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The inhibitory effect of Sambiloto (*Andrographis paniculata Nees*) leaf extract on growth of *Candida albicans*

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Abstract

Background: Linear gingival erythema (LGE) is a periodontal disease caused by fungal infection of the genus *Candida*. Based on the results of Nugraha's research, the most common *Candida* species in LGE was *Candida albicans* which reached 71.43%. Several studies have shown that sambiloto (*Andrographis paniculata Nees*) has antifungal activity because sambiloto contains active ingredients such as andrographolides, flavonoids, alkaloids, saponins, and tannins.

Objective: This study aims to determine the inhibitory effect of sambiloto (*Andrographis paniculata Nees)* leaf extract on growth of *Candida albicans*.

Methods: This research is an in-vitro laboratory experimental study. Sambiloto (*Andrographis paniculata Nees*) leaf extract was produced by the maceration method using 70% ethanol. The inhibition testing on *Candida albicans* cultures was carried out by spectrophotometric methods and colony counts.

Result: At concentration of 100%, 50%, 25%, and 12.5% of sambiloto leaf extract, no fungal colonies were found. While at concentration of 6.25% can inhibit fungal by 91.67%. Statistical tests on each concentration group showed a significance value of 0,001 ($p<0.05$).

Conclusion: Sambiloto (*Andrographis paniculata Nees*) leaf extract has an inhibitory effect on growth of *Candida albicans* with MIC values at concentration of 6.125% and MBC of 12.5%.

Keywords: Sambiloto (*Andrographis paniculata Nees*); *Candida albicans*; Inhibitory effect; Spectrophotometry; Colony counts.

1. Introduction

Oral cavity is the first entrance for materials needed by the body and a place for microorganism to infect diseases. Oral hygiene affects the occurance of the disease because the poor oral hygiene can lead to gingival diseases [1]. Gingival diseases not only caused by plaque, but also be caused by non-plaque factors such as by fungi can also contribute [2, 3].

Linear gingival erythema (LGE) is a periodontal disease that appears a distinct fiery red band along the margin of the gingiva extending 2-3 milimeters [4]. LGE caused by fungal infection of the genus *Candida*. Based on the results of Nugraha's research, there was a correlation between the LGE and *Candida* infection and the most common *Candida* species in LGE was *Candida albicans* which reached 71.43% [5].

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The use of traditional medicine has been practiced throughout the world because the use of traditional medicine is considered safer with relatively fewer side effects. Currenty, many herbal plants have a various health benefits such as Sambiloto (*Andrographis paniculata Nees*). Sambiloto or known as "King of Bitter" is a herbal plant that distributed in tropical Asian countries [6]. Several studies have shown that sambiloto has antifungal activity because sambiloto contains active ingredients such as andrographolide, flavonoids, alkaloids, saponins, and tannins [7].

On that account, the sambiloto (*Andrographis paniculata Nees*) leaf extract is expected to be an alternative therapy option and giving a therapeutic effect on patients with linear gingival erythema. In this study, the authors wanted to observe the inhibitory effect of sambiloto leaf extract on the growth of *Candida albicans*.

2. Material and methods

This study was conducted from Juli 2022 until Agustus 2022 at the Airlangga University Dental Medicine Research Center. Sambiloto (*Andrographis paniculata Nees*) leafs were extracted in the Laboratory of Pharmacy Faculty at Widya Mandala Surabaya University according to the protocol of the laboratory.

This study uses an in-vitro laboratory experimental study consisting of 9 treatment groups: Sambiloto leaf extract with concentrations of 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, 1.56%, 0.78%, and negative control. After doing the calculation using the Federer formula, it is found that the repetition should e held 3 times at a minimum, therefore the total sample would be 27. Materials and Equipment Preparation

The materials used in this experiment are sambiloto leaf extract, 70% ethanol, *Candida albicans* isolate, Saboraud Dextrose Agar (SDA), Saboraud Dextrose Broth (SDB), methylated spirit, and sterile aquades. The equipment used are blender, sieve, oven, knife, moisture balance, vacuum rotary evaporator, water bath, baker glass, aluminium foil, erlenmeyer, autoclave, spectophotometry, spirit burner, ose, incubator, anaerobic jar, test tube, test tube rack, petri dish, spreader, micropipette, and cuvet.

