In vitro study of antifungal effect of Carica papaya peel var. california extract against Candida albicans

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ABSTRACT

Candida albicans is mostly found in oral cavity as an opportunistic microbiota in some oral mucous diseases. The papaya peel extract is a rich source of antifungal compounds. The aimed of this study was to determine antifungal effect of carica papaya peel extract against Candida albicans. This study was experimental laboratory with post-test only control group design. The macerated ethanol extract of papaya peel were divided into six groups based on its concentrations. It consisted of 2.5%, 5%, 10%, 20% concentration of papaya peel extract, nystatin as positive control, and distilled water as negative control. All concentration of papaya peel extracts were effective against the growth of C. albicans. The minimum inhibitory concentration was 2.5%. Conclusion of this research shows that the extract of papaya peel var California has antifungal effect against C. albicans.

Keywords: antifungal, Candida albicans, Carica papaya.

Abstrak

Candida albicans merupakan suatu microbiota oportunistik yang paling banyak ditemukan pada beberapa penyakit mukosa mulut. Ekstrak kulit pepaya kaya akan komponen antijamur. Penelitian ini dilakukan untuk mengetahui efek antijamur ekstrak kulit pepaya terhadap Candida albicans. Penelitian ini merupakan suatu penelitian laboratorium dengan desain post-test only control group. Ekstrak kulit pepaya didapatkan dengan metode maserasi menggunakan etanol yang kemudian dibagi menjadi 6 kelompok dengan berbagai konsentrasi. Konsentrasi ekstrak yang digunakan adalah 2,5%, 5%, 10%, 20%, dengan nystatin sebagai kontrol positif dan akuades sebagai kontrol negatif. Hasil Penelitian menunjukkan bahwa semua konsentrasi ekstrak kulit pepaya efektif dalam menghambat pertumbuhan Candida albicans. Daya hambat minimum yaitu pada konsentrasi 2,5%. Kesimpulan penelitian ini adalah ekstrak kulit pepaya var. California memiliki efek antifungal terhadap Candida albicans.

Kata Kunci: antijamur, Candida albicans, Carica papaya.
1. Introduction

*Candida albicans* (*C. albicans*) is a normal microflora in the oral cavity and can turn into opportunistic pathogens that cause oral infections if there are underlying predisposing conditions. These predisposing conditions included decreased body defense, metabolic diseases, immunosuppressive therapy, and Acquired Immunodeficiency Syndrome (AIDS/HIV). As many as 50% of Oral Candidiasis cases is caused by *C. albicans*. Some clinical findings related to the infection included Pseudomembranous Candidiasis, Erythematous Candidiasis, Leukoplakia Candidiasis, Denture Stomatitis, Angular Cheilitis, Median Rhomboid Glossitis, and HIV-related Oral Candidiasis. Moreover, *C. albicans* also contributes to the formation of microbial acids which causes glucose and maltose fermentation and produces acids and gases. The presence of the fungus *C. albicans* can increase with the attachment of *Streptococcus mutans* to biofilms and dental caries substances. *C. albicans* have significant contribution to the pathogenesis of active caries in children.

Treatment of infections caused by *C. albicans* is antifungals. Along with the increasing cases of Candidiasis, treatment with synthetic materials has been widely developed. However, there are side effects of synthetic antifungal drugs such as nystatin, amphotericin B, ketoconazole, and fluconazole. Furthermore, there are side effects of synthetic antifungal drugs, in which continuous use can cause drug resistance. Therefore, other treatment modalities are needed to reduce these side effects by using herbal medicines as an alternative treatment.

Papaya (*Carica papaya*) is known to be one of the plants that has benefits in curing diseases. The parts of papaya plants that can be used as herbal medicines include the fruit, leaves, and flowers. Yogiraj et al. (2014) mentioned that parts of papaya plants including leaves, seeds, latex, and fruit have medical value. The peel of raw papaya fruit is known to contain flavonoids, phenols, tannins, saponins, and antioxidant properties as antibacterial and antifungal. Based on the research of Egbuonu et al. (2017), 10% ethanol extract of raw papaya peel of Agric variety can inhibited the growth of *C. albicans* 20 mm. This can be due to high active compound in papaya peel of Agric variety.

In Indonesia, there were various types of papaya, but the most frequent and the most common type of papaya was the Californian variety. Based on this explanation, further study needs to be conducted to understand the potential antifungal effect of raw papaya peel extract (*Carica papaya*) of California variety in Indonesia. This in vitro study was aimed to determine the antifungal effect and determine the minimum inhibitory concentration (MIC) of papaya peel extract of Californian variety against *C. albicans*.

