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Antifungal Activity of N-Hexane Extract of Duku Peel (*Lansium domesticum* Corr.) Against *Candida albicans* In Vitro

Humaira Fitriana[1], Trisnawaty K.[1*], Shanty Chairani[1]

[1]Dentistry Study Program, Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia
[*]Correspondence author email: trishfen@gmail.com

Abstract

Introduction: High colonization of *Candida albicans* in mouth can increase the risk of oral candidiasis. Duku peel (*Lansium domesticum* Corr.) has potential as antifungal material due to its terpenoid and phenolic content which can be extracted with n-hexane as solvent. **Purpose:** The purpose of this study is to determine the antifungal activity of n-hexane extract of duku peel against *Candida albicans*. **Methods:** This study used laboratorium experimental with post test only control group study design. Antifungal activity was determined using disc diffusion method on five sample groups: n-hexane extract of duku peel 15%, 20%, and 25%, nystatin as positive control, and aquadest as negative control. Antifungal activity was determined by measuring clear zone around the disc and measured with caliper in millimeter. The measurement of inhibition zone was analyzed with One Way ANOVA test. **Results:** There were no inhibition zone on n-hexane extract of duku peel with concentration of 15%, 20%, 25% and negative control while positive control had average inhibition zone diameter of 11,85 mm. **Conclusion**: N-hexane extract of duku peel with concentration of 15%, 20%, and 25% did not have antifungal activity against *Candida albicans*.

Keywords: Antifungal; *Candida albicans*; *Lansium domesticum*

Introduction

Candida albicans is the dominant normal flora in the oral cavity and can grow with varying numbers.[1,2] Colonization of *Candida albicans (C. albicans*) in the oral cavity can increase due to local factors such as poor oral hygiene, poor use of prostheses, and systemic factors such as immunocompromised conditions and diabetes mellitus.[3-5] High colonization of *C. albicans* in the oral cavity increases the risk of oral candidiasis.

Oral candidiasis treatment can be done by eliminating local factors and using antifungal drugs topically or systemically.[6] Nystatin is one of topical oral candidiasis drug. It contains ergosterol which can reduce fungal growth in the oral cavity. The use of nystatin has several side effects, such as nausea, vomiting, diarrhea, and abdominal pain so that other alternative ingredients are needed as antifungals.[7]

Natural substances from plants are widely used as an alternative medicines, including duku (*Lansium domesticum* Corr.). Duku can grow in various tropical regions, such as Indonesia. The Province of South Sumatra is the largest producer of duku in Indonesia, which

amounted to 53,399 tons in 2020.[8] Duku is used for its fruit to be eaten, while duku peel is generally discarded even though the it has several benefits.[9]

Duku peel has various active components, including aldehydes, flavonoids, saponins, terpenoids, and phenolics. They can be extracted from the duku peel using various solvents. Ethyl acetate solvent can extract aldehyde in duku peel, while methanol extract of duku peel contains flavonoids, triterpenoids, and saponins.[10,11] Acetone and n-hexane extracts of duku peel are also reported to contain terpenoids and phenolics.[12-14] Terpenoids, phenolics, saponins, and phenols in duku peel has antifungal activity.[12,15] Terpenoids, saponins, and phenols damages the cell membrane of *C. albicans*, while flavonoids can inhibit *C. albicans* cell metabolism.[16-21]

Methanol and ethyl acetate extracts of duku peel at concentrations of 50% and 75% were reported to inhibit the growth of *C. albicans*, but not as well as the positive control of nystatin as reported in the study of Darmadi et al.[22] Research by Ragasa et al. showed that the terpenoid content of the acetone extract of duku peel has antifungal activity against *C. albicans* but weaker than the positive control of clotrimazole.[12] There has been no study of duku peel using n-hexane solvent against *C. albicans* even though the solvent can extract terpenoids and phenolics that have antifungal activity. This is the basis for conducting research on antifungal activity.

