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Phytochemical Investigation and Antibacterial Activity Ethanol Extract of Papaya Seeds (*Carica papaya* L.) Applicated for Gel Product

(Tinjauan Fitokimia dan Aktivitas Antibakteri Ekstrak Etanol Biji Pepaya (*Carica papaya* L.) diaplikasikan untuk Sediaan Gel)

WAHYUNING SETYANI*, JACINDA YAKUB, OCTAVIANUS YANDRI, VIOLA RESTI KAWAN, THERESIA JENNY HARYANTO, I MADE MYASA DARMIKA

Faculty of Pharmacy, Sanata Dharma University, Yogyakarta

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Abstract: Papaya seeds has many benefits in medicine. One of the benefits that have been studied is it's antibacterial activity against *Staphylococcus aureus*. This study aims to determine the phytochemical compounds of papaya seed ethanol extract and their antibacterial activity against *Staphylococcus aureus* bacteria. The ethanol extract of papaya seeds was obtained through maceration method using 95% ethanol as a solvent. Maceration was carried out by weighing 85 g of simplicia powder, macerated using 500 mL of 95% ethanol then left for 4 days while stirring occasionally, then filtered to produce filtrate and residue. The remaining residue is then soaked again (remaserated) with 250 ml of 95% ethanol and left for 2 days. After 2 days, the sample is filtered to produce filtrate and residue. All filtrates were evaporated using a rotary evaporator to obtain a thick extract. Phytochemical screening results showed the presence of flavonoids, alkaloids, tannins, saponins, and phenolic compounds in ethanol extracts of papaya seeds. The antibacterial activity test of papaya seed ethanol extracts towards *Staphylococcus aureus* was carried out through in vitro by disc diffusion method with several concentrations. Antibacterial test showed that concentrations of 2%, 4%, and 6% of papaya seeds ethanol extracts was moderate antibacterial activity and at concentrations of 8% and 10% the antibacterial activity was classified as strong against *Staphylococcus aureus*.

Keywords: Ethanol extract of papaya seeds (*Carica papaya* L.), *Staphylococcus aureus*.

Abstrak: Biji pepaya memiliki banyak manfaat dalam pengobatan. Salah satu manfaat yang telah dipelajari adalah aktivitas antibakteri terhadap *Staphylococcus aureus*. Penelitian ini bertujuan untuk mengetahui senyawa fitokimia dari ekstrak etanol biji pepaya dan aktivitas antibakterinya terhadap bakteri *Staphylococcus aureus*. Ekstrak etanol biji pepaya diperoleh melalui metode maserasi menggunakan pelarut etanol 95%. Maserasi dilakukan dengan menimbang 85 g serbuk simplisia, dimaserasi menggunakan 500 mL etanol 95% kemudian dibiarkan selama 4 hari sambil diaduk sesekali, kemudian disaring untuk memperoleh filtrat dan residu. Sisa residu kemudian direndam kembali (diremaserasi) dengan 250 mL etanol 95% dan dibiarkan selama 2 hari. Setelah 2 hari, sampel disaring untuk memperoleh filtrat dan residu. Semua filtrat diuapkan menggunakan *rotary evaporator* untuk mendapatkan ekstrak kental. Skrining fitokimia menunjukkan adanya senyawa flavonoid, alkaloid, tanin, saponin, dan fenolik pada ekstrak etanol biji pepaya. Uji aktivitas antibakteri ekstrak etanol biji pepaya terhadap bakteri *Staphylococcus aureus* dilakukan secara *in vitro* dengan metode difusi cakram pada beberapa konsentrasi. Pada konsentrasi 2%, 4%, dan 6% aktivitas antibakteri ekstrak etanol biji pepaya tergolong sedang dan pada konsentrasi 8% dan 10% aktivitas antibakterinya tergolong kuat terhadap bakteri *Staphylococcus aureus*.

Kata kunci: Ekstrak etanol biji pepaya (*Carica papaya* L.), *Staphylococcus aureus*.

*Correspondence author
E-mail: wahyuningsetyani@usd.ac.id

INTRODUCTION

PAPAYA (*Carica papaya* L.) is one of the plants originated from Central America and West Indies and even areas around Mexico and Costa Rica. Papaya is known by various names such as bal, betik, pisang pelo (Sumatra), gedang, katerla gantung, kates (Java), hango, kampaja, ketes (Nusa Tenggara). Papayas are widely planted, both in the tropics and subtropics, in wet and dry areas or in plains and mountains up to 1000 meters above sea level. Almost all parts of papaya plants can be used for various purposes[4].

