Journal of Academic Biology and Biology Education
Vol. 2, No. 1, June 2025, pp. 147~156
ISSN: 3062-9705, DOI: 10.37251/jouabe.v2i1.2190

The Activity of Papaya Seeds (*Carica papaya L.*) Varieties of "Bangkok" and "California" in Inhibiting the Growth of Pathogenic Bacteria

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Article Info

Article history:

Received Feb 29, 2025
Revised Apr 17, 2025
Accepted Jun 19, 2025
Online First Jun 24, 2025

Keywords:

Antibacterial Activity
Bioactive Compounds
GC–MS
MIC
Papaya Seed Extract

ABSTRACT

Purpose of the study: The purpose of this study is to evaluate the antibacterial activity of ethanol extracts from Bangkok and California papaya (Carica papaya) seeds against *E. coli*, *Salmonella* sp., and *S. aureus*, and to identify the chemical compounds responsible for their antibacterial properties using GC–MS analysis.

Methodology: This study applied a Completely Randomized Design (CRD) using ethanol extracts of *Carica papaya* seeds. Equipment included GC–MS, rotary evaporator, autoclave, laminar flow, vortex mixer, hot plate, incubator, and micrometer. Media and reagents were NA, NB, MHA, ethanol, DMSO, and Amoxicillin. Tested bacteria were *E. coli*, *Salmonella* sp., and *S. aureus*. Data were analyzed with ANOVA and DMRT using SPSS.

Main Findings: Ethanol extracts of *Carica papaya* seeds ('Bangkok' and 'California') significantly inhibited *E. coli*, *Salmonella* sp., and *S. aureus* growth. The highest inhibition zones occurred at 20% concentration, with 'California' showing stronger activity against *Salmonella* sp. and 'Bangkok' against *S. aureus*. MIC was 20% for Gram-negative bacteria. GC–MS identified 20 compounds in 'Bangkok' and 24 in 'California', dominated by hexadecanoic acid and various alkaloids, flavonoids, terpenoids, and fatty acids.

Novelty/Originality of this study: This study provides new insights by comparing the antibacterial activity and chemical composition of ethanol extracts from *Carica papaya* seeds of 'Bangkok' and 'California' varieties. It identifies specific bioactive compounds through GC–MS and reveals variety-dependent antibacterial effects, advancing knowledge of papaya seeds as natural antibacterial agents and supporting their potential development as alternative antimicrobial resources.

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1. INTRODUCTION

Papaya (*Carica papaya*) varieties 'Bangkok' and 'California' are horticultural crops widely cultivated in several Asian countries. The high consumption of these papaya varieties generates an abundant supply of seeds that remain underutilized [1], [2]. Papaya seeds can be used in traditional medicine for various purposes, including as an anthelmintic, treatment for digestive disorders and diarrhea, male contraception, and as a raw material for herbal remedies [2], [3]. The potential uses of papaya seeds, particularly as antibacterial agents and in disease treatment, warrant further investigation [3]-[5]. Therefore, this study utilized seeds from the 'Bangkok' and 'California' papaya varieties, which are characterized by red flesh and black seeds.

Diseases caused by pathogenic bacteria are generally referred to as infections [6]-[8]. Antibiotics are commonly used to control the growth of pathogenic bacteria and to reduce infection transmission [9], [10]. However, prolonged antibiotic use can alter normal microbial flora and induce antibiotic resistance [11], [12]. In response, research efforts have increasingly focused on natural antibacterial agents to minimize the adverse effects of synthetic antibiotics [13]. One such natural source of antibacterial compounds is papaya seeds. Papaya seeds are medically beneficial because they contain secondary metabolites, including phenols, terpenoids, alkaloids, and saponins, all of which exhibit antibacterial activity [14], [15]. The specific compounds present in the seeds of 'Bangkok' and 'California' papaya varieties, however, remain unidentified.

Therefore, this study employed Gas Chromatography–Mass Spectrometry (GC–MS) analysis to determine the organic compounds present in the seeds of these varieties. The choice of solvent can influence the size of the inhibition zone due to differences in the solubility of secondary metabolites [16], [17]. Ethanol extract produced a larger inhibition zone against *Escherichia* coli and *Bacillus subtilis* compared to chloroform extract [18], [19]. Since the active compounds in papaya seeds are polar, ethanol is an effective solvent for extracting antibacterial constituents [20], [21]. Accordingly, this study utilized 96% ethanol as the extraction solvent.

