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Antifungal Effects of *Solanum Melongena* L Peel Extract Against Candida Albicans: *In Vitro Study*

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Abstract
Background and aim: Candida albicans (C. albicans) is a fungus that most commonly causes superficial mucosal infections in humans. If the host is immunosuppressed, C. albicans, which initially lived commensally, will turn into a pathogen and infect the host's body. The increased use of antifungals and side effects of synthetic/chemical drugs have made people turn to herbal medicine. Active compound that can be found in plants that act as an adjuvant therapy, such as Solanum melongena L. This study aims to determine the effectiveness of Purple eggplant peel extract compared with nystatin against the growth inhibition of C. albicans.
Methods: The method used well diffusion. Antifungal activity shown of the inhibition zone. Total samples were 25 samples divided into five groups; groups treated with Purple Eggplant peel extract 25 %, 50 %, 75%, positive control (nystatin) and negative control. Data was analysed with Kruskal-Wallis test.
Results: Purple Eggplant peel extract inhibited C. albicans growth characterized by an inhibition zone that formed. Means of diameter of inhibition zone were statistically significant different between groups (p-value = 0.000).
Conclusions: Purple Eggplant peel extract with 25%, 50%, and 75% concentration has the same ability as nystatin in inhibiting the growth of C. albicans.
Keywords: *Solanum Melongena L; Purple Eggplant Extract; Candida Albicans; Antifungal.*

Introduction

Candida albicans (C. *albicans*) is a fungus that most commonly causes superficial mucosal infections in humans. It is a component of the normal microflora, and about 30-50% of people have these microorganisms in their bodies. It is a species of the genus Candida that live in commensal colonies in the human body. The proliferation of these fungi is controlled by the host's immune system. If the host is immunosuppressed, C. *albicans*, which initially lived commensally, will turn into a pathogen and infect the host's body[1], [2]. The incidence of candidiasis is quite common. Insidens of Oral candidiasis has reached 2,000.00 cases/year,

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oesophageal candidiasis is 1,300,000 / year, and for vulvovaginal candidiasis, about 70-75% of women. Recurrent episodes of vulvovaginal candidiasis were found in 134,000 cases/year [3]. The increased use of antifungals is due to an increase in cases of C. *albicans* infection. This condition can lead to antifungal resistance. An example is widespread azole use, causing resistance to this agent[4]. Also, the side effects of synthetic/chemical drugs have made people turn to herbal medicine because they have minimal side effects and maximum therapeutic effects. Nowadays, in addition to treatment using chemical drugs, many researchers have also developed about herbal medicine. Active compound that can be found in plants that act as an adjuvant therapy. Purple eggplant (*Solanum melongena L)* is a potential source of antifungal agent [5]. This study aims to determine the effectiveness of Purple eggplant peel extract compared with nystatin against the growth inhibition of C. *albicans.*

Material and Methods

This research is true experimental research with the treatment of giving ethanol extract of purple eggplant peel (*Solanum melongena L*.) to C. *albicans*. Study aim is to test the effectiveness of the inhibition of this extract on the growth of C. *albicans* using the method disc diffusion, using extract concentrations of 25%, 50%, and 75%. This research was conducted in January 2020 at the Laboratory of the Biology, Universitas Negeri Makassar, Indonesia.

Procedure

Making of Extracts

We washed purple eggplant until clean. The peel of purple eggplant is separated from the fruit and then dried in a drying cabinet for ± 4-7 days, which is to prevent damage to its bioactive compounds, which are sensitive to direct sunlight. After the purple eggplant peel has dried, then blend until smooth and wrap it with aluminum foil. Sample extraction used 96% ethanol with the soxhlet extraction method. The soxhlet extraction method is a type of extraction with a liquid organic solvent that is carried out repeatedly at a specific temperature with a certain amount of solvent. The extract was diluted into a solution with a concentration of 25%, 50%, and 75%.

Preparation of Candida Medium

C. *albicans* was inoculated on Sabouroud Dextrose Agar (SDA) medium in a petri dish then incubated at 37^{o}C for one day in an incubator.