In some areas, papaya are used as traditional medicine such as intestinal worms, kidney stones, wound treatment, eczema (roots), antifever, abortivum, enlarged liver and spleen (seeds), hepatitis (flowers) [4]. As fresh fruit, papaya is consumed because of its good nutrition. The price is also relatively affordable compared to other fruits[8]. Papaya cultivation is increasing from year to year. Papaya cultivation is increasing in line with the amount of papaya seeds that are wasted.

Papaya seeds that become waste are still rarely used. In fact, papaya seeds are rich in benefits. Several studies have stated that papaya seeds have antibacterial benefits. The ethanol extract of ripe papaya seeds can be used to reduce the activity of *Staphylococcus aureus* with the highest inhibitory zone, which is 10 mm. In addition, research conducted by Torar *et al.*, states that at concentrations of 20%, 40%, 60%, and 80% ethanol extracts of papaya seeds have the activity of inhibiting *Pseudomonas aeruginosa* and *Staphylococcus aureus* in the medium category[5].

Papaya seeds extract (*Carica papaya* L.) extracted by maceration method using ethanol, chloroform, and benzene solvents contains secondary metabolites such as alkaloids, flavonoids, tannins, phenols and saponins[2]. The inhibition mechanism of each phytochemical compound is different, so it is expected that the inhibition result is wider. However, differences in varieties and conditions of papaya growth will affect the secondary metabolites produced. Therefore, this study will identify secondary metabolites of papaya seeds from the papaya plants cultivated in California varieties which can then be used for gel formulations to treat the bacterial infection of *Staphylococcus aureus*.

MATERIALS AND METHODS

MATERIALS. **Plant Materials**. Wet papaya seeds (*Carica papaya* L.) was obtained from Pandowoharjo hamlet education tourism village, Yogyakarta, Central Java, Indonesia.

Chemicals: All chemicals and solvents used were analytical grade. Sodium hydroxide 10%, ferric chloride, Mayer's reagent, Wagner's reagent, nutrient agar (Merck, Germany), and nutrient broth (Merck, Germany), micropipette (Pipetman Kit), autoclave, 10% DMSO, rotatory evaporator (buchi rotavator R-300) and nephelometer (poenix spec ref 440910).

METHODS. **Phytochemical Screening Procedure (Qualitative).** Test for flavonoids: Flavonoid test about 1 mL extract is added with a few drops of 10% NaOH. The appearence of orange shows the presence of flavonoids[3].

Test for Tannins. The extract is boiled with 20 mL of water and then filtered. A few drops of $FeCl_3$ were added to the sample. Positive reaction if greenish-brown or black-blue color is formed which indicates tannin[7].

Test for Saponin. The extract is boiled with 20 mL of water in a water bath. The filtrate is shaken and allowed to stand for 15 minutes. The formation of a stable foam showed positive samples containing saponins[3].

Test for Alkaloids. Alkaloid test about 1 mL of extract was added 2 drops of Mayer's reagent solution. The appearence of formation of white or yellow lumpy deposits shows the presence of alkaloids[3].

Test for Phenolic. The extract is diluted to 5 mL with distilled water. To that add a few drops of neutral 5% ferric chloride solution. The dark green color indicates the presence of phenolic compounds[5].

Phytochemical Screening Procedure (Quantitative). TLC test of Flavonoid. Stationary phase: Silica Gel 60 F_{254}, mobile phase: hexane: ethyl acetate : formic acid (6:4:0,2), standard : Quercetin 10mg/1mL ethanol, detector : sitroborate.

TLC Test of Tannin. Stationary phase: Silica Gel 60 F_{254}, mobile phase: ethyl acetate: formic acid: toluene: aquadest (6:1,5:3:0,5), standard : tannin 10mg/1mL ethanol, detector : $FeCl_3$.

