Antibacterial Activity And Determination Of Total Phenol And Flavonoid Of *Carica Papaya* L. Ethanol Extract

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Abstract
Infectious diseases are Indonesia's most common. Antibiotics are used to treat infections. Medicine needs new antibiotics due to antibiotic resistance. Antibiotics from plants are possible. Papaya leaf carpain alkaloids may be antibacterial. This study determined papaya leaf compound content, flavonoids' total phenol content, and the optimal concentration of papaya leaf ethanol extract that inhibits Staphylococcus aureus and Pseudomonas aeruginosa. The first stages of the research were the maceration of papaya leaf extract with 96% ethanol solvent, the phytochemical screening test (alkaloid, flavonoid, saponin, tannin, and steroid and triterpenoid tests), total phenol and total flavonoids tests, and the paper disc antibacterial activity test. Alkaloids, flavonoids, glycosides, tannins, steroids, and saponins were found in papaya leaf ethanol extract. Papaya leaf ethanol extract has 59.05 mgQE/g flavonoids and 56.80 GAE/g phenols. The antibacterial activity test of papaya leaf ethanol extract measured the diameter of the inhibitory zone on Staphylococcus aureus and Pseudomonas aeruginosa. Alkaloids, flavonoids, glycosides, tannins, steroids, and saponins were found in papaya leaf ethanol extract. Papaya leaf ethanol extract has 59.05 mgQE/g flavonoids and 56.80 GAE/g phenols. Papaya leaf ethanol extract had the best inhibition zone diameter for Staphylococcus aureus at 150 mg/mL (12.85 mm) and Pseudomonas aeruginosa at 150 mg/mL (13.10 mm).

Keywords: *Papaya leaf, Flavonoid, Fenol, Staphylococcus aureus, and Pseudomonas aeruginosa*

I. INTRODUCTION

The papaya plant (*Carica papaya* L.) is widely used in traditional medicine and is a member of the Caricaceae family. The papaya plant is a popular plant among humans. Papaya plants can thrive in various environments in Indonesia; their growth period is relatively brief. The chemical components in papaya leaves are antiseptic, anti-inflammatory, anti-fungal, and antibacterial [1]. The papaya leaf extract contains both papain and carpain alkaloids. The papain enzyme possesses proteolytic and antibacterial properties, while the carpain alkaloids have antibacterial properties [2]. Papain, chymopapain, cystatin, -tocopherol, ascorbic acid, flavonoids, cyanogenic glucosides, and glucosinolates are among the active components found in papaya leaves that might boost total antioxidant capacity and decrease levels of lipid peroxidation [3]. The papaya (*Carica papaya* L.) leaves contain the alkaloids carpainin, carpain, and pseudocarpain, as well as the vitamins C and E, choline, and carposid. Benzyl isothiocyanate is a glucosinolate found in papaya leaves. Papaya leaves include antibacterial substances such as tannins, alkaloids, flavonoids, terpenoids, and saponins [1], [4].Bacteria are included in single-celled microorganisms and reproduce by dividing. Bacteria vary in size, with a cross-section of about 0.7 – 1.5 and a length of about 1 – 6. The individual cells of bacteria can be shaped like ellipses, balls, rods, or spirals.

Each of these features characterizes the morphology of a species [5], [6]. *Staphylococcus aureus* and *Pseudomonas aeruginosa* are pathogenic bacteria that often infect human skin. *Staphylococcus aureus* is one of the most common pathogens, and the bacterium is a gram-positive bacterium. *Pseudomonas aeruginosa* is a bacterium belonging to the Gram-negative bacteria that are rod-shaped, aerobic, and opportunistic [7], [8]. *Pseudomonas aeruginosa* is usually found in moist environments. These bacteria form saprophytic colonies in healthy humans and can cause disease when the body's defences weaken [1], [9].Adverse consequences from irrational antibiotic usage, overuse and long-term use can result in antibiotic resistance in

microorganisms [10]. Another option for treating and preventing side effects is to use active plant-derived compounds. Papaya leaves contain carpain, carikaksantin, violaksatin, papain, saponins, flavonoids, tannins, and saponins, all of which have the potential to be developed [11]. This research aims to identify the chemical components found in papaya leaves, the total phenol and flavonoid content, and to investigate the antibacterial activity of an ethanol extract from papaya leaves.