Further research on papaya varieties has been conducted by Tumembow, Wowor, and Tambunan [22], who examined the effects of 'Hawai' and 'California' papaya on reducing debris indices. Their study revealed that 'Hawai' papaya had a more significant effect on lowering children's debris indices compared to the 'California' variety. These results suggest the need for further qualitative identification of the potential antibacterial activity of papaya seeds from different varieties, as each variety may possess distinct antibacterial properties against specific microorganisms.

This study utilized *E. coli*, *Salmonella* sp., and *Staphylococcus aureus* as test bacteria. According to the Indonesian National Standard (SNI, 2009), these species are common foodborne pathogens that can cause infectious diseases such as diarrhea, salmonellosis, food poisoning, and other infections. Contamination by *E. coli*, *Salmonella* sp., and *S. aureus* occurs readily because these bacteria are frequently found in the human and animal body as well as in the surrounding environment [23], [24].

Several previous studies have shown that *Carica papaya* seed extract has antibacterial activity against both Gram-positive and Gram-negative bacteria. GC–MS analysis of these studies revealed the presence of important bioactive compounds, including fatty acids, phenolic compounds, and other secondary metabolites, that support this antibacterial activity [25]. One compound widely reported to play an important role is benzyl isothiocyanate (BITC), a sulfur/isocyanate compound with significant potential as a natural antibacterial agent. Several studies have identified BITC in papaya seeds using advanced analytical methods such as HS-GC–MS and GC–MS to quantify its content [26]. In addition to bioactive compounds, the extraction method has also been shown to influence antibacterial effectiveness. The choice of solvent, such as ethanol, methanol, chloroform, or water, determines the metabolite profile obtained. Several studies have reported that ethanol and chloroform extracts often produce larger inhibition zones, while other studies have demonstrated high effectiveness of methanol, depending on the extraction procedure used [27].

However, studies specifically comparing the chemical composition and biological activity of papaya varieties are limited. While studies on physicochemical properties and seed oil profiles are available, few directly link the GC–MS profiles of specific varieties such as 'Bangkok' and 'California' to antibacterial activity against major foodborne pathogens, including *Escherichia coli*, *Salmonella sp*., and *Staphylococcus aureus* [28]. These limitations of previous research underlie the importance of this study. By analyzing the antibacterial activity of 96% ethanol extracts of papaya seeds from the 'Bangkok' and 'California' varieties, and identifying their active compounds using GC–MS, this research has the potential to provide new information that is relevant both academically and practically for the development of natural antibacterial agents.

The objectives of this study were to analyze differences in the antibacterial activity of ethanol extracts of papaya (*C. papaya*) seeds from the 'Bangkok' and 'California' varieties in inhibiting the growth of test bacteria, and to identify potential differences in the chemical compounds present in these extracts that may contribute to antibacterial properties. The findings of this research are expected to provide valuable information on the antibacterial potential of *C. papaya* seeds from the 'Bangkok' and 'California' varieties, supporting their use as natural antibacterial agents.

2. RESEARCH METHOD

2.1. Materials and Equipment

The equipment used in this study included test tubes, test tube racks, measuring cylinders, a vortex mixer, hot plate, magnetic stirrer, grinder, analytical balance, Bunsen burner, loop wire, micropipette, Petri dishes, micrometer screw gauge, matches, autoclave, cotton plugs, Erlenmeyer flasks, beakers, vials, incubator, labels, stationery, laminar air flow cabinet, Gas Chromatography–Mass Spectrometry (GC–MS) instrument, tissue paper, sterile discs (6 mm diameter), Whatman filter paper No. 1, stirring rods, aluminum foil, rotary evaporator, water bath, tweezers, watch glass, and a camera.

The materials used consisted of ripe seeds of *Carica papaya* 'Bangkok' variety obtained from local plantations in Sukabumi and ripe seeds of *Carica papaya* 'California' variety obtained from local plantations in Bogor. The chemical reagents included 96% ethanol, dimethyl sulfoxide (DMSO), 70% alcohol, Nutrient Agar (NA, OXOID), Nutrient Broth (NB, MERCK), Mueller Hinton Agar (MHA, HIMEDIA), Amoxicillin antibiotic (25 µg), and sterile distilled water. The bacterial cultures used as test organisms were *Escherichia coli* (ATCC 25922), *Salmonella* sp. (ATCC 19430), and *Staphylococcus aureus* (ATCC 25923), all obtained from the Bukalapak Microbiology Store (online supplier).

