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IN VITRO EVALUATION OF THE ANTIFUNGAL ACTIVITY OF ETHANOL EXTRACT COMBINATIONS OF SOURSOP (*Annona muricata*) AND RED DRAGON FRUIT (*Hylocereus polyrhizus*) PEELS AGAINST *Candida albicans*

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ABSTRACT

Candida albicans is a common commensal microorganism found on the skin, genital organs, oral cavity, and various other parts of the human body. However, its population can increase excessively due to inappropriate use of antibiotics without medical supervision and weakened immune function. One of the clinical manifestations caused by *C. albicans* overgrowth is pathological vaginal discharge, which may lead to more serious complications if left untreated. While commercial antifungal agents are available, they are often associated with side effects such as a burning sensation, nausea, and vomiting. Natural alternatives, including bioactive compounds from plant materials, are increasingly being explored for their antimicrobial properties. Soursop (*Annona muricata*) and red dragon fruit (*Hylocereus polyrhizus*) peels have been reported to contain such bioactive compounds with potential antimicrobial effects. The aim of this study was to evaluate the in vitro antifungal activity of combined ethanol extracts of soursop and red dragon fruit peels against *Candida albicans*. This laboratory-based experimental study used a one-way analysis of variance (ANOVA) to assess differences in antifungal activity among extract combinations. The results showed that combinations I (1:1), II (1:2), and III (2:1) of the extracts produced inhibition zones of 14.0 mm, 15.0 mm, and 20.1 mm, respectively. These inhibition zones are classified as strong to very strong based on standard antimicrobial activity categories. In conclusion, the combination of ethanol extracts from soursop and red dragon fruit peels demonstrated significant in vitro antifungal activity against *Candida albicans*. These findings support the potential use of natural plant-based antifungal agents as alternative or complementary treatments for candidiasis.

Keywords: *Dragon fruit peel and soursop peel; Candida albicans; inhibition zone; in vitro*

INTRODUCTION

Fungal infections are commonly encountered in tropical countries such as Indonesia. *Candida albicans* is a pathogenic fungus responsible for causing candidiasis, an infection primarily induced by fungi from the genus *Candida*, with *C. albicans* being the most prevalent species. This opportunistic yeast is part of the normal human microflora and can be found in various anatomical sites, including the oral cavity, genital organs, and skin. However, under certain conditions such as immunosuppression or dysbiosis, *C. albicans* can proliferate excessively and transition from a commensal organism to a pathogen. If not properly treated, candidiasis can progress to severe systemic infections that pose significant health risks [1].Candidiasis can be treated with a variety of antifungal agents, such as ketoconazole. However, the use of synthetic antifungal drugs is often associated with adverse side effects, including nausea, vomiting, and a burning sensation on the skin. In recent years, herbal medicines derived from natural ingredients have gained increasing popularity in Indonesia as alternatives to conventional therapies, owing to their perceived safety and lower risk of side effects. Among these natural sources, the peels of soursop (*Annona muricata*) and red dragon fruit (*Hylocereus polyrhizus*) have been identified as containing a variety of bioactive compounds. These include flavonoids, saponins, alkaloids, triterpenoids, tannins, and polyphenols, which are known for their antimicrobial and antifungal properties [2 3].

Dragon fruit peel contains various bioactive compounds that have been shown to inhibit the growth of *Candida albicans*. It is particularly rich in vitamin C, anthocyanins, betacyanins, and phenolic compounds, all of which contribute to its antioxidant and antimicrobial activities. These constituents may disrupt fungal cell walls or interfere with essential cellular processes, making dragon fruit peel a promising natural source for antifungal agents [4 5]. Extracts from dragon fruit (*Hylocereus polyrhizus*) and soursop (*Annona muricata*) peels have been shown to exhibit antimicrobial activity against various pathogens,

including *Streptococcus mutans* and *Candida albicans*. These effects are attributed to the presence of bioactive phytochemicals such as flavonoids, tannins, and phenolic compounds, which can disrupt microbial cell structures and inhibit their growth [6] [7] [8].Soursop and dragon fruit peels have been proven to possess the ability to inhibit the growth of *Candida albicans* in vitro due to their bioactive compound content. The combination of extracts may enhance their antimicrobial inhibitory activity. According to [9], a combination of noni fruit extract and soursop leaves can inhibit the growth of *Escherichia coli* and *Staphylococcus aureus*. Similarly, a combination of betel leaf and starfruit has been shown to inhibit the growth of *Staphylococcus aureus* [10].