TLC Test of Phenolic. Stationary phase: Silica Gel 60 F_{254}, mobile phase: ethyl acetate: formic acid: toluene: aquadest (6:1.5:3:0.5), standard : gallic acid 10 mg/ 1mL ethanol, detector : $FeCl_3$

TLC Test of Saponin. Stationary phase: Silica Gel 60 F_{254}, mobile phase: Chloroform: methanol: aquadest (64:50:1), standard: saponin 10mg/1mL ethanol, detector : Lieberman Burchard.

Antibacterial Activity Test. Testing the antibacterial activity of papaya seed ethanol extract to *S. aureus* was carried out using a 6 mm diameter paper disc. 0.1 mL culture of *S. aureus* in NB was added to 15 mL NA which was already solid. Ethanol extract

of papaya seeds as much as 20 µL was taken using micropipette on sterile paper discs with concentrations of 2%, 4%, 6%, 8% and 10% and then placed on NA media that had been inoculated with bacterial test. Incubation was carried out at 37 °C for 2 x 24 hours. The positive control used was 1% ampicillin disk while the negative control used was 10% Dimethyl sulfoxide. Observations were made on the formation of inhibitory zones around the paper disc[6].

RESULTS AND DISCUSSION

Phytochemical screening of ethanol extract of papaya seeds showed the presence of flavonoids, alkaloids,

Table 1. Phytochemical screening results (qualitative).

Test	Observation	
	Visual	Result
Flavonoid		(+) Orange color
Alkaloid		(+) White lumpy deposits
Saponin		(+) Foam
Phenolic		(+) Dark green
Tannin		(+) Greenish brown

(+) = Proven To Contain Related Compounds

Table 2. Phytochemical screening results (quantitative) of flavonoid.

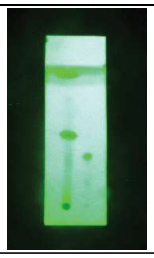
Observation	
Under UV (254 nm)	Under UV (254 nm)
	

Table 3. Phytochemical screening results (quantitative) of saponin.

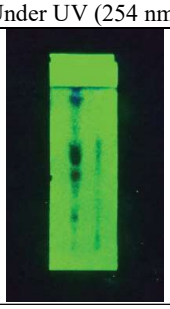
Observation	
Under UV (254 nm)	Under UV (254 nm)
	

Table 4. Saponin concentration (standard).

Saponin Concentration 50 mg/mL		
Standard	Level (mg/µL)	Abs
1	0.25	0.046
2	0.50	0.122
3	1.00	0.230
4	2.00	0.405

Table 5. Standard calibration of saponin.

Sample	Abs	Measured levels	Concentration (%)
1	0.498	2.4221	0.05
2	0.498	2.4221	0.05
3	0.498	2.4221	0.05

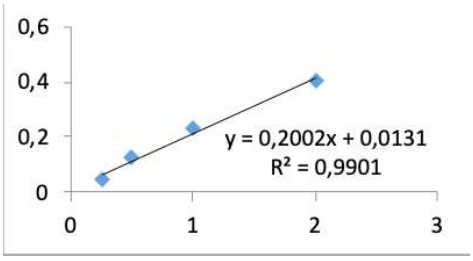


Figure 1. A standard curve of saponin.

Table 6. Phytochemical screening results (quantitative) of tanin.

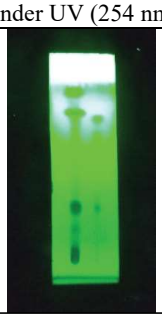
Observation	
Under UV (254 nm)	Under UV (254 nm)
	

Table 7. Phytochemical screening results (quantitative) of phenolic.

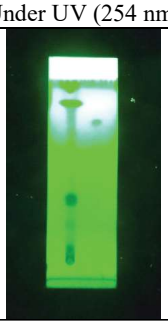
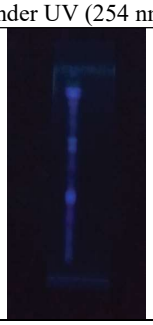
Observation	
Under UV (254 nm)	Under UV (254 nm)
	

Table 8. Phenolic concentration (standard).

Phenolic Concentration 10 mg/5mL		
Standard	Level (mg/µL)	Abs
1	1.001	0.123
2	2.002	0.179
3	6.006	0.682
4	8.008	0.914
5	10.01	1.065

Table 9. Standard calibration of phenolic.