2.1. Preparation of Sambiloto Leaf Extract

Sambiloto leaves were washed throughly then cut into pieces. Sambiloto leaves that have been cut are dried using an oven with a temperature of 50°C. The dried leaves measured by moisture balance. If the water content was less than 10%, the dried leaves sifted using blender and sieve to obtain simplicia powder.

481.5 gram of simplicia powder was macerated by immersing it in 70% ethanol with a powder to ethanol ratio of 1:4 then stirred several times and left for 24 hours. The sample is filtered with filter paper to obtain filtrate. The filtrate was evaporated using a vacuum rotary evaporator and water bath at 60°C for 24 hours. The concentrated extract was stored in a beaker glass and covered with aluminium foil.

2.2. Phytochemical Screening Test

Put 10 mL of sambiloto leaf extract into the UV-Vis spectrophotometer cuvette. Then, andrographolides, flavonoids, alkaloids, saponins, and tannins were measured with a wavelength of 224 nm for alkaloids, 330 nm for flavonoids, 235 nm for saponins, 225 nm for tannins, and 223 nm for andrographolides.

2.3. MIC and MBC Test on *Candida albicans*

The preparation of the pure isolates of *Candida albicans* obtained from the Research Center of the Faculty of Dental Medicine, Airlangga University. The suspension was made by taking *Candida albicans* from the culture medium using an ossicle, the putting it in a test tube containing 1 ml of sterile Saboraud Dextrose Broth (SDB) and then putting it in incubator, and incubated anaerobically for 2x24 hours at 37°C. Next, the suspension turbidity was comparable to the standard Mc Farland 0.5.

The suspension that had been standardized with Mc Farland turbidity 0.5 was put into each test tube containing sambiloto leaf extract with 8 different concentrations, namely 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, 1.56%, 0.78%, test tube containing fungi culture and media as negative control. After that, each test tube was incubated for 1x24 hours at 37°C. The test tubes were then measured to determine the inhibitory effect of sambiloto leaf extract on the growth of *Candida albicans* by observing MIC and MBC using a spectrophomoter and colony counter.

3. Results

Based on the result of the phytochemical screening that has been carried out, it shows that the percentage of the ingredients contained in sambiloto leaf extract is 2.01% for andrographolides, 5.22% for flavonoids, 4.11% for alkaloids, 5.90% for saponins, and 13.85% for tannins.

Table 1 The result of phytochemical screening

Active ingredient	Percentage (%)
Andrographolide	2.01%
Flavonoids	5.22%
Alkaloids	4.11%
Saponins	5.90%
Tannins	13.85%

The antifungal inhibitory effect of the sambiloto (*Andrographis paniculata Nees*) leaf extract was tested using spectrophotometric and colony count test methods to determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) on the growth of *Candida albicans*.

Figure 1 The result from serial dilution test of sambiloto (*Andrographis paniculata Nees*) leaf extract in Saboraud Dextrose Broth (SDB) media

Serial dilution was done to make the various concentrations of the 9 treatment groups. As can be seen in (Figure 1), tube 1 contained sambiloto (*Andrographis paniculata Nees*) leaf extract with a concentration of 100% in 5 mL with 0.1 mL of the suspension. Tube 2, 3, 4, 5, 6, 7, and 8 contained a mixture of 0.1 mL suspension with 5 mL sambiloto leaf extract in Saboraud Dextrose Broth (SDB) with each concentration of about 50%, 25%, 12.5%, 6.25%, 3.125%, 1.56%, and 0.78%. Tube (-) was a negative control, made from 5 mL Saboraud Dextrose Broth (SDB) and 0.1 mL of the suspension.

