

Antibacterial Activity of Papaya Seed (*Carica papaya* L.) Ethanol Extract With MAE And UAE Extraction Methods Against *Staphylococcus aureus*

Fini Hartanti, Devi Nisa Hidayati*

Department of Pharmacy Biology, Faculty of Pharmacy, Universitas Wahid Hasyim, Semarang
*corresponding author: email: devinisahidayati@unwahas.ac.id

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ABSTRACT

Papaya seeds extract was produced by the maceration method has been shown to have antibacterial activity against *S. aureus*, *P. aeruginous*, *S. thypi*, and *E. coli*. This study aims to compare the antibacterial activity of papaya seeds ethanol extract obtained using the Microwave Assisted Extraction (MAE) and Ultrasound-Assisted Extraction (UAE) extraction methods on the growth of *Staphylococcus aureus* bacteria. Papaya seed was extracted using MAE and UAE extraction methods using 70% ethanol as a solvent in concentrations of 20%, 40%, 60%, and 80%. The antibacterial activity test method used the disk diffusion method. The parameter observed was DDH (Diameter of Inhibitory Area) which was statistically analyzed using Two Way Anova at a 95% confidence level. The results showed that the papaya seeds ethanol extract produced from the MAE and UAE extraction methods forming significantly different had antibacterial activity against *Staphylococcus aureus* in all concentration series. UAE method is recommended use.

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1. Introduction

Staphylococcus aureus is a pathogenic bacterium in humans that can cause infection. Infectious diseases are one of the leading causes of public health problems in developed and developing countries. Respiratory Tract Infections and pneumonia are examples of infections caused by *Staphylococcus aureus* (Agustina et al., 2019). Antibiotics are usually used to treat infectious diseases. However, excessive use of antibiotics and not by the rules of use can cause side effects of antibiotic resistance. Papaya seeds can be used for plant material medication treatment of infectious. Papaya (*Carica papaya* L.) grows in tropical areas such as Indonesia. Some parts of the papaya plant have antibacterial activity, starting from the seeds, skin, and leaves (Roni et al., 2018). Papaya seeds contain various compounds, such as tannins, flavonoids, phenols, terpenoids, alkaloids, carpains, and saponins (Wijayanti & Nisa Febrinasari, 2017). The ethanol extract from maceration method of papaya seeds has been shown antibacterial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa* (Torar et al., 2017), *Salmonella typhi* (Lestari et al., 2018), and *Escherichia coli* (Roni et al., 2018).

MAE and UAE are extraction methods that utilize ultrasonic waves. The use of MAE and UAE is faster and more efficient compared to conventional methods, saves energy, and reduces extraction costs (Fauzi'ah & Wakidah, 2019). The use of various extraction methods will affect the effectiveness of the extraction and retraction of compounds and also affect the content of chemical compounds, which can affect the antibacterial activity. Research conducted by Fauzi'ah & Wakidah (2019) stated that other parts of the papaya plant, which were papaya leaves extracted using the UAE method, contained saponins, flavonoids, terpenoids, alkaloids, and phenolic compounds. Ethanol extract of papaya leaves from the MAE extraction method is more efficient

and produces higher yields than the Soxhlet extraction method (Kumari & Maitra, 2020). The phytochemical screening test showed that the ethanol extract of papaya leaves extracted by MAE contained alkaloids, flavonoids, saponins, steroids, and glycosides. Previous research only knew the activity of papaya seed extract against *Staphylococcus aureus* bacteria, so it is necessary to know the use of modern methods to obtain much better activity with a shorter extraction time.

2. Materials and Methods

Papaya seeds (*Carica papaya* L.) obtained from Pongangan Village, Gunungpati District, Semarang City, Central Java, 70% ethanol, Muller Hinton Agar (MHA) media (Merck), Nutrient Broth (NB) media (Merck), media Nutrient Agar (NA), sterile distilled water, DMSO, disc paper, *Staphylococcus aureus* bacteria (Culture from Microbiology Laboratory, Faculty of General Medicine, University of Muhammadiyah Semarang), 0.9% NaCl solution (Brataco), standard solution Mc. Farland I (10^8 CFU/mL) and chloramphenicol antibiotic disk 30 g/disk.

2.1. Preparation of samples

3.73 kg of young papaya seeds aged 3-5 months were harvested, then carried out wet-processing, sorting of seeds, and drying at 40°C with oven, to produce 0.735 kg of powder with 19.70% of yield and 2.1% of moisture content with moisture balance.

2.1.1. Extraction

MAE method is performed by inserting 250 grams into a microwave tube plus 2500 mL of 70% ethanol solvent, then put into the MAE tool then extracted within 60 minutes at a temperature of 70 °C (Kumari & Maitra, 2020). In both UAE methods, as much as 250 grams were put into a glass beaker plus 2500 mL 70% ethanol solvent, then stirred until mixed and then covered with aluminium foil so that the solvent did not evaporate put into the UAE tool. Extraction was carried out within 60 minutes at 30°C with an extract: solvent ratio of 1:10 (Utami et al., 2020). Filtrate of the MAE and UAE methods were concentrated using a rotary evaporator to obtain a thick extract.