II. METHODS

2.1 Materials

Papaya leaves (*Carica papaya* L.) were obtained from Padang Bulan, North Sumatra, and transported to the laboratory in new condition after the papaya leaves were cleaned thoroughly. Made Simplisia to be tested.

2.2 Extraction Process

The papaya leaves used in this study were from a garden in Padang Bulan, Medan. The papaya leaves are separated from the stems and other components. Following their cleansing and rinsing, papaya leaves are dried. The dried papaya leaves are ground to a fine powder [12]. Using a rotary evaporator, evaporate at 40°C until a concentrated extract is obtained [12]. 500 g of papaya leaf powder was extracted using the maceration technique with 5 L of 96% ethanol solvent, agitated for the first 6 hours, allowed to stand for 18 hours with intermittent stirring, and filtered with maceration filter paper No. I. Using 2.5 L of the 96% ethanol obtained from the second maceration, repeat the extraction process on the pulp and combine the results[13].

2.3 Phytochemical Screening

The goal of the phytochemical screening test is to assess the secondary metabolite content of the papaya leaf extract. This phytochemical screening test begins with the saponin test, which requires 0.5 grams of a papaya leaf extract from the extraction results, 0.5 milliliters of heated water, and one minute of shaking. If the solution foams, add 1% HCl and wait 10 minutes; if the foam remains, the extract includes saponins. Flavonoid test, specifically 0.5 grams of a papaya leaf extract from the extraction results added with 1-2 ml of hot water and a small amount of magnesium (Mg) powder and shaken until mixed; then added 4-5 drops of 37% HCl and 4-5 drops of 95% ethanol% and shaken until mixed [14]. Observing the color change in the extract solution, the extract contained flavonoids if a red, yellow, or orange hue occurred. 0.5 grams of a papaya leaf extract from the extraction results are combined with 1-2 millilitres of water and two drops of 1% FeCl to determine the tannin concentration. When the extract solution generates a bluish-green tint, it is determined to contain tannins [15].

2.4 Determination of Fenol Content

To get 500 ppm gallic acid, 25 mg was weighed, dissolved in 1 methanol, and added to 50 mL of distilled water. Pipetting 0.4 mL of a 500 ppm gallic acid standard solution into a 10 mL flask yielded 20 ppm. After pipetting 0.5 mL Folin-Ciocalteau, 1-minute vortexing, and 1 mL 20% Na_2CO_3, the mixture was incubated for 35 minutes. From 400 to 800 nm, a UV-Vis spectrophotometer measured the maximum wavelength of a 500 ppm gallic acid reference solution. After using a 0.5 mL pipette, 0.5 mL Folin-Ciocalteau, and one minute of vortexing, 1 mL of 20% Na_2CO_3 was added. 738 nanometers was the longest wavelength detected by a UV-Vis spectrophotometer. Combining 10 mg of condensed papaya leaf extract, 1 mL of methanol, and 10 mL of distilled water yields a 1000 ppm concentration. Incubate for 35 minutes after pipetting 0.5 mL of a 1000 ppm concentrated solution, 1 mL of distilled water, 0.5 mL of Folin-Ciocalteau, 1 minute of vortexing, and 1 mL of 20% Na_2CO_3. UV-Vis spectrophotometry at a maximum wavelength of 738 nm measured the absorbance of each reagent (blank) solution concentration [16].

2.5 Determination of Flavonoid Content

10 mg of quercetin in 100 mL of methanol yields a 100 ppm solution. Pipette a 2 ml 20 ppm solution into a 10 ml flask. Incubate 0.5 mL of 100 ppm quercetin standard mother liquor with 0.1 mL $AlCl_3$, 0.1 mL CH3COONa, and 2.8 mL distilled water for 25 minutes. 10 mg of viscous extract was weighed and diluted in 10 mL methanol to reach 1,000 ppm. Transfer 0.5 mL of the solution to a pipette, add 0.1 mL of $AlCl_3$ and CH_3COONa and incubate for 25 minutes in 2.8 mL of distilled water. UV-Vis spectrophotometry measured

absorbance at 430 nm [17]. UV-Vis spectrophotometers measured the highest wavelength between 400 and 800 nanometers.