2. Methods

**Preparations of Papaya Peel Extract**

Raw papaya of Californian variety was bought from farmers and washed with tap water. The papaya skin was peeled with peeler then air dried for 3 hours and dried in the oven for 3 days at 40°C. The dried papaya peel then mashed up using blender to form powder. The obtained powder was then extracted using maceration method, immersed in 96% ethanol solution at room temperature and stored in a place free of sunlight. The solution was immersed for 3 days while stirred occasionally, then...
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filtered with Whatman paper. After filtering, ethanol filtrate was then evaporated with a rotary evaporator at 40°C with rotational speed of 77 rpm, to eliminate excess solvents to obtain thick extract of papaya peel. The extract *Carica papaya* var. California peel was made into 4 concentration; 2.5%, 5%, 10%, and 20% that were used in this study.

**Phytochemical Test of Californian Papaya Peel Extract**  
The evaluation of bioactive compound of *Carica papaya* var. California peel was done by phytochemical Screening. The result of phytochemical screening of *Carica papaya* var. California peel were positive for alkaloids, steroid, and tannin.

**Preparations of C. albicans Media**  
Six grams of Saboraud Dextrose Agar (SDA) were put into Erlenmeyer flask and 180 ml of sterile distilled water was added. The media was heated to dissolve the whole agar. The media was autoclaved at 121°C for 15 minutes. Afterwards, the media was poured into petri dishes and allowed to stand until the media solidified.

**Preparations of C. albicans Suspension**  
Some fungal colonies were taken from *C. albicans* culture on SDA media using a transfer loop. The transfer loop was dipped into a test tube containing 0.9% NaCl until homogeneous. The test tube was covered with cotton and incubated for 24 hours at 37°C. The solution was then compared to Mac Farland 0.5 standard. If the turbidity is still lacking, *C. albicans* can be added, otherwise then the solution can be diluted again. The obtained *C. albicans* suspension turbidity was in accordance to Mac Farland 0.5 standards or proportional to the number of fungal 1.5 x 108 CFU/ml. *C. albicans* suspension was then taken by dipping a cotton swab into the solution and then scraping it into 5 petri dishes containing SDA media evenly.

**Antifungal Effect Test (Disc Diffusion)**  
This study was aimed to determine the antifungal effect of papaya peel extract of California variety against clinical isolates of *C. albicans* using agar discs diffusion test method (Kirby-Bauer) with 6 mm diameter disc paper. Five petri dishes containing *C. albicans* in the media was prepared. Paper disc was soaked for 15 minutes in each treatment extract concentrations of 2.5%, 5%, 10%, 20%, positive control, and negative control. Then the disc paper was taken using nippers and placed above the agar that has been smeared with *C. albicans*. Five petri dishes containing *C. albicans* were placed with 4 variations of the extract concentration (2.5%, 5%, 10%, and 20%), positive control, and negative control. Then the petri dishes were incubated for 24 hours at 37°C. The formation of clear zone was observed in the petri dishes and were measured using calipers. Clear zone area that formed showed the amount of inhibition of papaya peel extract against *C. albicans*. The primary data results obtained were analyzed and described descriptively.

3. **Results**  
Phytochemical test results of the ethanol extract of Californian papaya peel in showed that the ethanol extract of papaya peel contains alkaloids, tannins, and steroids. These results was in line with the previous study of Lydia et al. (2016) and Shiddique et al. (2017).12,15
However, it was different from studies conducted by Dada et al. (2016) and Chukwuka et al. (2013), which stated that papaya peel extracts contained phytochemical compounds such as saponins and flavonoids, while this study did not indicate the presence of these compounds. Based on the study by Chukwuka et al. (2013), raw papaya peel extract has high saponin content, which according to Alfiah et al. (2015), saponin is a compound that works by disrupting the stability of the fungal cell membrane which lead to cell membrane damage and causes various important components from the fungal cell to be released, such as protein, nucleic acids and nucleotides. According to Alves et al. (2016), flavonoids work by inhibiting the synthesis of fungal cell walls because it has trimethoxflavone (TMF) which inhibits fungal growth. Flavonoid activity also prevents ergosterol from binding to the fungal cell membrane. Ergosterol is an important sterol in fungal filaments and yeast for fungal nutrition.

The difference in the phytochemical results of the papaya peel extract might be caused by the different of papaya varieties. According to Abdullah et al. (2010), the various types of papaya caused the active compounds contained also vary, the difference in the amount and type of content of the active compound also depends on the age of the plant. The statement was supported by Chukwuka et al. (2013), in terms of differences in age (maturity stage) of the papaya. Raw papaya peel has a higher active compound content compared to mature or very mature stage of papaya.