2.2. Experimental Design

This study employed a Completely Randomized Design (CRD) with two factors. The first factor was the variety of papaya seeds, namely 'Bangkok' and 'California'. The second factor was the concentration of papaya seed extract, consisting of four levels: 5%, 10%, 15%, and 20%. Each treatment was replicated three times using different sterile paper discs. The experimental workflow is illustrated in Figure 1.

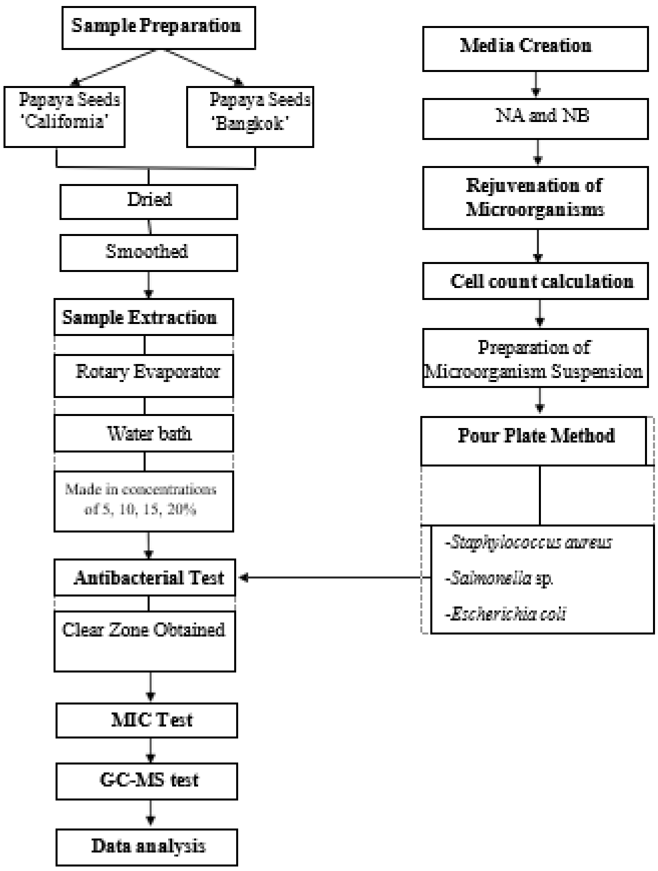

Figure 1. Workflow of the Antibacterial Activity Test of Papaya Seed Extract

2.3. Research Procedures

1. Preparation of Culture Media

Media were prepared according to the manufacturer's instructions, weighed, and dissolved in distilled water. Liquid media were homogenized using a magnetic stirrer, while solid media were heated on a hot plate. After sealing the flask mouth with cotton plugs, the media were sterilized in an autoclave at 121 °C and 1 atm for 15 minutes.

2. Rejuvenation of Microorganisms

The test bacteria *Escherichia coli* (ATCC 25922), *Salmonella* sp. (ATCC 19430), and *Staphylococcus aureus* (ATCC 25923) were inoculated onto slanted Nutrient Agar (NA) and incubated for 24–48 hours at 37 °C.

3. Cell Density Determination

Bacterial cultures were serially diluted (10^{-1} to 10^{-6}) using sterile NaCl solution. The pour-plate method was applied by inoculating 0.1 mL of the 10^{-4}–10^{-6} dilutions into Petri dishes containing 12 mL of NA medium, followed by incubation for 24 hours at 37 °C. Colony counts were performed to ensure a cell density of 10^5–10^8 cells/mL in the mid-log phase.

4. Preparation of Papaya Seed Extract

A total of 5 kg of papaya seeds were cleaned, dried at 50 °C, and ground into powder. Approximately 250 g of the powder was macerated with 96% ethanol (1:4 w/v) for 4 days and filtered (filtrate 1). The residue was re-macerated for 2 days with ethanol (filtrate 2). Both filtrates were combined and concentrated using a rotary evaporator (50 °C, 15–20 psi), followed by further concentration in a water bath to obtain a thick extract.

5. Antibacterial Activity Assay

A 0.1 mL aliquot of mid-log-phase bacterial suspension was mixed with 10 mL of Mueller Hinton Agar (MHA) in a Petri dish. Papaya seed extracts were prepared at concentrations of 5%, 10%, 15%, and 20% using dimethyl sulfoxide (DMSO) as the solvent. Sterile discs were soaked in the extract solutions, dried, and placed on the agar surface. Amoxicillin (25 µg) served as a positive control, while DMSO served as a negative control. Plates were incubated for 24–48 hours at 37 °C, and the diameters of the inhibition zones were measured.

