND	0
Bacillus subtilis (mm)	8.50 ± 1.5	7.00 ± 1.0	3.50 ± 0.5	1.00 ± 1.0	0	0	18.00 ± 4.0	ND	0
Staphylococcus aureus (mm)	0	0	0	0	0	0	26.00 ± 0.5	ND	0
Candida albicans (mm)	6.50 ± 1.5	4.50 ± 0.5	1.00 ± 1.0	1.00 ± 2.0	1.50 ± 1.5	1.00 ± 1.0	ND	18.00 ± 0.0	0

Minimum inhibitory concentration of the extracts against the isolates: Table-4 indicates that the aqueous extract of unripe *C. papaya* exhibited a MIC of 12.5 mg/mL on all the isolates except for *Sal-monella* sp., which showed no activity. For the ethanol extract, the MIC was 12.5 mg/mL for *K. pneumoniae* and *C. albicans*, 50 mg/mL for *B. subtilis*, 25 mg/mL for *Salmonella* sp., and 12.5 mg/mL for *C. albicans.*

Table 4: Minimum inhibitory concentration of the extract on the isolates

Isolates	Aqueous extract (mg/mL)	Ethanol extract (mg/mL)
Staphylococcus aureus	12.5	-
Bacillus subtilis	12.5	50
Klebsiella pneumoniae	12.5	12.5

Salmonella typhi	-	25
Candida albicans	12.5	12.5

Minimum bactericidal concentration of the extracts against the isolates: The MBC for the aqueous extracts on all the isolates was 12.5 mg/mL, except for *Salmonella* sp., which showed no bactericidal activity, as indicated in Table-5. For the ethanol extract, the MBC was 50 mg/mL for *B. subtilis*, 25 mg/mL for *S. typhi*, and 12.5 mg/mL for *K. pneumoniae* and *C. albicans*. The ethanol extract had no bactericidal activity against *S. aureus*.

Table 5: Minimum bactericidal concentration of extract on the isolates

Isolates	Aqueous extract (mg/mL)	Ethanol extract (mg/mL)
Staphylococcus aureus	12.5	-
Bacillus subtilis	12.5	50
Klebsiella pneumoniae	12.5	12.5
Salmonella sp.	-	25
Candida albicans	12.5	12.5

Bioactive compounds of the extracts: The purpose of the current investigation was to identify the hytocomponents most likely to be present in the aqueous and ethanol extracts of unripe C. papaya fruit (AqEUF). The GC-MS detection technique was employed to identify the phytocompo-nents present in both extracts.

This research uses ethanol and aqueous solvents on clinical isolates (*S. typhi*, *S. aureus*, *K. pneumoniae*, *Escherichia coli*, and *C. albicans*) to support the wide spectrum of activity seen in *C. papaya* fruit. The papaya, also known as pawpaw, was chosen for this research because of its remarkable nutritional and therapeutic qualities, which are well known worldwide. The papaya plant, including its leaves, seeds, ripe and unripe fruits, and juice, has been used for medicinal purposes since ancient times [9]. Locally, it has been used to treat urinary tract infections. The chemical characteristics of papaya may be responsible for the antibacterial and inhibitory effects of papaya extracts [10]. Research has shown that the extraction solvent used affects the antibacterial activity of papaya peel extract [11]. According to the findings (Tables-2 and 3), the concentration of 200 mg/mL of *C. papaya* aqueous extract showed the largest zone of inhibition for all the isolates (except for *Salmonella* sp.), while 6.25 mg/mL showed the lowest. There was no zone of inhibition for *Salmonella* sp. This indicates that the extract has no antibacterial activity on the *Salmonella* sp. isolate under investigation. The study [12] was demonstrated the antibacterial effect of the aqueous extract of unripe papaya on *S. typhi*, is inconsistent with our conclusion. On *S. aureus*, the ethanol extract of unripe papaya exhibited no activity. The study was which documented the antibacterial activity of an ethanol extract of unripe papaya against *S. aureus*, is at odds with our conclusion. *B. subtilis* exhibited a zone of inhibition at a dosage of 200 mg/mL and the lowest at 6.25 mg/mL. At 200 mg/mL, the maximum IZD was seen for *K. pneumoniae*, whereas the lowest IZD was observed at 6.25 mg/mL. Tables-4 and 5 show that the aqueous extract of *S. aureus*, *B. subtilis*, *K. pneumoniae*, and *C. albicans* had an MIC and an MBC of 12.5 mg/mL. For the ethanol extract, the MIC and the MBC of *S. typhi*, *B. subtilis*, *K. pneumoniae*, and *C. albicans* were 50, 12.5, 25, and 12.5 mg/mL, respectively. The aqueous extract of unripe papaya fruit was shown to have inhibitory effects on *C. albicans*, *B. subtilis*, *K. pneumoniae*, and *S. aureus*. This result is consistent with the research conducted [13] who also documented the antibacterial properties of papaya pulp water extract. This outcome is consistent with the findings [14]. These findings also agree with those of who observed that papaya peels exhibited antifungal properties against *C. albicans*. In addition, *B. subtilis*, *Enterobacter cloacae*, *Escherichia coli*, *S. typhi*, *S. aureus*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, and *K. pneumoniae* were inhibited by the aqueous extract from a blend of meat, peel, and seed of unripe *C. papaya* [15]. Unripe fruits are used as an antiseptic to remove germs from the intestines and facilitate the absorption of vitamins and minerals, particularly vitamin B1 [16]]. The study's findings indicate that the papaya fruit's aqueous extract has more antibacterial activity than its ethanol extract. The results [17] showed that the ethanol extract was more powerful than the distilled water extract, are not supported by our investigation.