Methods

This research is an in vitro laboratory analytic experimental research with post test only control group design. The research was conducted on September 2022 at the Biochemistry Laboratory and Biotechnology Laboratory of the Faculty of Medicine, Sriwijaya University to create duku peel extract and then on October 2022 at the Palembang Health Institute Center to conduct antifungal tests. The research subjects were colonies of C. *albicans* ATCC 10231 obtained from the Palembang Health Laboratory Center.

Duku fruit was originated from Banyuasin, South Sumatra. It was selected with these criteria: the fruit is ripe with a slightly soft texture; the peel was not blackened, not contaminated by pests, not affected by diseases so it does not have blackish spots, and overall in good condition.[23] The samples were divided into five groups: 15%, 20%, 25% concentration of n-hexane extract of duku peel, nystatin as positive control, and distilled water as negative control. The test was conducted seven times, which was calculated using Federer's formula.[24]

A total of 20 kg of duku peel was separated from the fruit flesh and dried using an oven for three days at 40ºC then pulverized using a blender into powder. N-hexane solvent was added to the duku peel powder until it was soaked and allowed to left for three days at room temperature (25ºC). The result of maceration was filtered with Whatman paper no. 40 to separate the residue. The filtered solution was evaporated with a rotary evaporator to separate the solvent, then dried in a waterbath (40ºC) until it became a thick extract.

Seven petri dishes of *C. albicans* and thirty-five paper discs were prepared. Each petri dish contained five paper disks that had been dipped in 15%, 20%, and 25% concentrations of n-hexane extract of duku peel, nystatin as positive control and distilled water as negative control. Petri dishes were incubated at 37ºC for 24 hours in an incubator. Inhibition zone measurement was performed after 24 hours of incubation by observing the presence of a clear zone around the paper disc. The diameter of the inhibition zone was measured as shown in Figure 1 using a caliper in millimeters. The inhibition zone calculation formula was measured using the following formula

$$\frac{(Dv-Dc) + (Dh-Dc)}{2}$$

□ = inhibition zone Dh = horizontal diameter

Dv = vertical diameter Dc = disc diameter

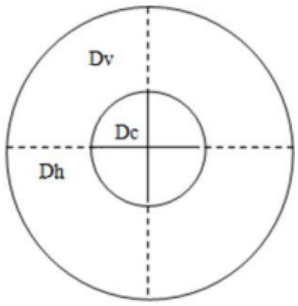

Figure 1. Ilustration of Inhibition Zone[25]

Data analysis was carried out using a parametric test because the variables in this study were numerical. The Shapiro-Wilk normality test and the Levene homogeneity test were performed to see the normality and homogeneity of the data distribution. Normal distributed data has a value of $p > 0.05$. One Way Analysis of Variance (One Way ANOVA) data analysis was performed because the data was normally distributed and homogeneous.

Results

The results in Table 1 showed that there were no inhibition zone in the negative control group, 15%, 20%, and 25% concentration of n-hexane extract of duku peel while the positive

control group had average inhibition zone diameter of 11.85 mm. These results indicated that the three concentrations of n-hexane extract of duku peel in this study did not have antifungal activity against *C. albicans*. As can be seen in figure 1 below, a clear inhibition zone was observed for positive control group only.

Table 1. Average Inhibition Zone Diamter of N-Hexane Extract of Duku Peel against *C. albicans*.

Sample	Average inhibition zone diameter (mm)
N-hexane extract of duku peel 15%	0
N-hexane extract of duku peel 20%	0
N-hexane extract of duku peel 25%	0
Negative control	0
Positive control	11,85

