Antimicrobial assay of papaya seed ethanol extract (*Carica papaya Linn*) and phytochemical analysis of its active compounds

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Antimicrobial assay of papaya seed ethanol extract (*Carica papaya Linn*) and phytochemical analysis of its active compounds

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Abstract. Natural drugs usage as alternative treatment has been increased in decades. This is due to the 'back to nature' life style of people and the side effect of natural drugs are relatively low. The prices of these natural drugs are cheaper as well. Papaya seed is one of the natural product with antimicrobial effect. The objectives of this research were to determine *Minimum Inhibitory Concentration* (MIC) of papaya seed ethanol extract to inhibit the growth of *Candida albicans* and *Vibrio cholera* and to analyze its active compounds. This research was performed using experimental design laboratory. The ethanol extract of papaya seed was obtained by maceration. The antimicrobial assay of papaya seed ethanol extract was performed using agar plate diffusion. The MIC determination of papaya seed ethanol extract was performed by 20, 15, 10, 5 and 1% concentration. The MIC of papaya seed ethanol extract against *Candida albicans* and *Vibrio cholerae* were in 5% concentration (diameter of inhibition zone=0.75 mm) and 15% (diameter of inhibition zone =14.75 mm), respectively. The phytochemical assay of papaya seed ethanol extract showed there were alkaloids, flavonoids, steroids, polyphenols, tannins and saponins. It means that papaya seed ethanol extract has a good potential as antimicrobial therapy agent.

1. Introduction

Infectious desease are the biggest threat for human health and almost 50,000 death cases occure daily due to the infectious diseases[1]. The resistance of various infection agents over synthetic drugs can be the main reason of the antibiotic alternatives development [2]. Plants have main advantage to be effective and cheaper drug altenative[3].

Papaya (*Carica papaya L.*) is a major cultivated plant in Indonesia and this plant has a great economical value. According to several previous researches, the papaya seed which is used to be treated as waste, has several great benefits for health. All of parts of papaya tree, from root, leaf, flower, fruit to the seed have great medical values[4]. Traditionally, papaya seed can be used for treatment against roundworm (*Ascaris lumbricoides*), indigestion, diarrhea, skin disease, colds, men contraception and this seed can also be used as oil source containing certain amount of fatty acids [5]. The objectives of this research were to determine MIC (*Minimum Inhibitory Concentration*) of papaya seed ethanol extract to inhibit the growth of *Candida albicans* and *Vibrio cholera* and to analyze its active compounds.

2. Materials and methods

2.1. Material and microorganisms

Papaya plant (*Carica papaya L.*) was harvested from Turen, Malang. This plant was identified in the UPT Balai Konservasi Tumbuhan Kebun Raya Purwodadi, Malang. The fungi of *Candida albicans* was collected from Biochemistry Laboratory of Science and Technology Faculty, Universitas Airlangga, Surabaya. *V. cholerae* was collected from Microbiology Laboratory of University of Wijaya Kusuma Surabaya.

2.2. Extraction of papaya seed extract

The ripe papaya fruits were cut into two pieces and the seeds were collected. The fresh papaya seeds were washed twice using water and dried at room temperature for 4 weeks. The seeds were grinded in a blender to reduce the size. A 500 g papaya seed powder was macerated using 96% EtOH for approximately 3 days. The extract was filtered using clean white fabric and Buchner. The filtrate was evaporated at 40°C, resulted in a sweet-smelling concentrated extract and brown coloured. This extraction process was repeated 4 times. The solid powder obtained from the extraction was weighed, kept in a vaccum container and refrigerat.

2.3. Antibacterial activity assay

Antibacterial activity assay was performed using disk diffusion assay in Nutrient Agar.One single colony of bacteria from working plate was picked and incubated in 10 mL of liquid broth (Nutrient Broth) for 18-24 h at 37°C with shaking in a waterbath at 100 rpm. A 5 mL of bacterial culture was collected and the optical density (OD) was measured at wavelenght of 620 nm. The OD should be less than 1.0.

The clear zone (*halo*) surrounding the well indicated antibacterial activity of the samples. The diametres of *halos* were measured using calipers. The MIC test was performed to the papaya seed sample that indicated a positive result using the same method.

2.4. Antifungal activity assay

Antifungi activity assay was performed using the same assay as antibacterial assay, disk diffusion assay in SDA (Sabroud Dextrose Agar) medium.