6. Minimum Inhibitory Concentration (MIC) Test

For MIC determination, 1 mL of extract, 3.9 mL of Nutrient Broth (NB), and 0.1 mL of bacterial suspension were mixed in test tubes and incubated for 24 hours at 37 °C. Subsequently, 10 µL of the culture was inoculated onto NA medium. The MIC was defined as the lowest extract concentration that showed no visible bacterial growth. The percentage of bacterial growth inhibition was calculated using the following formula:

$$\%Inhibition = 100\% - \left(\frac{N_t}{N_0} \times 100\%\right) \quad \text{... (1)}$$

Description:

- N_t = Number of bacterial colonies after incubation
- N_0 = Initial number of bacterial colonies

7. GC–MS Analysis

The extract (5000 µL) was analyzed using a SHIMADZU QP 2010 Gas Chromatography–Mass Spectrometry (GC–MS) system. The operating conditions were set as follows: oven temperature at 40 °C, injector temperature at 210 °C, and a split ratio of 100. The GC spectrum was used to determine the retention times of the compounds, while the MS detector identified the relative molecular masses. The resulting chromatographic peaks were compared with reference data from the literature to identify the composition of the active compounds.

2.4. Data Analysis

The inhibition zone diameters obtained from the antibacterial activity tests were statistically analyzed using Analysis of Variance (ANOVA) with SPSS software at a 95% confidence level. The hypotheses tested were as follows:

- H_0: The treatments applied to the test bacteria show no significant differences in antibacterial activity.
- H_1: The treatments applied to the test bacteria show significant differences in antibacterial activity.

The decision criteria were:

- If $P < 0.05$, H_0 is rejected and H_1 is accepted, indicating a significant effect of the treatments.
- If $P > 0.05$, H_0 is accepted and H_1 is rejected, indicating no significant effect of the treatments.

When significant differences were detected, Duncan's Multiple Range Test (DMRT) was performed as a post hoc analysis to determine specific differences among treatment groups.

3. RESULTS AND DISCUSSION

3.1. Antibacterial Activity Test

The antibacterial activity test aimed to determine the ability of a substance to inhibit the growth of microorganisms. Based on data analysis using ANOVA, the treatments applied (papaya varieties and extract concentrations) had a significant effect on the inhibition of the test bacteria ($P < 0.05$). This finding indicates an interaction between the papaya variety and the extract concentration on the inhibition zone diameter of the tested bacteria. Subsequent Duncan's Multiple Range Test revealed that the 5% extract concentration exhibited a relatively high mean inhibition zone value compared with other concentrations, measuring 6.90 mm. The differences in inhibition zone diameters for the antibacterial activity of *Carica papaya* 'Bangkok' seed extract are presented in Table 1.

Tabel 1. Average Inhibition Zone Values of 'Bangkok' Papaya Seed Extract

Concentration %	*E.coli* (mm)		*Salmonella sp.* (mm)		*S. aureus* (mm)	
	Mean	St. Dev	Mean	St. Dev	Mean	St. Dev
5	6.38	0.02	6.46	0.11	6.48	0.15
10	6.46	0.05	6.54	0.05	6.58	0.13
15	6.49	0.01	6.62	0.06	6.63	0.04
20	6.64	0.06	6.70	0.03	6.81	0.02
Positive Control (+)	12.65	0.38	10.67	1.40	10.19	1.25
Negative Control (–)	2.05	3.55	4.10	3.55	2.05	3.55

Note: The calculation of the inhibition zone diameter includes the diameter of the paper disc (6 mm).

The results presented in Table 1 show that the 5% extract of *Carica papaya* 'Bangkok' seeds was capable of inhibiting the growth of the tested bacteria. Among the three bacterial strains, the highest inhibition zone produced by the 'Bangkok' seed extract was observed against *Staphylococcus aureus*, followed by *Salmonella* sp. and *Escherichia coli*. At the 5% extract concentration, the inhibition zone diameters were 6.48 mm for *S. aureus*, 6.46 mm for *Salmonella* sp., and 6.38 mm for *E. coli*. The greatest inhibition zones were obtained at the 20% extract concentration, measuring 6.81 mm for *S. aureus*, 6.70 mm for *Salmonella* sp., and 6.64 mm for *E. coli*. The antibacterial activity of the *C. papaya* 'California' seed extract is presented in Table 2.