Bioactive compounds were identified in the extracts (Tables-6 and 7). The major phytocomponents present in the aqueous extract of *C. papaya* in terms of their relative abundance were cyclotrisiloxane, hexamethyl, cyclohexasiloxane dodecamethyl, pentasiloxane, do-decamethyl, dihydroxyphenylglycol derivatives, thiocyanic acid, and bis(2-ethylhexyl) phthalate. Their concentration ranged from 3.90% to 9.71%. The phytocomponents present in the ethanol extracts of *C. papaya* are 3-methoxy-2,2 dimethyloxirane, 3-furaldehyde, 2-amino5-hexamethylbenzoic acid, 2-furancarboxaldehyde 5-methyl, 2-methoxy methylene-bicyclo [2.2.21] heptane, bis(2-ethylhexyl) phthalate. The concentrations range from 2.17% to 48.87%. The present investigation revealed that aqueous and ethanol extracts of C. *papaya* were composed of various bioactive substances that can be used as substances for the production of novel therapeutic agents. To verify these compounds' biological and therapeutic properties and learn more about their mode of action, they may be separated and subjected to further empirical assessment.

Table 6: Gas chromatography-mass spectrometry results of the aqueous extract of unripe fruit of *Carica papaya*

Peak	Retention time (secs)	Area PCT (%)	Compounds identified	Molecular weight	Molecular formula
1.	7.714	2.21	Cyclotrisiloxane, hexamethyl-	265.35	C15H23NO3
2.	7.822	1.32	2-Indolizinecarboxylic acid, 1-phenyl-	237.26	C15H11NO2
3.	7.874	1.32	2-Furancarboxaldehyde, 5-methyl-	110.1106	C6H6O2