2.1.2. Papaya Seed Ethanol Extract Antibacterial Activity Test

The tools and materials were sterilized in an autoclave at 121°C for 15 minutes for the antibacterial activity test. MHA, NA and NB media were manufactured by heating until dissolved while stirring until homogeneous on an electric bath. The ethanol extract of papaya seeds was made into an 80% stock solution by weighing 4 grams and then dissolved using 5 mL DMSO step by step until dissolved. The stock solution was then diluted with concentrations of 20%, 40%, and 60% w/v using DMSO. DMSO was using negative control; the antibiotic chloramphenicol 30 µg/disk was using positive control. Each test solution and negative control was taken 10 µL, dripped on sterile disc paper, then waited for saturation in 10 minutes. Then the paper disc was affixed to the Mueller Hinton Agar media, which already contained the test bacteria. The media that had been pasted with paper discs was then incubated at 37°C for 24 hours. The test results were observed by looking at the Inhibitory Zone Diameter (DDH) formed around the paper disc after 24 hours.

2.2. Data Analyzes

Clear zone was formed around the paper disc indicates antibacterial activity from papaya seeds ethanol extract, which was expressed as the Inhibitory Zone Diameter (DDH). The data were analyzed statistically using SPSS version 16.0. DDH data from each concentration based on MAE and UAE extraction methods were carried out by homogeneity test (Levene's test) and normality test (Shapiro Wilk). DDH data is said to meet the normality and homogeneity test requirements if ($p > 0.05$), then continue the test with Two Way Anova at 95% confidence level and Independent T-Test.

3. Results and Discussion

Papaya seeds used in the study were 3.73 kg after the drying process and produced 0.735 kg of powder with 19.70% powder yield and 2.1% simplicia water content. The yield of papaya seed ethanol extract can be seen in Table 1.

Based on papaya seed ethanol extract (EEBP) yield, the MAE extraction method produced a higher extract yield than the UAE method. This is because the MAE extraction process uses the addition of heat, which helps the process of increasing the extraction. Another factor that can affect the extraction speed is high temperature. High temperatures can increase the desorption of active compounds from plants resulting in cell damage to the material (Jain et al., 2009). In the MAE method, stirring occurs where it aims to make the interaction between the solvent and the simplicia to achieve a faster equilibrium concentration of the extracted material into the liquid (Istiqomah, 2013). Ethanol extract of papaya leaves resulting from the MAE extraction method produces a yield of 12.55% more than the yield of the Soxhlet extraction method of 7.17% (Kumari & Maitra, 2020).

The organoleptic results of the thick extract of papaya seeds obtained from the MAE and UAE extraction methods have almost the same characteristics: the extract has a thick texture, blackish brown color, and a distinctive smell of papaya seeds. The following pictures display the thick extract of papaya seeds from the MAE and UAE extraction methods in Figure 1.

Papaya seed ethanol extract have antibacterial activity against *Staphylococcus aureus* bacteria was carried out using the agar diffusion method. The test solution used was an ethanol extract of young papaya seeds with a solution concentration of 20%, 40%, 60%, and 80% were obtained from the two extraction methods, MAE and UAE. Both ways were used to compare the Inhibitory Zone Diameter (DDH) formed around the paper disc. The results of the antibacterial activity test of papaya seed ethanol extract can be seen in Figure 2.

Table 1. Yield Results of Papaya Seed Ethanol Extract Extraction Methods MAE and UAE

Extraction Methods	Extract Yield
MAE	11,08 %
UAE	7,99 %



Figure 1. Papaya Seed Ethanol Extract Results MAE (A) and UAE (B)

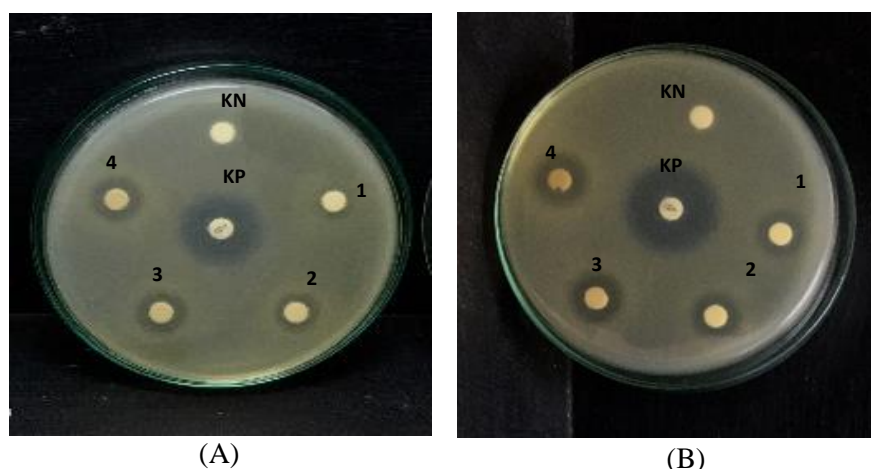


Figure 2. Display of Antibacterial Activity Test Results of Papaya Seed Ethanol Extract Results of MAE (A) and UAE (B) Extraction Methods. Concentration extract: 20% (1), 40% (2), 60% (3), 80% (4); DMSO (KN) and chloramphenicol 30 µg/disk (KP)