Previous research has shown that combining plant extracts can inhibit microbial growth more effectively than using a single extract, due to the synergistic interactions between the bioactive compounds of both extracts. However, no studies have been conducted on the combination of soursop peel and dragon fruit peel extracts in inhibiting the growth of *Candida albicans.*The objective of this study was to determine the in vitro antifungal activity of the combination of soursop and dragon fruit peel ethanol extracts against *Candida albicans*. It is expected that this research will provide a foundation for the development of new antifungal agents derived from natural ingredients to treat fungal infections. The purpose of this study was to determine the effectiveness of ethanol extracts of soursop and dragon fruit peels on the growth of *Candida albicans* in vitro.

MATERIALS AND METHODS

This study was a laboratory experimental research conducted at the Microbiology Laboratory of Syedza Saintika University from February to April 2024. Data obtained were analyzed using SPSS software with a one-way ANOVA test to determine significant differences among treatment groups. The tools and materials used in this study included Petri dishes, test tubes, a Bunsen burner, autoclave, labels, micropipettes, an analytical balance, paper discs, test tube racks, forceps, Nutrient Agar (NA) medium, ethanol extracts of soursop and dragon fruit peels, ketoconazole, 0.9% NaCl solution, distilled water (aquadest), 70% alcohol, and Sabouraud Dextrose Agar (SDA) medium.

Soursop (*Annona muricata*) and dragon fruit (*Hylocereus polyrhizus*) peels were first cleaned, shade-dried, and ground into fine powder. The powdered materials were extracted using the maceration method by soaking them in 96% ethanol for 72 hours at room temperature with occasional stirring. After maceration, the mixture was filtered, and the filtrate was concentrated using a rotary evaporator to obtain a thick extract. The crude extracts were then diluted with DMSO to obtain final concentrations of 5%, 25%, and 50%, prepared both as individual extracts and in combination.*Candida albicans* was used as the test microorganism. The fungal isolate was reactivated on SDA media and incubated at 37°C for 24 hours. A fungal suspension was prepared by adjusting the turbidity to match the 0.5 McFarland standard using 0.9% NaCl solution. Antifungal activity was tested using the disc diffusion method. SDA media was sterilized, poured into sterile Petri dishes, and allowed to solidify. The surface of the agar was evenly inoculated with *C. albicans* suspension using a sterile cotton swab. Sterile paper discs (6 mm diameter) were each loaded with 20 µL of the extract at the specified concentrations and then placed on the inoculated agar surface.Ketoconazole was used as a positive control, and DMSO served as a negative control. The plates were incubated at 37°C for 24 to 48 hours. After incubation, the diameter of the inhibition zones around each disc was measured in millimeters using a ruler or caliper. All treatments were conducted in triplicate to ensure accuracy and reliability. The mean inhibition zone diameters were statistically analyzed using a one-way ANOVA to assess differences among the treatment groups.

RESULT

A. Univariate Analysis

1.Antifungal Activity Test of Soursop (*Annona muricata*) Peel Ethanol Extract Against the Growth of *Candida albicans* In Vitro

Table 1. Inhibition Zone Diameter of Soursop (*Annona muricata*) Peel Ethanol Extract Against the Growth of *Candida albicans* In Vitro

Number	Variation of Concentration	Mean Inhibition Zone (mm)	Category
1	5%	3.5	Weak
2	25%	5.6	Moderate
3	50%	8.1	Moderate
4	K(-)	0	-
5	K(+)	10.3	Strong