The spectrophotometry test was conducted to determine wheter the sambiloto (*Andrographis paniculata Nees*) leaf extract actually has antifungal activity against *Candida albicans* by observing the differences in general between all the absorbance value.

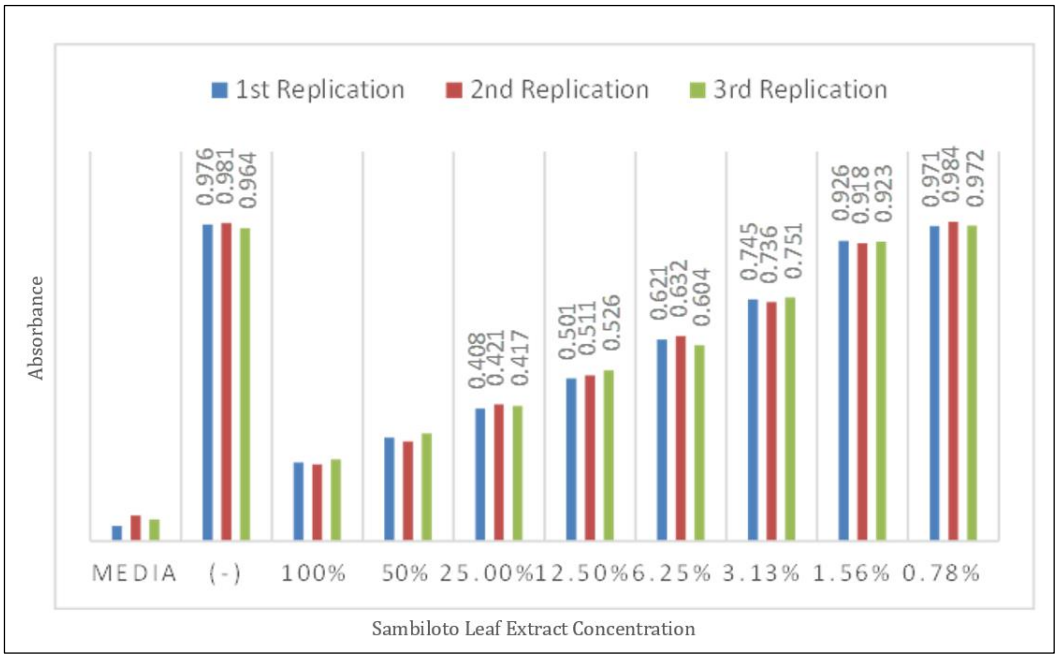

Figure 2 Absorbance results using a spectrophotometry

After conducting the spectrophotometry test, the dilution test results were then cultured into Saboraud Dextrose Agar (SDA) to do a colony count test. Each plate represents each tube with the concentrations.