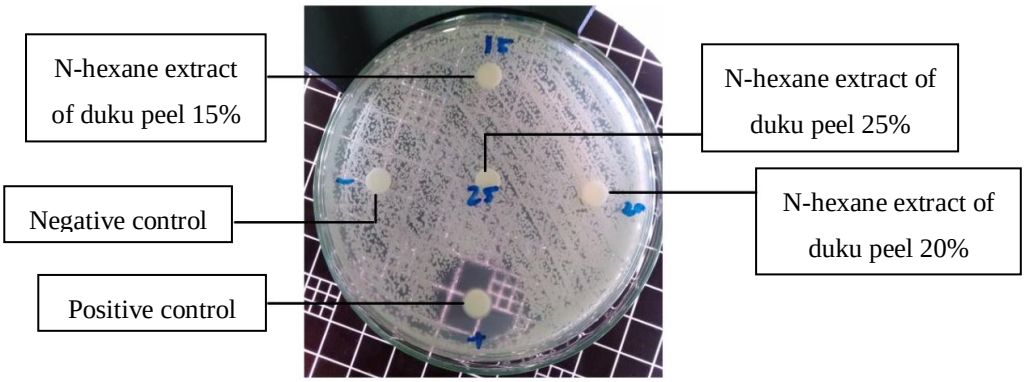

Figure 2. Antifungal test result of n-hexane extract of duku peel against *C. albicans*

Discussion

The study results showed that there was no clear zone n-hexane extract of duku peel at concentrations of 15%, 20%, and 25%. This could be due to usage of out-of-season duku. The duku in this study was purchased in September, while in general the duku blooms in September to October and can be harvested six months later.[23] Plants harvested out of season tends to contain less active substance than plants harvested in season. Research by Smita et al. showed that seaweed has more total flavonoid content in seaweed harvested pre-monsoon than post-monsoon.[26] Research by Liu et al. also showed that tomatoes harvested during the harvest season have more vitamin C and lycopene content than tomatoes off-season due to differences in sun exposure and temperature during tomato growth.[27]

The drying method of simplisia can also affect the phytochemical content. Duku peel in this study was dried using an oven at 40°C. Research by Luliana et al. reported that senggani leaves dried using wind had higher antioxidant activity than drying using ovens and sunlight but did not affect the phytochemical content of senggani leaves.[28] Meanwhile, research by Warnis et al. reported that the total flavonoids in Moringa leaves were higher in leaves dried using ovens than it was dried at room temperature because the use of ovens accelerates the drying process and air circulation is better.[29] High drying temperatures can also affect the phytochemical content. Research by Kusuma et al. showed that dried cocoa peels at high temperature resulted in a decrease in antioxidant activity due to damaged secondary compounds.[30]

Duku peel extract in this study did not have antifungal activity against *C. albicans*. This may be caused by amount of active substances in concentrations of 15%, 20%, and 25% cannot inhibit the growth of *C. albicans*. Darmadi et al. who extracted duku peel in methanol and ethyl acetate solvents at concentrations of 50% and 75% showed antifungal activity but there was no antifungal activity in the extract of duku peel at a concentration of 25%.[22] The relation between concentration and the amount of active substance is known to be directly proportional: the higher the concentration, the higher active substances contained.[31] The concentration of n-hexane extract of duku peel in this study, 15%, 20%, and 25%, is likely to contain smaller amount of active substance hence it did not show antifungal activity. The disadvantage of this study is that the n-hexane extract of duku peel did not tested for phytochemical screening. In this study, the phytochemical compounds extracted as a whole in the duku peel while the research of Ragasa et al. reported that the terpenoid content of the acetone extract of duku peel showed antifungal activity.[12] Extraction of phytochemical substances as a whole can have different antimicrobial activities than extractions of particular substance.[32]

Extraction of duku peel in this study used n-hexane as solvent without fractionation. This may be the cause that n-hexane extract of duku peel did not show antifungal activity against *C. albicans*. Fractionation can increase the phytochemical content of the extract.[33] Previous research by Salim et al. showed that the acetone extract of the n-hexane fraction can be used to extract terpenoids and saponins in duku peel.[14]

Conclusion

The current study showed that n-hexane extract of duku peel with concentration of 15%, 20%, and 25% did not have antifungal activity against *Candida albicans*.

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