2.6 Antibacterial Activity

The paper disc diffusion method was used to test for antibacterial activity. Antibacterial test results for bacterial growth formed around the paper disc by measuring the diameter of the inhibition zone. Each extract at a different concentration (100 mg/mL; 75 mg/mL; 50 mg/mL; 25 mg/mL; 5 mg/mL; and 1 mg/mL) [18] was dripped onto a paper disc. The suspension of the test bacteria was put in the Nutrient agar medium. The cup containing the bacterial test suspension was left for a few moments, and then the disc paper dripped with EEDP was placed in the cup. Incubated at 37ºC for 24 hours, the diameter of the formed inhibition zone was measured with a calliper. The test was carried out with three repetitions [19].

III. RESULT AND DISCUSSION

3.1 Extraction od Papaya Leaves

The results of maceration of papaya leaf simplicia powder (*Carica papaya* L.) as much as 1190 grams using 96% ethanol solvent, then evaporated with a rotary evaporator at 40ºC, obtained a viscous extract of 258 grams (21.60% yield).

3.2 Phytochemical Screening

Flavonoids, alkaloids, saponins, tannins, and terpenoids are found in papaya leaf ethanol extract. Table 1 displays the findings of the phytochemical screening.

Table 1. Result of Phytochemical Screening of Papaya Leaf Extract

Chemical Compounds	Result
Alkaloids	+
	+
	+
Flavonoids	+
Glycoside	+
Saponins	+
Tannins	+
Triterpenoids/Steroids	+

The ethanol extract of papaya leaves contains alkaloids, flavonoids, glycosides, saponins, tannins, and triterpenoids, according to the results of the phytochemical screening study. This builds on prior research using the maceration procedure with 96% ethanol solvent to develop phytochemical molecules that are secondary metabolite positive [19]. Alkaloids, flavonoids, tannins, and saponins are antibacterial compounds [18].

3.3 Determination of Phenol Content

The results of determining the total phenolic content in the ethanol extract of papaya leaves can be seen in Table 2.

Table 2. Total phenol levels in papaya leaf ethanol extract

Sample Weight (g)	Sample Volume (mL)	Absorbance	Concentration (µg/mL)	Total Rate (mgGAE/g extract)	Mean of Rate (mgGAE/g extract)
0,0103	10	0.708	55.89	54.26	57.80
0,0101	10	0.688	54.26	53.72	
0,0109	10	0.898	71.33	65.44	

Use the gallic acid standard curve to calculate papaya leaf ethanol extract phenolic content. Papaya leaf ethanol extract had 57.80 GAE/g phenolics. Polyphenols are a class of plant-based chemical compounds. This chemical is distinguished by the presence of many phenol groups in its molecule. Since polyphenols are polar, polar solvents can extract these chemicals [20], [21]. Polyphenols contribute to plant pigmentation, such as leaf color. The polyphenol concentration can shield cells from damage caused by free radicals [22]. One of the causes of degenerative diseases, including cancer, atherosclerosis, stroke, rheumatism, and heart disease, is free radicals [10], [23], [24].

3.4 Determination of Flavonoid Content

The results of determining the total levels of flavonoids in the ethanol extract of papaya leaves can be seen in Table 3.

Table 3. Total Flavonoid levels in papaya leaf ethanol extract

Sample Weight (g)	Sample Volume (mL)	Absorbance	Concentration (µg/mL)	Total Rate (mgGAE/g extract)	Mean of Rate (mgGAE/g extract)
0,0103	10	0.746	59.47	57.74	59.05
0,0101	10	0.743	59.22	58.63	
0,0109	10	0.829	66.29	60.80	

According to the table above, it can be concluded that the total level of flavonoids in the ethanol extract of papaya leaves is 59.05 mg QE/g extract. Flavonoids such as quercetin are known as potential antioxidants. The compound used as a standard in determining flavonoid levels is quercetin because quercetin is a flavonol class of flavonoids. Flavonoids which are useful as antioxidants, act as messenger molecules in interactions between cells; anti-inflammatory by interrupting the effects of arachidonic acid metabolic pathways, influencing prostaglandin production and histamine release; antitumor by interrupting tumor promoter activity; and antivirals are thought to interrupt nucleic acid synthesis [25].