Table 2. Average Inhibition Zone Values of California Papaya Seed Extract

Concentration %	*E.coli* (mm)		*Salmonella sp.* (mm)		*S. aureus* (mm)	
	Mean	St. Dev	Mean	St. Dev	Mean	St. Dev
5	6.34	0.04	7.28	0.12	6.43	0.18
10	6.54	0.08	7.45	0.08	6.48	0.18
15	6.60	0.08	7.61	0.04	6.54	0.12
20	6.76	0.04	7.82	0.09	6.69	0.14
Positive Control (+)	12.65	0.38	10.67	1.40	10.19	1.25
Negative Control (–)	2.05	3.55	4.10	3.55	2.05	3.55

Note: The calculation of the inhibition zone diameter includes the diameter of the paper disc (6 mm).

The results presented in Table 2 show findings similar to those obtained from the seeds of the 'Bangkok' papaya variety. A 5% concentration of the *Carica papaya* 'California' seed extract was sufficient to inhibit the growth of the tested bacteria. Among the tested organisms, the largest inhibition zone was observed against *Salmonella* sp., followed by *Staphylococcus aureus* and *Escherichia coli*. At a concentration of 5%, the inhibition zones measured 7.28 mm for *Salmonella* sp., 6.43 mm for *S. aureus*, and 6.34 mm for *E. coli*. The highest inhibition was recorded at a concentration of 20%, with inhibition zones of 7.82 mm for *Salmonella* sp., 6.69 mm for *S. aureus*, and 6.76 mm for *E. coli*.

Previous research by Taufiq et al. examined the antibacterial activity of ethanol extracts of papaya seeds against *E. coli* and *Salmonella typhi*. At concentrations of 1, 2, 3, 4, 5, 10, 15, and 20%, the inhibition zones against *E. coli* were 1.20, 1.26, 1.30, 1.40, 1.48, 1.57, 1.65, and 1.85 mm, respectively. No inhibition was observed against *S. typhi* at concentrations of 1–4%, whereas concentrations of 5, 10, 15, and 20% produced inhibition zones of 1.23, 1.42, 1.59, and 1.72 mm, respectively. Both the present study and that of Taufiq et al. confirm that inhibition zones increase proportionally with higher extract concentrations.

When comparing the two papaya seed varieties, the inhibition zones produced by the 'California' extract against *Salmonella* sp. were consistently larger than those of the 'Bangkok' extract. At concentrations of 10%, 15%, and 20%, the 'California' extract also showed greater inhibitory activity against *E. coli* than the 'Bangkok' extract. In contrast, the 'Bangkok' extract produced larger inhibition zones against *S. aureus* compared to the 'California' extract. These differences indicate that the antibacterial activity of the two papaya varieties varies depending on the bacterial species, which may be attributed to differences in bacterial resistance to bioactive compounds [29], [30].

Previous research by Fitri and Widiyawati [31] reported the antibacterial efficacy of *Phyllanthus niruri* (meniran herb) extract, with an inhibition zone of 6.3 mm against *Salmonella* sp. at a concentration of 10%. In comparison, the papaya seed extracts in this study produced inhibition zones of 6.54 mm ('Bangkok') and 7.45 mm ('California') at the same concentration, indicating that *Salmonella* sp. is more sensitive to papaya seed extracts than to *P. niruri* extract.

Amoxicillin was used as a positive control to compare the inhibition zones of the papaya seed extracts. The inhibition zones produced by both the 'California' and 'Bangkok' extracts were smaller than those of

Amoxicillin. This difference is expected because Amoxicillin contains a pure active compound. As a broad-spectrum semisynthetic antibiotic derived from penicillin, Amoxicillin inhibits the growth of both Gram-positive and Gram-negative bacteria. It contains a β-lactam ring, a structural analog of natural L-alanine–D-alanine, which binds covalently to penicillin-binding proteins (PBPs) at their active sites. Once bound, Amoxicillin blocks the transpeptidase reaction, leading to cell wall rupture and bacterial cell death [32], [33].

The antibacterial activity of both the 'Bangkok' and 'California' papaya seed extracts is closely associated with the presence of bioactive compounds in the seeds. Papaya seed extract contains several groups of active compounds, including alkaloids, steroids, tannins, flavonoids, saponins, triterpenoids, and essential oils [34], [35]. However, the specific compounds present in the 'Bangkok' and 'California' varieties have not yet been identified. Therefore, a gas chromatography–mass spectrometry (GC–MS) analysis was conducted in this study to determine the chemical constituents of the two papaya seed varieties.