Table 2. Results of Antibacterial Activity of Papaya Seed Ethanol Extract Extraction Methods MAE and UAE Against *Staphylococcus aureus*

Extraction Methods	Treatment	Inhibitory Zone Diameter (mm)			
		I	II	III	Average±SD
MAE	EEBP 2000 µg/disk	9.79	9.80	9,85	9,81±0,32
	EEBP 4000 µg/disk	10.34	10.64	10.69	10.56±0,19
	EEBP 6000 µg/disk	11.10	11.80	11.74	11.55±0,39
	EEBP 8000 µg/disk	11.15	11.99	12.28	11.81±0,59
	Chloramphenicol 30 µg/disk	20.30	21.05	20.65	20.67±0,37
	DMSO	-	-	-	-
UAE	EEBP 2000 µg/disk	10.80	10.68	10.15	10.54±0,34
	EEBP 4000 µg/disk	11.86	11.49	11.82	11.72±0,20
	EEBP 6000 µg/disk	12.54	12.45	12.00	12.33±0,29
	EEBP 8000 µg/disk	12.61	12.72	12.42	12.58±0,15
	Chloramphenicol 30 µg/disk	20.75	20.85	20.10	20.57±0,41
	DMSO	-	-	-	-

Information:

EEBP 2000 µg/disk equivalent to EEBP Concentration 20%

EEBP 4000 µg/disk equivalent to EEBP Concentration 40%

EEBP 6000 µg/disk equivalent to EEBP Concentration 60%

EEBP 8000 µg/disk equivalent to EEBP Concentration 80%

EEBP = Papaya Seed Ethanol Extract

(-) = No obstacle

Antibacterial activity test of papaya seed ethanol extract (EEBP) using the MAE and UAE extraction methods against *Staphylococcus aureus* bacteria showed the presence of Radical Inhibitory Power (DDH) which was characterized by a clear area around the paper disc that was not overgrown with bacteria (Djarot et al, 2020). The ethanol extract maceration method of papaya seeds has been shown to have antibacterial activity against *Staphylococcus aureus*, it ranged 6,00-7,00 mm (Torar et al., 2017). Table II shows the average DDH results above show that the MAE method obtained DDH values ranging from 9.81-11.81 mm, while for the UAE method, it ranged from 10.54-12.58 mm. DDH data on antibacterial activity test of ethanol extract of papaya seeds using MAE and UAE methods showed the higher the concentration, the greater the inhibitory power. This is by the statement of, that the higher the attention of an antibacterial agent, the stronger the antibacterial activity. Increasing the concentration of substances causes an increase in the content of active compounds that function as antibacterial so that the ability to kill bacteria is also greater (Bernier and Surette, 2013).

The results of the One Way Anova statistical test followed by the Pos Hoc test with MAE and UAE methods in all concentration series resulted in significant differences in DDH values, meaning that there was a difference in the diameter of the inhibitory zone between concentrations of 20%, 40%, 60%, 80%, and 30 g chloramphenicol because significance value <0.05. The Two Way Anova statistical test showed a significant difference between the two extraction methods

because it had a significance value of 0.00 ($p < 0.05$). Meaning that the difference in the extraction method gave a significant difference to the DDH value.

The results of the research that has been performed show that there are differences in DDH between the MAE extraction method and the UAE extraction method so in future studies, it is recommended to use the UAE extraction method to test the antibacterial activity of papaya seed ethanol extract. In UAE, extraction does not use heat, so it is possible that the active compounds contained in the plant are not damaged by heating. The UAE extraction mechanism involves two physical phenomena, which are diffusion through the cell wall of plant parts and the breakdown of the cell wall using ultrasonic waves, which then release the cell contents into the extraction medium (Endarini, 2016).

4. Conclusion

There was a significant difference between the MAE and UAE extraction methods against *Staphylococcus aureus* bacteria. Papaya seeds ethanol extract from the MAE and UAE extraction methods had antibacterial activity against *Staphylococcus aureus*. UAE method is recommended use.

Author Contributions: Devi Nisa Hidayati conceived and designed the study. Devi Nisa Hidayati and Fini Hartanti performed all data analyses, interpreted the results, revised the paper, and approved the final manuscript. Devi Nisa Hidayati wrote the manuscript.

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Competing Interests

The authors declare no conflict of interest.

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