2.5. Determination of MIC (Minimun Inhibition Concentration)

MIC is defined as the lowest concentration of antimicrobial that still inhibit the visible growth of microorganisms. MIC determines the minimum concentration of the active compounds needed to inhibit the growth of microorganisms. This research used *V. cholerae* and *C. albicans* as microorganism. The determination of MIC was performed using serial dilution of EtOH in papaya seed of 20%, 15%, 10%, 5% and 1%. This assay was performed using paper disc method (Kirby bauer). The bacteria was inoculated in a Nutrient Agar solid medium and the fungi was inoculated in a SDA medium. The paper discs were placed on the surface of medium. The 50 µL extracts of various concentrations were dropped on the paper discs, then were incubated for 1x24 h at 37°C. The negative control used was aquadest. The positive control used was chloramphenicol (for antibacterial assay) and Nystatin (for antifungal assay). MIC was determined by measuring the diametres of inhibitory zones around the paper discs using calipers.

2.6. Chemical compounds identification of papaya seed extract(6)

2.6.1. Alkaloid assay. A 2 mL of sample was diluted in 2 mL of 2% HCl, heated for 5 min and filtered. The filtrate was obtained and given 2-3 drops of Mayer reagent. The formation of white precipitate indicates the presence of alkaloids in the sample.

2.6.2. Flavonoid assay. A 2 mL of sample was diluted in 2 mL of MeOH. The powder of Magnesium (Mg) and 5 drops of concentrated HCl were added to the solution. The formation of red or orange coloured solution indicates the presence of flavonoid compounds. The saponins assay was performed by diluting 2 mL of sample to aquadest in a test tube. A 10 drops of KOH was added to the solution and heated in a waterbath at 50°C for 5 min. The test tube was shaken for 15 min. The formation of solid and stable foam (~1 cm height for 15 min) indicates the presence of saponins.

2.6.3. Steroid and terpenoid assay. A 1 mL of Liberman-Buchard reagent was added to 2 mL of sample. The presence of terpenoids were indicated by the alteration of solution colour to dark blue or blackish-green.

2.6.4. Polyphenol assay. A 2 mL of sample was diluted in 10 mL of aquadest, heated for 5 min and filtered. The filtrate was obtained and given 4-5 drops of 5% $FeCl_3$ (b/v). The presence of phenol were indicated by the alteration of solution colour to dark blue or blackish-green. The tannins assay was performed by adding $FeCl_3$ reagent to 2 mL of sample. The presence of tannins were indicated by the alteration of solution colour to dark blue or blackish-green.

2.6.5. Tannins assay. The tannins assay was performed by adding $FeCl_3$ reagent to 2 mL of sample. The presence of tannins were indicated by the alteration of solution colour to dark blue or blackish-green.

3. Results and discussion

3.1. Papaya seed extraction

The papaya seed extraction was perfored using maceration method that was a cold extraction method using 96% EtOH as solvent. Maceration was used as extraction method since this method was simple, did not need vast amount of solvent and the concentrated extract could form rapidly. This research obtained 83.49 g of papaya seed total dry weight and resulted in concentrated brownish-yellow extract of 7.1 g (total yield of 8.4%).

3.2. Antimicrobial activity of papaya seed extract

Antimicrobial activities from papaya seed extract were identified by the presence of clear zones at the certain diametres around the extract wells called inhibitory zones. The existed inhibitory zone diametre was measured using calipers vertically, horizontally and diagonally. The mean was calculated and presented in milimetre [7]. The results of papaya seed extract antimicrobial activities were exhibited in Figure-1.