Positive and Negative Control

The positive control used in this study was nystatin. Nystatin comes from a group that is generally used in the therapy of infections caused by Candida sp. Nystatin has an antifungal effect that is a very effective fungistatic and fungicide. Nystatin will bind to sterols, especially ergosterol, in the cytoplasmic membrane of fungal cells and change the membrane's permeability so that vital components in fungal cells, such as ions and small molecules, will be lost until the fungal cell experiences death. Another antifungal effect possessed by nystatin is that it causes oxidative damage to fungal cells [6], [7]. A 10% Dimethyl Sulfoxide (DMSO) solution was used as a negative control. DMSO is a solvent that dissolves polar and non-polar compounds that do not have antibacterial and antifungal effects [8].

Determination of antifungal activity using the Disc Diffusion method

Sterile paper discs and nystatin discs 100 units/mg were placed with sterile tweezers. Then the extract solution of purple eggplant peel with a concentration of 25%, 50%, and 75% was dropped on the disc paper as much as 25 µl using. After being left for 1 hour for the solution to absorb into the disc paper, the Candida albicans medium was tightly closed and incubated at

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37oC for 24 hours. Observations were made on the growth medium by measuring the zone of inhibition formed around the disc paper using a ruler.

Phytochemical Test of Extract

The phytochemical test of qualitative analysis was conducted. Test the active ingredient flavonoid content, with 0.1 gram of the Purple Eggplant peel extract with 10 ml ethanol, then add a few drops of concentrated HCl and 0.05 grams of magnesium metal. The presence of flavonoids, indicated by the formation of yellow in 3 minutes. For the presence of tannin active ingredients, 1 ml of purple eggplant peel extract is reacted with a solution of $FeCl_3$ 10%, if there is a change in colour to dark blue or greenish-black, shows the presence of tannin. Test the content of the active ingredient Alkaloid, extract of the purple eggplant peel extract is reacted with 1 ml of Wagner's reagent, and a brown precipitate is formed. For the presence of saponin, 1 gram of purple eggplant peel extract is soaked and boiled for 2-3 minutes over a bunsen fire. Then, shake firmly. Positive if the stable foam is formed[9].

Statistical Analysis

Data about the diameter of the inhibition zone was presented as mean and its standard deviation. The data was not normally distributed with the Shapiro-Wilk test. Kruskal-Wallis H test was used to determine the mean differences of diameter inhibition zone among groups.

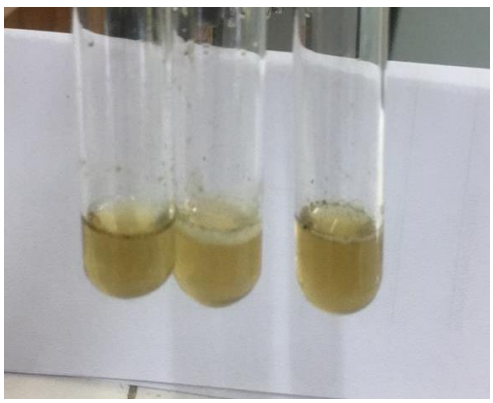
Results

Phytochemicals Screening

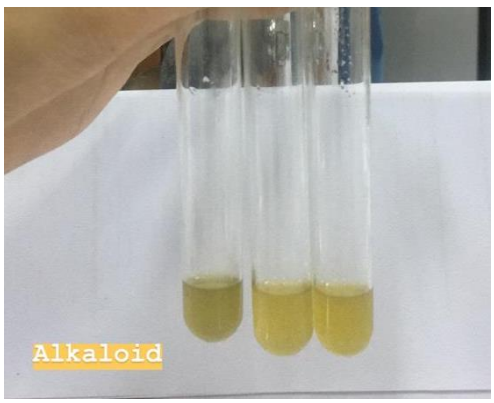
Phytochemical test of the Purple Eggplant peel extract was done to identify the content of the active compound in the extract. Phytochemical screening carried out in this study was screening for flavonoids, alkaloids, tannins, and saponins. The data obtained can be seen in Table 1.