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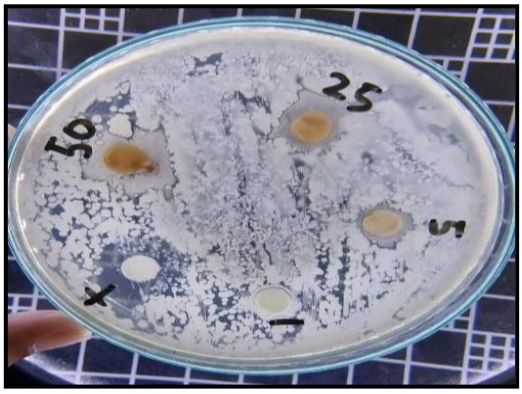

Figure 1. Inhibition Zone of Soursop Peel Extract

Table 1 shows that the largest inhibition zone was produced by the positive control, ketoconazole, followed by soursop peel extract at concentrations of 50%, 25%, and 5%. The inhibitory strength of the soursop peel extract ranged from weak to strong. Figure 1 illustrates the formation of clear zones resulting from the administration of ketoconazole and soursop peel extract, indicating their potential to inhibit the growth of *Candida albicans*.

Antifungal Activity Test of Dragon Fruit Peel Ethanolic Extract (*Hylocereus polyrhizus*) Against *Candida albicans* Growth In Vitro

Table 2. Inhibition Zone Diameter of Dragon Fruit (*Hylocereus polyrhizus*) Peel Ethanolic Extract Against *Candida albicans* Growth In Vitro

Number	Variation of Concentration	Mean Inhibition Zone (mm)	Category
1	5%	2.8	Weak
2	25%	5.5	Moderate
3	50%	10.1	Strong
4	K(-)	0	-
5	K(+)	12.5	Strong

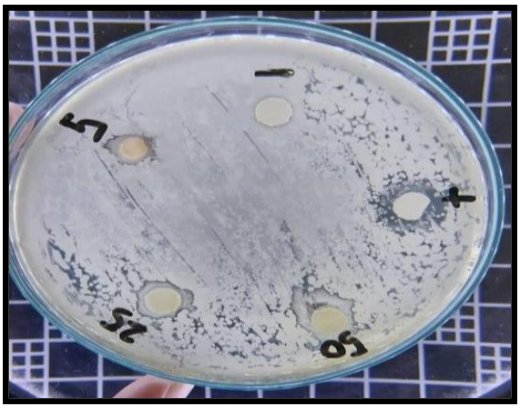

Figure 2. Inhibition Zone of Dragon Fruit Peel Extract (*Hylocereus polyrhizus*)

Tabel 2. shows that the largest inhibition zone was produced by the positive control, ketoconazole, followed Dragon Fruit Peel Extract (*Hylocereus polyrhizus*) at concentrations of 50%, 25%, and 5%. The inhibitory strength of the Dragon Fruit Peel Extract (*Hylocereus polyrhizus*) ranged from weak to strong. Figure 2 illustrates the formation of clear zones resulting from the administration of ketoconazole and Dragon Fruit Peel

Extract (*Hylocereus polyrhizus*), indicating their potential to inhibit the growth of *Candida albicans*.

B. Bivariate Analysis

1. Antifungal Activity Test of the Combination of Soursop Peel (*Annona muricata*) and Dragon Fruit Peel (*Hylocereus polyrhizus*) Ethanolic Extracts Against *Candida albicans* Growth In Vitro

Table 3. Inhibition Zone Diameter of the Combination of Soursop Peel (*Annona muricata*) and Dragon Fruit Peel (*Hylocereus polyrhizus*) Ethanolic Extracts Against *Candida albicans*

Number	Variation of Concentration	Mean Inhibition Zone (mm)	Category
1	1:1	14.5	Strong
2	1:2	15.3	Strong
3	2:1	20.1	Very Strong
4	K(-)	0	-
5	K(+)	21.8	Very strong

Figure 3. Inhibition Zones fof the Combination of Soursop Peel and Dragon Fruit Peel Extracts

Based on Table 3, the combination of soursop peel and dragon fruit peel extracts at a 1:1 ratio produced an average inhibition zone of 14.5 mm, which falls within the category of strong inhibition. The 1:2 ratio combination yielded an average inhibition zone of 15.3 mm, also categorized as strong. In contrast, the 2:1 ratio resulted in an average inhibition zone of 20.1 mm, which is classified as very strong inhibition. The negative control (K−) showed no inhibitory activity, with an average inhibition zone of 0 mm, whereas the positive control (K+) produced an average inhibition zone of 21.8 mm, indicating very strong antifungal activity.