4.	8.171	2.16	Cyclotetrasiloxane, octamethyl-	296.6158	C8H24O4Si4
5.	8.309	6.94	Cyclotetrasiloxane, octamethyl-	296.6158	C8H24O4Si4
6.	9.837	4.75	Cyclopentasiloxane, decamethyl-	370.7697	C10H30O5Si5
7.	10.197	9.71	Cyclohexasiloxane, dodecamethyl-	370.7697	C10H30O5Si5
8.	10.540	0.77	Cyclopentasiloxane, decamethyl-	370.7697	C10H30O5Si5
9.	10.781	2.33	Cyclopentasiloxane, decamethyl-	370.7697	C10H30O5Si5
10.	11.376	0.81	Fumaric acid, 3,5 difluorophenyl dodecyl ester	116.072	HO2CCH=CHCO2H
11.	12.125	0.19	4,6′-Dimethoxy-2′-(tert.butyldimethylsilyl)oxychalcone	338.5	C21H26O2Si
12.	12.240	0.15	Ethanone, 1-phenyl-2-(2-phenyl-4H-1-benzopyran-4-yl)-	196.2445	C14H12O
13.	12.337	1.04	1,7-Di(3-ethylphenyl)-2,2,4,4,6,6-hexamethyl-1,3,5,7-tetraoxa-2,4,6-trisilaheptane	448.8	C22H36O4Si3
14.	12.589	0.25	1,3,5-Benzetriol, 3TMS derivative	342.6534	C15H30O3Si3
15.	13.058	0.18	Cyclohexasiloxane, dodecamethyl-	444.9236	C12H36O6Si6
16.	13.172	7.97	Cyclohexasiloxane, dodecamethyl-	444.9236	C12H36O6Si6
17.	13.281	1.11	Morphine, 2TMS derivative	429.6999	C23H35NO3Si2
18.	14.151	0.45	1H-Benzo[4,5] furo[3,2-f]indole	207.23	C14H9NO
19.	14.277	0.30	Cyclotrisiloxane, hexamethyl	265.35	C15H23NO3
20.	14.700	1.75	Cycloheptasiloxane, tetradecamethyl	519.0776	C14H42O7Si7
21.	14.843	0.82	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-	503.07	C14H42O6Si7
22.	15.032	0.56	Phenol, 3,5-bis(1,1-dimethylethyl)	206.3239	C14H22O
23.	15.244	0.63	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl	503.07	C14H42O6Si7
24.	15.433	5.59	Pentasiloxane, dodecamethyl-	384.8393	C12H36O4Si5
25.	15.673	0.15	2-(Acetoxymethyl)-3 (methoxycarbonyl)biphenylene	282.29	C17H14O4
26.	15.719	0.35	Phenol, 2,5-bis(1,1-dimethylethyl)	206.3239	C14H22O
27.	15.913	0.16	1H-Indole, 1-methyl-2-phenyl-	207.275	C15H13N
28.	16.016	0.27	Cyclotrisiloxane, hexamethyl-	265.35	C15H23NO3
29.	16.119	0.17	5-Methyl-2-phenylindolizine	207.27	C15H13N
30.	16.274	0.20	2,3,4 Trimethoxyphenylacetonitrile	207.2258	C11H13NO3
31.	16.669	0.30	3-Methyl-2-phenylindole	207.2704	C15H13N
32.	17.172	1.93	Norepinephrine, (R)-, 4TMS derivative	457.9023	C20H43NO3Si4
33.	17.275	0.49	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl	503.07	C14H42O6Si7
34.	17.435	4.85	3,4-Dihydroxyphenylglycol, 4TMS derivative	458.8871	C20H42O4Si4
35.	19.026	0.77	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl	503.07	C14H42O6Si7
36.	19.135	3.90	Thiocyanic acid, 5. alpha. -cholestan-3.beta.-yl ester	386.66	C27H48O
37.	20.445	1.05	Terbutaline, N-trifluoroacetyl-O, O,o-tris(trimethylsilyl)deriv.	481.7293	C19H34F3NO4Si3
38.	20.657	2.86	Cyclononasiloxane, octadecamethyl-	667.3855	C18H54O9Si9
39.	21.584	0.24	(E)-2 bromobutyloxychalcone	359.3	C19H19BrO2
40.	21.881	1.08	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-	503.07	C14H42O6Si7
41.	22.065	2.85	2,3-Dihydroxybenzoic acid, 3TMS derivative	154.12	(HO)2C6H3CO2H
42.	23.220	0.50	Heptasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-	503.07	C14H42O6Si7
43.	23.346	2.76	Cyclononasiloxane, octadecamethyl-	667.3855	C18H54O9Si9
44.	24.050	0.39	Thiophen-2-methylamine, N-(2-fluorophenyl)-	125.14	C7H8FN
45.	24.531	2.84	Tetracosamethyl-cyclododecasiloxane	889.8473	C24H72O12Si12
46.	25.555	7.23	Bis (2-ethylhexyl) phthalate	390.5561	C24H38O4
47.	25.641	3.53	Trisiloxane, 1,1,1,5,5,5 hexamethyl-3,3-bis[(trimethylsilyl)oxy]-	408.9	C17H44O3Si4

48.	26.688	3.10	1,2 Bis(trimethylsilyl)benzene	222.47	C12H22Si2
49.	27.672	2.77	1,1,1,3,5,5,5-Heptamethyltrisiloxane	221.50	C7H21O2Si3
50.	28.611	2.05	1,2-Bis(trimethylsilyl)benzene	222.47	C12H22Si2

Table 7: Gas chromatography-mass spectrometry results of the ethanol extract of papaya