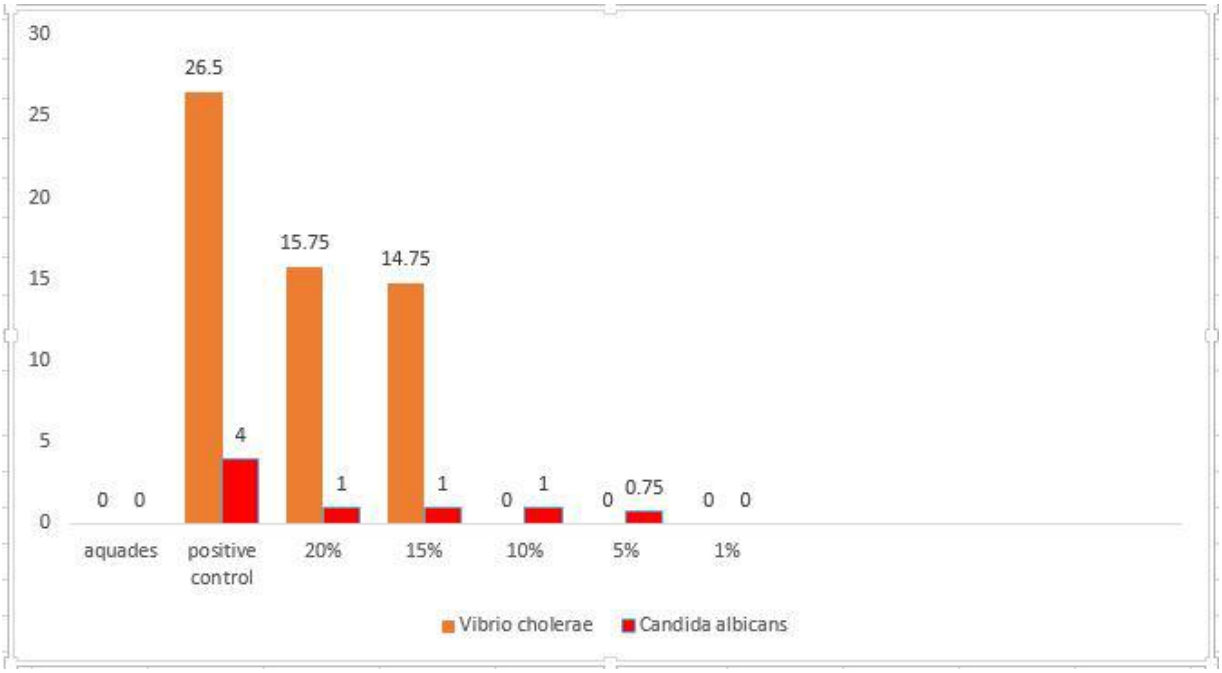

Figure-1. The graph of concentration of papaya seed extract (%) against diameter of clear zones (mm)

The results showed that papaya seed extract possessed high antimicrobial activities against *V. cholerae* and low antimicrobial activities against *C. albicans.* Davis and Stout (1971)[8] have previously categorized the assessment of bacterial inhibition activity based on the clear zones diametres into 3 categories which are, very strong (clear zone > 20 mm), strong (clear zone 10-20 mm), medium (clear zone 5-10 mm) and weak (clear zone < 5 mm). This results were in accordance with the research results of Setiawan et al., (2017)[9], reported that bacang mango leaf extract had low antifungal activities against *C. albicans.* The combination of red betel leaf and avocado seed extract also had low antifungal activities against *C. albicans* [10].

3.3. Minimum Inhibition Concentration (MIC)

Determination of MIC was performed to find out the minimum inhibitory concentration of papaya seed extract in various concentrations. The methanol concentrations used for the papaya seed extract in this research were 20%, 15%, 10%, 5% and 1%. The positive control used in the experiment was chloramphenicol for *V. cholerae* and nystatin for *C. albicans.*

Figure 1 showed antimicrobial activities decreased with the decreasing concentration of papaya seed extract. The minimum concentration of of papaya seed extract to inhibit the growth of *V. cholerae* and *C. albicans* were respectively 15% (inhibitory zone=14.75 mm) and 5% (inhibitory zone=0.75 mm). According to statistical analysis, the ethanol extract of papaya seed had significantly different inhibition activities amongst the groups ($p<0.05$).

The inhibitory abilities of papaya seed ethanol extract against the growth of *C.albicans* and *V. cholerae* could be due to its active compounds with antimicrobial activities such as alkaloids, flavonoids, stereoids, polyphenols and tannins, as demonstrated in Table 1.

3.4. Phytochemical analysis

Phytochemical assay was performed to examine the secondary metabolites of the papaya seed extract such as alkaloids, flavonoids, stereoids, polyphenols, saponins and tannins. The results of papaya seed extract phytochemical analysis are exhibited in Table 1.

Table 1. Qualitative phytochemical composition of papaya seed extract.

Chemical compounds	Remarks
Alkaloids	+
Flavonoids	+
Tannins	+
Saponins	+
Phenols	+
Steroids	+

+ represent present
- represent absent

Carica papaya seed contains antibacterial compunds that inhibit the growth of Gram positive and Gram negative bacteria[11]. Sukadana et.al(2008) [5] in Maria Martiasih (2014)[12]had reported that papaya seed contained a potential antibacterial triterpenoid aldehyde compound. Triterpenoid has ability to disrupt cell pores so the membrane permeability was disrupted as well. Another reported antibacterial compound in papaya seed was carpaine alkaloid. Carpaine digests proteins from microorganisms and changes it into peptone. According to Sabir (2005)[13], flavonoid contents in *Trigona sp*. propolis inhibited the growth of *Streptococcus mutans in vitro.* Flavonoid denaturates proteins and disrupts the cell membrane leading to the death of bacterial cell. Mustikasari and Ariyani (2010)[14] stated that alkaloids had antimicrobes activity by disrupting the microbial cell wall. According to Zhu (2000)[15], steroids that were isolated from Damar leaves could be toxic for microbes and had antifungal effect so the steroids could be useful in medical field. Each of the secondary metabolites has different mechanisms as antibateria or antifungi [16].

4. Conclusions

Papaya seed extract possessed high antimicrobial activities against *V. cholerae* and low antimicrobial activities against *C. albicans.* The phytochemical analysis of papaya seed extract demonstrated that this extract contained bioactive compounds such as alkaloids, flavonoids, saponins, tannins, etc. This results showed that papaya seed has a potential therapheutic effect.

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