**Inhibition Test of C. albicans Using Discs Diffusion Method**

This study consisted of six treatment groups, i.e. papaya peel extract with concentrations of 2.5%, 5%, 10%, 20%, positive control, and negative control with 5 replications. The results of the inhibition zone measurements of all treatments are written in millimeters (mm) that can be seen in Table 1.

| Group       | Total | Mean (mm) | SD  | Min* | Max** |
|-------------|-------|-----------|-----|------|-------|
| 2.5%        | 5     | 1.56      | 2.96| 0    | 6.8   |
| 5%          | 5     | 0.88      | 0.52| 0    | 1.4   |
| 10%         | 5     | 1.2       | 1.64| 0    | 4     |
| 20%         | 5     | 0.8       | 0.83| 0    | 2     |
| Control (+) | 5     | 5.67      | 3.14| 3    | 10.7  |
| Control (-) | 5     | 0         | 0   | 0    | 0     |

*Minimum  **Maximum

Based on Table 1, it can be seen that the highest inhibitory zone of papaya peel extract is found at 2.5% concentration with mean of 1.56 mm (SD ± 2.96). However, the inhibition zone decreased when the extract concentration was increased to 5%, 10%, and 20%, the inhibition zone decreased to 0.8 mm (SD ± 0.83). The positive controls had an average inhibition zone greater than all extract concentrations while the negative controls did not show any inhibitory zone. The inhibition zone diameter of California papaya peel extract against the growth of clinical C. albicans isolates can be seen in Figure 2.
Inhibition zones are formed due to the presence of active substances contained in papaya peel such as tannins, alkaloids, and steroids. Tannin is a polyphenol group compound that works with the target of fungal cell wall polypeptide which causes damage to the fungal cell wall and causes increase to fungal cell permeability, thus fungal growth is inhibited. Alkaloids also have antimicrobial activity due to pH > 7 with alkaline properties that can inhibit the RNA and DNA polymerization and fungal cell protein synthesis. Therefore, the growth of \textit{C. albicans} can be suppressed. Steroids can inhibit fungal growth, through the cytoplasm of the fungal cell or interfere with the growth and development of fungal spores. Steroids have lipophilic properties that can inhibit spore germination.

4. Discussion

Based on the results of the inhibition zone, it can be seen that increasing the concentration of papaya peel extract does not always increasing the diameter of the inhibition zone. The results of this study are similar to study conducted by Fitriani et al. (2012), that the increased concentration of the Salam leaf extract (\textit{Syzygium polyanthum (wight) walp.}) was not always able to increase the inhibitory zone against \textit{C. albicans}. This is because the higher the concentration of the extract the greater the molecules that were formed. Therefore, there is no direct contact between the active compound and the fungi due to the difficult diffusion process. According to Setiorini et al. (2014), determination of Minimum Inhibitory Concentration (MIC) is based on the lowest concentration of extract that produced inhibition zone. Based on the results, the MIC of ethanol extract of papaya peel against \textit{C. albicans} is 2.5%.

According to the theory suggested by Davis and Stout (1971), the diameter of the inhibitory zone formed in the in vitro test of 0 to 4 mm was categorized as weak. Thus, the antifungal effect from this study was relatively weak. The weak antifungal effect in this study was different from the study conducted by Egwuonu et al. (2017), which showed that the 10% ethanol extract of raw papaya peel of Agric variety can inhibit the growth of \textit{C. albicans} by 20 mm. The large inhibitory zone suggested that the ethanol extract of raw papaya peel of Agric variety have a relatively strong antifungal effect. Hilzon CF (2016) stated that papaya peel extract with 30% concentration inhibited \textit{C. albicans} zone by 13 mm. The antifungal effect was categorized as strong, while this study...
showed that 20% papaya peel extract had weak antifungal effect. Californian papaya peel has a small antifungal effect compared to other parts of papaya such as leaves and seeds.\textsuperscript{25,26}

The weak inhibition of papaya peel extract indicated the lack of concentration of the compounds contained in the extract, thus the effect antifungal was not optimal. The active compound which is absorbed in the disc paper is suspected to be not optimal due to an imperfect dilution process. The extract that was used was too thick and sticky thus cannot dissolve completely in distilled water, causing granules within the extract. The differences in the inhibition zone in this study and previous studies might be caused by various factors, one of these can be caused by differences of types and amounts of active compounds present in this study, that can influence the result of antifungal effect. The different types and amounts of active compounds are influenced by the variety, stage, and environment in which papaya grows, such as ambient temperature, humidity, adequate sunlight, and water availability. The quality of an extract depends by the type and the number of chemical compounds it contains, the way it was extracted, and the selection of the solvent.\textsuperscript{24}

The difference in antifungal effects in this study compared to previous studies might have been caused of \textit{C. albicans} isolates that are different from \textit{C. albicans} isolates used by previous studies. The inhibitory zone of positive controls observed from this study, was not in accordance with inhibitory zone in general positive controls. It might be assumed that the \textit{C. albicans} isolate in this study was not a standard isolate but a clinical isolate and might have started resistance caused by drug storage factors.

5. Conclusion

Based on these results, the ethanol extract of raw papaya peel (\textit{Carica Papaya}) of Californian variety has a weak antifungal effect with MIC value of 2.5% against \textit{Candida albicans} in vitro.

Other dilution agents should be used to dissolve the thick and sticky extract so that the extract can be dissolved homogeneously and phytochemical quantitative tests are required to determine the number of compounds contained in Californian papaya peel extracts

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