Peak	Retention time (secs)	Area percentage (%)	Compound identified	Molecular weight	Molecular formula
1.	5.139	4.29	3-Methoxy-2,2-dimethyloxirane	102.1317	C5H10O2
2.	5.551	0.67	Benzene, (2,2-dimethoxyethyl)-	166.2071	C10H14O2
3.	5.831	4.58	3-Furaldehyde	96.0841	C5H4O2
4.	6.363	0.74	2(3H)-Furanone, 5-methyl-	126.1531	C7H10O2
5.	6.604	0.46	4-Cyclopentene-1,3-dione	96.0841	C5H4O2
6.	6.810	0.34	3-Methylpyrazolobis(diethylboryl)h	236.2281	C12H26B2N2O
7.	6.901	5.48	2-Amino-5-methyl benzoic acid	methyl benzoic	C8H9NO2
8.	7.084	0.68	1H-Imidazole, 4,5-dihydro-2-methyl	84.1197	C4H8N2
9.	7.187	2.34	Oxime-, methoxy-phenyl-Penta-2,4-dienamide	151.16	C8H9NO2
10.	7.622	0.57	2 {[(Trimethylsilyl)oxy] carbonyl} phenyl 2 [(trimethylsilyl)oxy]benzoate	310.53	C15H26O3Si2
11.	7.685	0.88	Cyclotrisiloxane, hexamethyl-	265.35	C15H23NO3
12.	7.828	6.96	2-Furancarboxaldehyde, 5-methyl	110.1106	C6H6O2
13.	8.160	1.27	Pentasilsiloxanedecamethyl-	384.8393	C12H36O4Si5
14.	8.263	7.83	-Chloro-8-methoxy-N,N-dimethyl-5-nitroquinolin-2-amine	121.61	C5H12ClN
15.	8.927	0.34	Cyclotrisiloxane, hexamethyl-	265.35	C15H23NO3
16.	9.734	0.46	4-Indolyl acetate	175.18	C10H9NO2
17.	9.848	0.53	3,3-Diisopropoxy-1,1,1,5,5,5-hexamethyl disiloxane	324.63	C12H32O4Si3
18.	10.363	1.48	1,1-Diisobutoxy-butane	202.3336	C12H26O2
19.	10.535	0.35	3,3-Diisopropoxy-1,1,1,5,5,5-hexamethyl disiloxane	324.63	C12H32O4Si3
20.	10.712	0.36	Cyclopentasiloxane, decamethyl-	370.7697	C10H30O5Si5
21.	10.781	1.68	Cyclopentasiloxane, decamethyl	370.7697	C10H30O5Si5
22.	11.370	0.29	1-Decene	140.27	C10H20
23.	12.526	0.46	1-Hydroxyanthraquinone, trimethylsilyl ether	224.21	C14H8O3
24.	13.132	0.32	Trans-4-(2-(5-Nitro-2furyl)vinyl)-2-quinolinamine		C15H11N3O3•C3H6O3
25.	13.321	1.04	Cyclohexasiloxane, dodecamethyl	444.9236	C12H36O6Si6
26.	14.723	0.39	3,4-Dihydroxybenzyl alcohol,tris(trimethylsilyl)-	356.68	C16H32O3Si3
27.	15.238	0.31	Cyclopentasiloxane, decamethyl-	370.7697	C10H30O5Si5
28.	15.484	0.47	Cycloheptasiloxane, tetradecamethyl-	519.0776	C14H42O7Si7
29.	15.541	0.40	2-Amino-4-hydroxy-6,8-dimethyl-7(8H)-pteridinone	231.68	C8H14ClN5O
30.	16.606	0.28	1,2,5-Oxadiazol-3-amine, 4-(4-methoxyphenoxy)-	207.19	C9H9N3O3
31.	17.184	0.30	1,2-Bis(trimethylsilyl)benzene	222.47	C12H22Si2
32.	17.458	0.38	3-Amino-2-phenazinol ditms	355.6	C18H25N3OSi2
33.	17.950	0.52	Heptane, 1,7-dibromo-	257.994	C7H14Br2
34.	18.957	0.28	Trans-4-Dimethylamino-4′-methoxychalcone	281.349	CHNO
35.	19.209	0.36	Morphine, 2TMS derivative	429.6999	C23H35NO3Si2
36.	19.570	48.87	2-Methoxymethylene-bicyclo[2.2.1]heptane	110.1968	C8H14
37.	20.880	0.28	Cyclotrisiloxane, hexamethyl-	265.35	C15H23NO3
38.	22.133	0.25	Cyclotrisiloxane, hexamethyl-	265.35	C15H23NO3
39.	24.634	0.35	Benzo[h]quinoline, 2,4-dimethyl-	207.2704	C15H13N
40.	25.584	2.17	Bis(2-ethylhexyl) phthalate	390.5561	C24H38O4

Conclusions

This study demonstrates the potential of aqueous and ethanol extracts from unripe *C. papaya* fruit as potent antimicrobial agents against selected bacterial and fungal isolates, including *S. aureus*, *B. subtilis*, *K. pneumoniae*, and *C. albicans*. The aqueous extract exhibited higher antibacterial activity than the ethanol extract, highlighting its potential potency against certain pathogenic mi-crobes. However, both extracts showed limited or no inhibitory activity against *S. typhi*, indicating a selective antimicrobial effect. The identification of bioactive phytocomponents, such as cyclot-risiloxane, hexamethyl, and bis(2-ethylhexyl) phthalate, in the aque-ous and ethanol extracts, suggests that these compounds may contribute to the observed antimicrobial effects. This study aligns with previous findings on the antimicrobial properties of *C. papaya*,

reinforcing its value in traditional medicine and its potential as a natural alternative to synthetic antimicrobial agents, especially in the context of rising antimicrobial resistance. Further research is recommended to isolate and characterize the specific active compounds within *C. papaya* that are responsible for its antimicrobial action. Such studies could pave the way for developing plant-based antimicrobial agents, supporting the global effort to mitigate the threat of antimicrobial resistance in healthcare.

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