3.2. Minimum Inhibitory Concentration (MIC) Test

The Minimum Inhibitory Concentration (MIC) test was performed to determine the lowest concentration of the extract capable of inhibiting the growth of the test bacteria. The MIC value was defined as the lowest extract concentration at which no visible bacterial growth was observed. The MIC results for the *Carica papaya* 'Bangkok' seed extract are presented in Table 3.

Table 3. MIC Test Results of Bangkok Papaya Seed Extract

Test Bacteria	Extract Concentration (%)	Number of Bacteria (cells/mL) Incubated 24 h (N)	% Inhibition
E. coli ($N_0 = 9.87\times10^8$ cells/mL)	5	1.18×10^8	38.05
	10	8.32×10^7	91.97
	15	3.04×10^7	96.56
	20*	No growth	100
Salmonella sp. ($N_0 = 9.87\times10^8$ cells/mL)	5	1.35×10^7	85.69
	10	1.67×10^7	91.95
	15	6.70×10^6	99.32
	20*	5.10×10^6	99.29
S. aureus ($N_0 = 9.87\times10^8$ cells/mL)	5	2.87×10^7	93.28
	10	5.95×10^6	99.34
	15	2.87×10^6	99.72
	20*	1.67×10^6	98.27

*) MIC value = lowest concentration that can inhibit bacterial growth.

The MIC value of *Carica papaya* 'California' seed extract at a concentration of 20% was not obtained against *E. coli* and *S. aureus*, whereas an MIC value was observed against *Salmonella* sp. This result indicates that *Salmonella* sp. was more sensitive to the 'California' seed extract. The MIC findings were consistent with the antibacterial activity assay, demonstrating a similar inhibition pattern. Based on the MIC results presented in Tables 3, the 'Bangkok' and 'California' papaya seed extracts exhibited different inhibitory effects on the tested bacteria. The 'California' seed extract was more effective against *Salmonella* sp., whereas the 'Bangkok' seed extract showed greater inhibitory activity against *E. coli* compared with the other tested bacteria. These differences may be attributed to variations in the chemical constituents of the 'Bangkok' and 'California' papaya seeds that influence their antibacterial activity.

The data in Tables 3 show that the MIC was achieved at a concentration of 20% for Gram-negative bacteria (*E. coli* and *Salmonella* sp.), while no MIC value was observed for the Gram-positive bacterium *S. aureus*. Antimicrobial mechanisms can be classified into four categories: (1) inhibition of cell wall synthesis, (2) disruption of cell membrane function, (3) inhibition of protein synthesis, and (4) inhibition of nucleic acid synthesis [36], [37]. These results suggest that the antibacterial mechanism of papaya seed extracts likely does not involve inhibition of cell wall synthesis or disruption of cell membrane integrity. Instead, the extracts are presumed to interfere with bacterial metabolism, particularly through the inhibition of nucleic acid and protein synthesis. Since each bacterial species has different metabolic pathways, the variation in chemical constituents between the 'Bangkok' and 'California' seeds may explain their selective inhibitory effects.

A previous study by Hudaya et al. [38] using an aqueous extract of torch ginger (*Etlingera elatior*) flowers determined antibacterial activity by calculating the percentage of bacterial inhibition. Their results showed that *S. aureus* exhibited 95.63% inhibition at a concentration of 15%, while *E. coli* showed 92.41% inhibition at a concentration of 50%. In comparison, the present study revealed higher inhibition percentages for both papaya seed extracts. At a concentration of 15%, the 'Bangkok' seed extract achieved inhibition rates of 97.23% against *S. aureus* and 96.56% against *E. coli*, whereas the 'California' seed extract exhibited inhibition rates of 98.61% and 97.63%, respectively. These findings indicate that the ethanol extracts of *C. papaya*

'Bangkok' and 'California' seeds are more effective in inhibiting the growth of *S. aureus* and *E. coli* than the aqueous extract of torch ginger flowers.

3.3. GC–MS Analysis of *Carica papaya* Seed Extracts

GC–MS analysis was performed to identify the chemical constituents present in the seeds of *Carica papaya* L. varieties 'Bangkok' and 'California'. The peaks observed in the chromatographic spectra were compared to known retention times and mass spectral data from established literature to determine the types of compounds present and their respective percentage areas. The number of chemical constituents in the papaya seed extracts was inferred from the total number of peaks detected in the chromatograms. Each spectral peak was assigned a specific peak number, which was then matched to reference data for compound identification.