Table 1. Phytochemical Screening Results of Purple Eggplant peel extract (*Solanum melongena L.*)

Phytochemical Test	Results	Interpretation
Flavonoid	Yellow colour in 3 minutes	Positive
Alkaloid	No brown sediment	Negative
Tannin	Blackish green	Positive
Saponin	Stable emulsion	Positive



Flavonoid (+)



Alkaloid (-)

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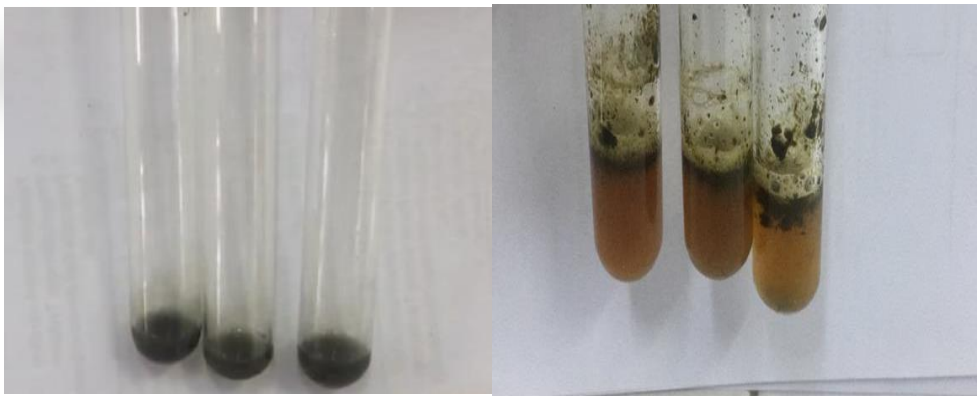

Tanin (+) **Saponin (+)**

Figure1. Phytochemical test for qualitative analysis of active substances in Purple Eggplant peel extract

It has been shown that the active substances contained in Purple Eggplant peel extract based on phytochemical test qualitative analysis is positive containing Flavonoids, Saponins, and Tanins (Table 1, Figure 1)

Antifungal activity of Purple Eggplant peel extract

The Purple Eggplant peel extract test results against C. *albicans* showed antifungal activity. This is evidenced by the formation of an inhibition zone around the disc paper that has been given the extract.

Tabel 2. The results of the diffusion test for the antifungal activity of Purple Eggplant peel extract against C. *albicans*

	Diameter of Inhibition Zone (mm) in petri dish						
Groups	I	II	III	IV	V	Means ± SD	P – value*
Positive control	22.4	28.6	22.9	23.8	22.9	24.12±2.55	0.000
Negative control	6.0	6.0	6.0	6.0	6.0	6.0±0.00	
Purple Eggplant peel extract 25 %	8.8	8.6	10.1	9.9	10	9.46±0.72	
Purple Eggplant peel extract 50 %	11.1	11.3	13.6	12.1	12.9	12.20±1.06	
Purple Eggplant peel extract 75 %	14.1	14.7	15.9	15.7	15.3	15.14±0.74	

***Data expressed as mean ± SD and p-value with Kruskal-Wallis test. Positive control (nystatin), negative control (DMSO)**

A Kruskal-Wallis test was conducted to determine if there were differences in diameter of inhibition zone between groups. Means of diameter of inhibition zone were statistically significant different between groups (p-value = 0.000).

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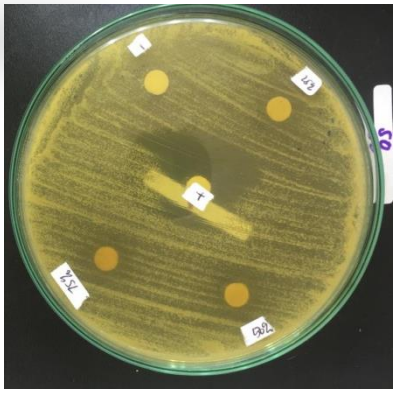
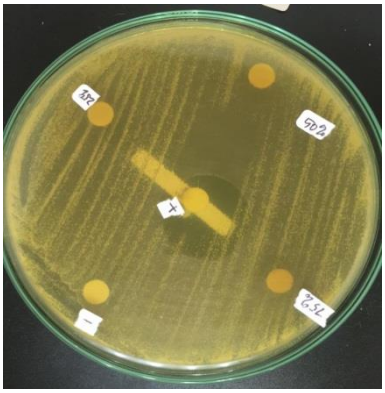
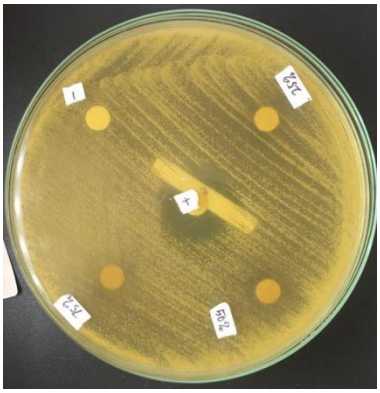