3.5 Antibacterial Activity of Papaya Leaf Ethanol Extract against *Staphylococcus aureus* and *Pseudomonas aeruginosa*

The result of antibacterial activity can be seen in Table 4.

Table 4. Results of antibacterial activity test of papaya leaf ethanol extract against bacteria *Staphylococcus aureus*

Concentration (mg/mL)	Diameter Inhibitory Zones (mm)			
	I	II	III	X±SD
1	8.7	8.3	8.4	8.46 ± 0.20
5	8.9	8.8	8.7	8.80 ± 0.10
25	9.5	9.0	9.0	9.16 ± 0.28
50	11.0	10.8	10.8	10.86 ± 0.11
75	11.4	11.5	10.9	11.26 ± 0.32
100	12.0	11.8	11.5	11.76 ± 0.25
125	12.5	12.0	12.2	12.23 ± 0.40
150	12.7	12.8	12.85	12.78 ± 0.32

The concentration of the ethanol extract of papaya leaves has an effect on the diameter of the inhibition zone; the wider the zone that results from increasing the concentration of the extract, the smaller the diameter of the zone. At an extract concentration of 150 mg/mL, the papaya leaf ethanol extract with the biggest diameter (12.78 mm) inhibited the development of Staphylococcus aureus bacteria. The concentration with lowest concentration is 1 mg/mL, which is considered inactive.The antibacterial activity strength can be categorized into four groups: 9 mm (inactive), 9-12 mm moderately active, 13-18 mm active, and 18 mm very active. Based on the criteria for the degree of antibacterial activity, it was determined that at concentrations of 50 mg/mL, 75 mg/mL, and 100 mg/mL, the antimicrobial activity of the ethanol extract of papaya leaves is somewhat active (9 - 12 mm) [26].

Table 5. Results of antibacterial activity test of papaya leaf ethanol extract against bacteria *Pseudomonas aeruginosa*

Concentration (mg/mL)	Diameter Inhibitory Zones (mm)			
	I	II	I	X± SD
1	9.5	9.9	9.5	9.60 ± 0.23
5	9.7	10.1	9.9	9.90 ± 0.20
25	10.2	10.3	10.4	10.30 ± 0.10
50	12.0	11.6	11.0	11.53 ± 0.25
75	12.0	11.8	11.2	11.60 ± 0.43
100	12.4	11.6	11.8	12.02 ± 0.41
125	12.5	12.8	13.0	12.76 ± 0.37
150	13.10	13.0	13.2	13.10 ± 0.25

The antibacterial activity of the ethanol extract of papaya leaves with the largest diameter to inhibit the growth of Pseudomonas aeruginosa was only partially active at a concentration of 150 mg/mL and a diameter of 13.10 mm. This was the case even though the extract had a diameter of 13.10 mm. The concentration of the extract affects the diameter of the inhibition zone; the greater the concentration of the extract, the greater the diameter of the inhibition zone. Antibacterial activity strength can be classified into four groups: 9 millimeters (inactive), 9-12 millimeters (moderately active), 13-18 millimeters (active), and 18 millimeters (very active). Based on the criteria for antimicrobial activity potency, it was determined that at 50 mg/mL, 75 mg/mL, and 100 mg/mL, the ethanol extract of papaya leaves possesses moderate antibacterial activity (9 - 12 mm) [26].Due to the action of bioactive components in the ethanol extract of papaya leaves, papaya leaf extract can limit the establishment of bacterial colonies if clear spaces appear around the discs. It is believed that papaya leaves include alkaloids, flavonoids, tannins, saponins, and steroids as bioactive substances [27].