The bioactive compounds of ethanol extracts from 'Bangkok' and 'California' papaya seeds that contribute to antibacterial activity are summarized in Tables 4 and 5. Classification of these compounds was performed using the Human Metabolome Database (HMDB; http://www.hmdb.ca/).

Table 4. Antibacterial Compounds Identified in the Seed Extract of *Carica papaya* 'California' Variety

Compound Name	Area (%)	Compound Class
Acetic acid	0.12	Carboxylic acid
Ethanol, 2-butoxy	0.94	Alkaloid
Benzeneethanamine, N-(phenylmethyl)	0.15	–
9-Octadecenoic acid (Z)	0.07	Unsaturated fatty acid
Methyl ester of Benzylcarbamic acid	0.46	Flavonoid
Neophytadiene	0.40	–
Phenol, 2,4-bis	0.19	–
Dodecanoic acid	0.13	Terpenoid
4-Nitro-Phenylacetic acid	0.07	–
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	0.07	–
Tetradecanoic acid, methyl ester	0.63	–
Tetradecanoic acid	0.46	Steroid
Tetradecanoic acid, ethyl ester	0.42	–
Hexadecanoic acid, methyl ester	69.72	Saturated fatty acid
Hexadecanoic acid	12.27	Saturated fatty acid
Hexadecanoic acid, ethyl ester	0.56	–
Hexadecanoic acid, 15-methyl-, methyl ester	0.56	–
Total	87.52	

Table 5. Antibacterial Compounds Identified in the Seed Extract of *Carica papaya* 'Bangkok' Variety

Compound Name	Area (%)	Compound Class
Ethanol, 2-(2-butoxyethoxy)	0.10	Alkaloid
Phenol, 2,4-bis(1,1-dimethylethyl)	0.19	–
2,4-Methoxy-6-propylbenzoic acid	1.52	Flavonoid
Methyl ester of Benzylcarbamic acid	0.33	–
Dodecanoic acid	0.53	Terpenoid
1-Pentadecene	0.25	–
4-Nitro-Phenylacetic acid	0.07	–
Tetradecanoic acid, methyl ester	1.86	–
Tetradecanoic acid	0.66	–
9-Hexadecenoic acid, methyl ester	6.41	–
Hexadecanoic acid, methyl ester	23.14	Unsaturated fatty acid
Hexadecanoic acid, palmitic acid	42.56	Unsaturated fatty acid
Hexadecanoic acid, ethyl ester	2.02	–
Hexadecanoic acid, 15-methyl-, methyl ester	0.29	–
Total	78.13	

Based on the GC-MS analysis, the ethanol extracts of papaya seeds from the 'Bangkok' and 'California' varieties contain different chemical compounds. The ethanol extract of 'Bangkok' papaya seeds contained 20 compounds, whereas the ethanol extract of 'California' papaya seeds contained 24 compounds. These differences in chemical composition are influenced by genetic and environmental factors. Environmental factors that may affect the chemical composition of papaya seeds include temperature, light intensity, nutrient availability, and plant hormones.

According to Tables 4 and 5, the GC-MS analysis revealed that the ethanol extracts of 'Bangkok' and 'California' papaya seeds possess different groups of compounds with antibacterial activity. The ethanol extract of 'Bangkok' papaya seeds contained four compound groups—alkaloids, terpenoids, flavonoids, and saturated fatty acids—whereas the ethanol extract of 'California' papaya seeds contained seven groups: carboxylic acids, alkaloids, unsaturated fatty acids, flavonoids, terpenoids, steroids, and saturated fatty acids.

The GC-MS analysis also showed that the ethanol extracts of both 'Bangkok' and 'California' papaya seeds contained fatty acids, but with different types. The fatty acids identified in the 'Bangkok' variety included tetradecanoic acid methyl ester, tetradecanoic acid, 9-hexadecenoic acid methyl ester, hexadecenoic acid methyl ester, palmitic acid, hexadecenoic acid ethyl ester, and hexadecanoic acid 15-methyl-methyl ester. In contrast, the fatty acids in the 'California' variety included hexadecanoic acid methyl ester, hexadecanoic acid, hexadecanoic acid ethyl ester, hexadecanoic acid 15-methyl-methyl ester, and 9-octadecenoic acid (Z). These variations in fatty acid composition may result in differences in their antibacterial activity.