Sample	Abs	Measured levels	Concentration (%)
1	0.257	23.6818	2.30
2	0.257	23.6818	2.30
3	0.257	23.6818	2.30

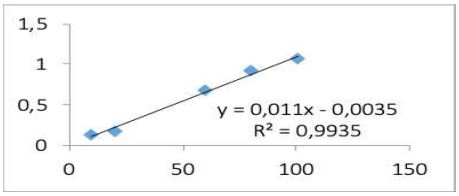
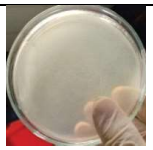


Figure 2. A **standard curve of phenolic.**

saponins, tannins, and phenolic.

Phytochemical screening results showed that the ethanol extract of papaya seeds (*Carica papaya* L.) positively contained alkaloids, flavonoids, tannins, phenolic compounds and saponins. The results of phytochemical screening are in accordance with research conducted by Lohidas *et al*. which states that papaya seed ethanol extract contains secondary metabolites such as alkaloids, flavonoids, tannins, phenolic compounds and saponins. In addition, the results of phytochemical screening in this study are also in accordance with research conducted by Torar *et al.* which states that papaya seeds positive ethanol extract contains secondary metabolite compounds such as alkaloids, and flavonoids which are proven to have antibacterial activity.

Table 10. Antibacterial activity of papaya seed (*Carica papaya* L.) ethanol extract.

Picture (Documentation)	Inhibition Zone Average (mm)
	2% (7.67 mm) 4% (8.67 mm) 6% (9.33 mm) 8% (13.00 mm) 10% (16.50 mm) Ampicillin 1% (14 mm) DMSO 10% (0 mm)
	Bacterial growth according to Mc. Farland II (6 x 10^8 CFU/mL)
	No Contamination

Antibacterial activity test of papaya seeds ethanol extract conducted by using the disc diffusion method. The concentration of ethanol extract of papaya seeds are 2%, 4%, 6%, 8%, and 10% in order to know the increase in activity along with the increasing concentrations. In addition, the positive control used was ampicillin 1% which generally can be used to treat *Staphylococcus aureus* infection and the negative control was DMSO 10%. The results of the antibacterial activity test of papaya seed ethanol extract showed inhibition of the *Staphylococcus*

aureus bacteria which was shown by the irradical inhibitory zone. The inhibition zone produced was classified as moderate at a concentration of 2%, 4%, and 6%, namely 7.67 mm; 8.67 mm; and 9.33 mm respectively, which were classified as strong at concentrations of 8% and 10%, which were 13 mm; and 16.5 mm. Positive control (Ampicillin 1%) was shown inhibition zone about 14 mm. Antibacterial activity test results in this study differed from previous studies, where previous studies stated that the ethanol extract of papaya seeds had moderate activity in inhibiting the activity of *Staphylococcus aureus*, the difference in these results can occur due to differences in the origin of papaya that is used so as to allow for differences in the amount of secondary metabolite content in the papaya.

The antibacterial activity of the extract comes from the content of phytochemical compounds. Flavonoids work by forming complex compounds with extracellular proteins that interfere with the integrity of the bacterial cell membrane[9]. Alkaloids function as antibacterial by disrupting the peptidoglycan constituent in bacterial cells, so that the cell wall layer is not formed intact and causes the death of these cells[9]. Saponins have soap-like properties which are strong surface active compounds, so they can reduce surface tension. Absorbing saponins on the cell surface will cause damage to cell membranes by increasing membrane permeability or cell leakage causing cell death due to loss of essential cell ingredients[9]. The antibacterial activity of tannins is based on their ability to shrink cell walls or cell membranes so that they interfere with bacterial cell permeability. Disruption of cell permeability results in cells not being able to carry out living activities so that their growth is stunted or even dies. Besides tannin also has antibacterial power by precipitating protein, because it has the same effect as phenolic[9].

CONCLUSION

The phytochemical screening of papaya seeds ethanol extract, showed the presence of alkaloid, flavonoid, tannin, saponin, and phenolic compounds. In addition, tested papaya seed ethanol extract has antibacterial activity against *Staphylococcus aureus* which is classified as moderate at concentrations of 2%, 4%, and 6% and classified as strong at concentrations of 8% and 10%. The preliminary investigation and the antibacterial activity test of the papaya seed ethanol extract will be further help as basis for preparing of gel product formulation.

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