IV. CONCLUSION

The conclusion of this study shows that the ethanol extract of papaya leaves has phenol, flavonoid and antibacterial levels. Papaya leaf extract is a plant that grows around the community and has many benefits.

REFERENCE

[1] M. Muhammad, N. Nasri, V. E. Kaban, D. Satria, and H. Cintya, 'Antibacterial Potential Ethanol Extract of Papaya Leaves (Carica papaya Linn.) Towards Salmonella typhi', ***BEST Journal (Biology Education, Sains and Technology)***, vol. 5, no. 2, pp. 265–270, 2022.

[2] J. F. Rehena, 'Uji Aktivitas Ekstrak Daun Pepaya (Carica papaya. LINN) sebagai Antimalaria in vitro', ***Jurnal Ilmu Dasar,*** vol. 11, no. 1, pp. 96–100, 2010.

[3] U. B. S. Dimas, Y. M. D. Arina, and M. N. Amin, 'Pengaruh Ekstrak Daun Pepaya Terhadap Jumlah Sel Limfosit Pada Gingiva Tikus Wistar Jantan Yang Mengalami Periodontitis (The effect of papaya leaves extract to the number of lymphocytes cells to the male-wistar rat's gingiva that undergo periodontitis)', *Pustaka Kesehatan*, vol. 2, no. 1, pp. 50–57, 2014.

[4] M. Parle and A. Gurditta, 'Basketful benefits of papaya', ***Int. Res. J. Pharm,*** vol. 2, no. 7, pp. 6–12, 2011.

[5] D. Meilani and M. Y. Kusumastuti, 'Formulasi Gel Ekstrak Etanol Daun Afrika (Vernonia amygdalina Del) sebagai Antibakteri Staphylococcus aureus dan Pseudomonas aeruginosa', *Prosiding SainsTeKes*, vol. 1, pp. 1–6, 2019.

[6] M. J. Pelczar and E. C. S. Chan, 'Dasar-Dasar Mikrobiologi Jilid I. Jakarta'. Ui Press, 2008.

[7] W. W. Davis and T. R. Stout, 'Disc plate method of microbiological antibiotic assay: II. Novel procedure offering improved accuracy', *Applied microbiology*, vol. 22, no. 4, pp. 666–670, 1971.

[8] D. J. Dr, 'Duke's Phytochemical and Ethnobotanical Databases', *Artemisia annua*, 2005.

[9] G. J. Tortora, B. R. Funke, and C. L. Case, 'Microbiology: An Introduction San Francisco'. Benjamin Cummings, 2001.

[10] B. J. Howard and J. C. Rees, 'Host-parasite interactions: mechanisms of pathogenicity', *Clinical and Pathogenic Microbiology. 2nd ed. USA: Mosby-Year Book Inc*, 1994.

[11] E. Septiningsih, 'Efek Penyembuhan Luka Bakar Ekstrak Etanol 70% Daun Pepaya (Carica Papaya L.) Dalam Sediaan Gel Pada Kulit Punggung Kelinci New Zealand', PhD Thesis, Universitas Muhammadiyah Surakarta, 2009.

[12] N. Nasri, V. E. Kaban, K. Gurning, H. D. Syahputra, and D. Satria, 'Aktivitas Antibakteri Ekstrak Etanol Daun Pepaya (Carica papaya Linn.) Terhadap Bakteri Pseudomonas aeruginosa', ***INSOLOGI: Jurnal Sains dan Teknologi,*** vol. 1, no. 3, Art. no. 3, Jun. 2022, doi: 10.55123/insologi.v1i3.438.

[13] R. I. Kemenkes, 'Farmakope Herbal Indonesia Edisi II', *Jakarta: Kementrian Kesehatan RI*, 2017.

[14] H. M. Nasution, R. Yuniarti, Z. Rani, and A. Nursyafira, 'Phytochemical Screening And Antibacterial Activity Test Of Ethanol Extract Of Jengkol Leaves (Archidendron Pauciflorum Benth.) IC Nielsen Against Staphylococcus Epidermidis And Propionibacterium Acnes', ***International Journal of Science, Technology & Management,*** vol. 3, no. 3, pp. 647–653, 2022.