Compounds with antibacterial properties in the ethanol extract of 'Bangkok' papaya seeds consisted of 14 organic compounds, accounting for 78.13% of the total area, whereas the 'California' variety contained 17 organic compounds, representing 87.52% of the total area. These results indicate that the 'California' variety contains a greater number of antibacterial compounds. However, this does not necessarily mean that the 'California' extract has stronger antibacterial activity compared to the 'Bangkok' extract, as the inhibitory effect depends on the specific test bacteria and the types of compounds present. The GC-MS results further revealed that the ethanol extracts of both papaya seed varieties were dominated by hexadecanoic acid. Hexadecanoic acid accounted for 44.56% of the total area in the 'Bangkok' variety and 69.72% in the 'California' variety. Hexadecanoic acid, a saturated fatty acid, is known to possess antioxidant and antibacterial activities [39], [40].

Among the alkaloids with antibacterial activity, the 'Bangkok' variety contained ethanol, 2-(2-butoxyethoxy) and phenol, 2,4-bis(1,1-dimethylethyl), while the 'California' variety contained ethanol, 2-butoxy and benzenemethanamine, N-(phenylmethyl). Alkaloids have the ability to inhibit the activity of several microorganisms by altering them into derivative compounds such as peptones [41], [42].

The flavonoid compounds in the ethanol extract also differed between the two varieties. The 'California' variety contained only methyl ester of benzylcarbamic acid, whereas the 'Bangkok' variety contained two flavonoid compounds: 2-4-methoxy-6-propylbenzoic acid and methyl ester of benzylcarbamic acid. Flavonoids can inhibit bacterial growth and generally act as antioxidants, making them widely used as pharmaceutical ingredients.

Similarly, the terpenoid compounds varied between the two extracts. The 'Bangkok' variety contained three terpenoid compounds dodecanoic acid methyl ester, 1-pentadecene, and 4-nitrophenylacetic acid—while the 'California' variety contained five terpenoid compounds: neophytadiene, phenol, 2,4-bis, dodecanoic acid, 4-nitrophenylacetic acid, and 3,7,11,15-tetramethyl-2-hexadecen-1.

4. CONCLUSION

The study demonstrated that seed extracts of *Carica papaya* varieties 'Bangkok' and 'California' exhibit different antibacterial activities depending on the type of test bacteria. These differences are closely related to the diversity of bioactive compounds present in each extract and the characteristics of the bacterial strains tested. Minimum inhibitory concentration (MIC) testing at the highest concentration (20%) revealed that the 'Bangkok' seed extract showed inhibitory activity only against *Escherichia coli*, while the 'California' seed extract was effective only against *Salmonella* sp. Furthermore, the chemical composition of the two varieties also differed, with the 'Bangkok' seeds containing 20 identified compounds and the 'California' seeds containing 24 compounds. The antibacterial activity of both extracts is attributed to the presence of key bioactive groups, including alkaloids, carboxylic acids, terpenoids, flavonoids, steroids, saturated fatty acids, and unsaturated fatty acids. These findings highlight the potential of papaya seeds as a natural source of antibacterial agents and underline the importance of varietal differences in determining their bioactivity. Based on these results, it is recommended to conduct further experiments by increasing the concentration of ethanol extracts from both papaya seed varieties in order to obtain more precise MIC values for each tested bacterium. In addition, subsequent testing against a broader range of microorganisms is encouraged to determine the wider antibacterial spectrum and identify the most effective targets for each extract.

ACKNOWLEDGEMENTS

The authors would like to express their deepest gratitude to all parties who have supported the completion of this research. Sincere appreciation is extended to the laboratory staff and technicians for their valuable assistance during the experimental and GC-MS analysis processes. The authors also thank the academic supervisors and colleagues for their constructive suggestions and guidance throughout the study. Special thanks are given to the funding institution and the university for providing the facilities and resources necessary to carry out this research. Finally, the authors are grateful to their families and friends for their encouragement and continuous support during the preparation and completion of this work.

AUTHOR CONTRIBUTIONS

Conceptualization, R.A.R. and H.A.; Methodology, R.A.R. and H.A.; Software, R.A.R.; Validation, R.A.R., H.A. and N.S.J.; Formal Analysis, R.A.R.; Investigation, R.A.R.; Resources, H.A. and N.S.J.; Data Curation, R.A.R.; Writing – Original Draft Preparation, R.A.R.; Writing – Review & Editing, R.A.R., H.A. and N.S.J.; Visualization, R.A.R.; Supervision, H.A. and N.S.J.; Project Administration, H.A.; Funding Acquisition, N.S.J.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

Not applicable.

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