DISCUSSION

The ethanolic extract of soursop (*Annona muricata*) peel has been demonstrated to inhibit the growth of *Candida albicans* in vitro. At a concentration of 50%, the extract produced an inhibition zone measuring 10.1 mm, which is categorized as having moderate antifungal activity. Similarly, the ethanolic extract of sweet orange (*Citrus sinensis*) peel contains a variety of bioactive compounds, including triterpenoids, saponins, polyphenols, and tannins, all of which are known to contribute to antifungal activity. These compounds are believed to disrupt fungal cell walls, alter membrane permeability, and interfere with essential metabolic pathways in *C. albicans* [11]. Saponin compounds are known to reduce surface tension on microbial cell membranes, leading to increased permeability and eventual cell lysis. In addition, tannins possess cytotoxic properties that can damage fungal cell structures by precipitating proteins and disrupting cellular integrity, thereby inhibiting fungal growth and survival [12] [13] [14].The results of this study are consistent with findings reported by which demonstrated that soursop extract is capable of inhibiting the growth of *Staphylococcus epidermidis*. The bioactive compounds present in soursop peel and seeds have been shown to possess antimicrobial, antidiabetic, and antioxidant properties, contributing to their potential use as natural therapeutic agents [2].

Dragon fruit (*Hylocereus polyrhizus*) peel extract has been shown to effectively inhibit the growth of *Candida albicans*. The peel contains a wide range of bioactive compounds, including ascorbic acid, oleic acid, decanoic acid, phenolic

compounds, esters, vitamin C, alkaloids, tannins, flavonoids, and steroids. These constituents are believed to contribute to its antifungal activity through mechanisms such as disruption of cell membranes, inhibition of enzymatic activity, and interference with fungal metabolic pathways [15 416]

Dragon fruit (*Hylocereus polyrhizus*) peel extract has demonstrated the ability to inhibit the growth of *Candida albicans* in vitro. The antifungal activity of the extract has been reported to range from weak to strong, depending on the concentration used and the specific extraction method. According to previous research, this antifungal effect is attributed to the presence of various phytochemicals with antimicrobial properties, such as flavonoids, tannins, and phenolic compounds [1718] Dragon fruit (*Hylocereus polyrhizus*) peel extract has been shown to exhibit antimicrobial activity against a variety of pathogenic microorganisms, including *Propionibacterium acnes*, *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*. This broad-spectrum activity is attributed to its rich content of bioactive compounds such as flavonoids, tannins, phenolic acids, and vitamins, which contribute to its potential as a natural antimicrobial agent.Each individual extract, at various concentrations, has been shown to inhibit the growth of *Candida albicans* in vitro, with inhibition levels ranging from weak to strong. The findings of this study indicate that the combination of soursop (*Annona muricata*) peel and dragon fruit (*Hylocereus polyrhizus*) peel extracts exhibits enhanced antifungal activity, with inhibition zones categorized as strong to very strong. This increased effectiveness may be attributed to the synergistic action of the bioactive compounds present in both extracts, which collectively enhance their ability to suppress the growth of *Candida albicans*.

The combination of extracts may have a synergistic effect in inhibiting microbial growth. According to research [19] According to Yulia & Prima (2023), the combination of sweet orange peel and kepok banana peel extracts can inhibit the growth of *Candida albicans* in vitro. According to [20]. The combination of *Trigona* sp. propolis extract and ginger has shown inhibitory effects against *Salmonella Typhimurium*..

CONCLUSION AND SUGGESTIONS

The conclusion of this study is that the combination of soursop (*Annona muricata*) peel and dragon fruit (*Hylocereus polyrhizus*) peel extracts effectively inhibits the growth of *Candida albicans* in vitro. The observed antifungal activity of the combined extracts falls within the categories of strong to very strong inhibition. It is recommended that future research include comprehensive phytochemical screening of each extract to identify and characterize the specific bioactive compounds responsible for the antifungal effects.

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