[15] Q. Lathifah, 'Uji efektifitas ekstrak kasar senyawa antibakteri pada buah belimbing wuluh (Averrhoa bilimbi L.) dengan variasi pelarut', PhD Thesis, Universitas Islam Negeri Maulana Malik Ibrahim, 2008.

[16] M. Škerget, P. Kotnik, M. Hadolin, A. R. Hraš, M. Simonič, and Ž. Knez, 'Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities', *Food chemistry*, vol. 89, no. 2, pp. 191–198, 2005.

[17] C.-C. Chang, M.-H. Yang, H.-M. Wen, and J.-C. Chern, 'Estimation of total flavonoid content in propolis by two complementary colorimetric methods', ***Journal of food and drug analysis,*** vol. 10, no. 3, 2002.

[18] N. Nasri, V. E. Kaban, H. D. Syahputra, and D. Satria, 'Aktivitas Antibakteri Ekstrak Etanol Daun Alpukat (Persea americana Mill) Terhadap Escherichia coli, Salmonella typhi, dan Pseudomonas aeruginosa', ***Herbal Medicine Journal,*** vol. 5, no. 1, pp. 13–19, 2022.

[19] V. E. Kaban, N. Nasri, H. Dharmawan, and D. Satria, 'Formulasi dan Uji Efektivitas Sabun Pencuci Tangan dari Ekstrak Daun Pandan (Pandanus amaryllifolius Roxb.) Terhadap Bakteri Salmonella sp.', ***Herbal Medicine Journal,*** vol. 5, no. 1, pp. 8–12, 2022.

[20] J. B. Harbone, 'Metode Fitokimia: Penuntun Cara Modern Menganalisa Tumbuhan, Terjemah Padmawinata, K', *Dan Soediro, I., Edisi Ii, Penerbitan ITB, Bandung*, 1987.

[21] A. Syah, 'EFEKTIVITAS TANAMAN PEPAYA (Carica papaya L.) TERHADAP PENYEMBUHAN LUKA: A NARRATIVE REVIEW', PhD Thesis, Skripsi, Universitas Muhammadiyah Magelang, 2021.

[22] A. Roni, M. Maesaroh, and L. Marliani, 'Aktivitas antibakteri biji, kulit dan daun pepaya (Carica papaya L.) terhadap bakteri Escherichia coli dan Staphylococcus aureus', *Kartika:* ***Jurnal Ilmiah Farmasi****,* vol. 6, no. 1, pp. 29–33, 2019.

[23] F. Pourmorad, S. J. Hosseinimehr, and N. Shahabimajd, 'Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants', ***African journal of biotechnology,*** vol. 5, no. 11, 2006.

[24] Y. Y. Andriani, I. Rahmiyani, S. Amin, and T. Lestari, 'kadar fenol total ekstrak daun dan biji pepaya (Carica papaya L) menggunakan metode spektrofotometri UV-VIS', ***Jurnal Kesehatan Bakti Tunas Husada: Jurnal Ilmu-ilmu Keperawatan, Analis Kesehatan dan Farmasi,*** vol. 15, no. 1, pp. 73–78, 2016.

[25] P. P. A. Bangun, A. P. Rahman, and H. Syaifiyatul, 'Analisis kadar total flavonoid pada daun dan biji pepaya (carica papaya l.) Menggunakan metode spektrofotometer Uv-Vis', ***Jurnal Ilmiah Farmasi Attamru (JIFA),*** vol. 2, no. 1, pp. 1–5, 2021.

[26] T. M. de A. Alves *et al.*, 'Biological screening of Brazilian medicinal plants', *Memórias do Instituto Oswaldo Cruz*, vol. 95, pp. 367–373, 2000.

[27] N. K. Jati, A. T. Prasetya, and S. Mursiti, 'Isolasi, identifikasi, dan uji aktivitas antibakteri senyawa alkaloid pada daun pepaya', ***Indonesian Journal of Mathematics and Natural Sciences,*** vol. 42, no. 1, pp. 1–6, 2019.