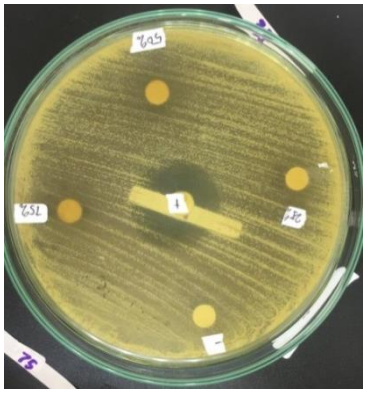
Petri dish I



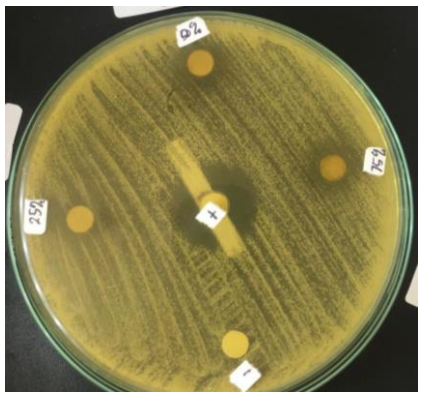
Petri dish II



Petri dish III



Petri dish IV



Petri dish V

Figure 2. Results for antifungal activity of Purple Eggplant peel extract against C. *albicans* with positive and negative control

Discussion

Purple Eggplant (*Solanum melongena L)* contains active ingredients which are provide antibacterial and antifungal activity. This plant is considered a functional food, because in some previous studies reported higher concentration of flavonoids and phenolic acids [10]–[12]. Our results showed that the Purple Eggplant peel extract inhibited C. *albicans* growth characterized by an inhibition zone that formed (Figure 2). The ability of Purple Eggplant peel extract to

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inhibit the growth of C. *albicans* due to the active compound in this plant. These chemical compounds include flavonoids, tannins, and saponins (Table 1, Figure 1). These compounds have a mechanism that damages cell membranes in inhibiting the growth of C. *albicans*.

Flavonoids are metabolites in the Purple Eggplant peel that can denature fungal cell proteins. The mechanism of flavonoids is by denaturing proteins, disrupting the lipid layer, and causing cell wall damage. The lipophilic properties of these flavonoids will bind phospholipids to fungal cell membranes and interfere with cell membrane permeability. Flavonoids work by binding to extracellular proteins present in the cell wall of the fungus, then damaging the cell wall of the fungus and inhibiting the work of the enzyme. Protein denaturation disrupts cell formation so that it changes the composition of protein components which causes increased cell membrane permeability. It causes the leakage of intracellular material; cells become deficient in ATP, impaired cell metabolism, stunted growth, and cell lysis [13]. Cell damage also causes detachment of the fungal cell wall and decreased germ tube formation and hyphae [14].

Tannins are active compounds that act as antifungals. The antifungal mechanism of tannins is due to their ability to inhibit the synthesis of chitin used for cell wall formation in fungi and damage cell membranes so that fungal growth is inhibited. The inhibitory ability of chitin synthesis possessed by tannins is due to the high polymerase present in the hydroxyl group in ring B in the chemical structure of the tannin. Tannins can also inhibit ergosterol biosynthesis in the membrane of fungal cells. This causes the cell permeability to increase, the cytoplasm out of the cell, and the cell volume decreases so that the cell shrinks. Cells become deficient in ATP, impaired cell metabolism, stunted growth, and cell lysis. Tannins can also inhibit the yeast budding process and inhibit pseudohifa formation [15].

Saponins can cause fungi cell lysis by disrupting the stability of the cell membrane. Saponins act as a surfactant that is polar in shape and will reduce the surface tension of the sterol membrane from the cell wall of C. *albicans*, causing interference with membrane permeability which results in the entry of materials or substances needed to be disrupted, eventually, the cell swells and lysis. [16].

Conclusions

Purple Eggplant peel extract contains active substances flavonoids, tannins, and saponins. Purple Eggplant peel extract with 25%, 50%, and 75% concentration has the same ability as nystatin in inhibiting the growth of C. *albicans*.

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Conflict of Interest

Authors declare that there is no conflict of interest within this research and publication.

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