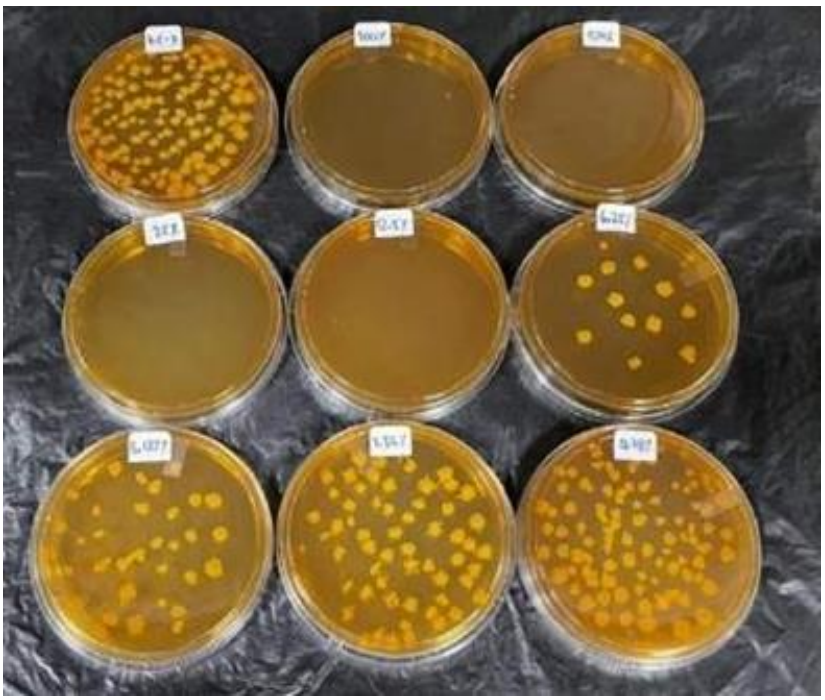

Figure 3 The result of colony count test in Saboraud Dextrose Agar (SDA)

The MIC and the MBC value were determined based on the average number of fungal colonies on SDA media. The results of the colony count test can be seen in Figure 4.

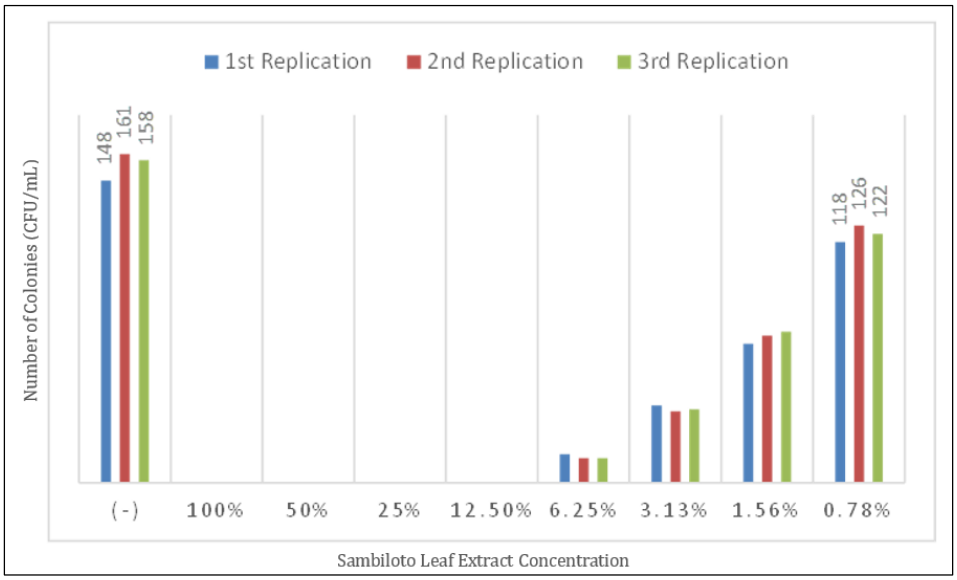

Figure 4 The result of colony count test (CFU/ml)

In order to acknowledge the MIC and MBC value, the calculation of the fungal inhibition percentage and average colony growth was conducted. The table following contains the calculation result:

Table 2 Average colony growth and fungal inhibition percentage

Group		**Average Colony Growth(CFU/ml)**	**Fungal InhibitonPercentage (%)**
Negative Control		156	0
Concentrations	100%	0	100
	50%	0	100
	25%	0	100
	12.5%	0	100
	6.25%	13	91.67
	3.125%	36	76.92
	1.56%	71	54.49
	0.78%	122	21.79

As can be seen in (Table 2), sambiloto leaf extract at the concentration of 100%, 50%, 25%, and 12.5%, no fungal colonies were found. While at concentration of 6.25%, 3.125%, 1.56%, and 0,78% shows growth of *Candida albicans*. At the concentration of 6.25%, sambiloto leaf extract can inhibit fungal by 91.67%.

In this study, statistical data analysis and determination of MIC and MBC were done using the data from *Candida albicans* colony growth on Saboraud Dextrose Agar (SDA) media. The data obtained was then tested for statistical analysis with the normality test using the Shapiro-Wilk test. The test results show that all treatment groups have a value $p > 0.05$, thus the data is normally distributed. The test is continued with the homogeneity test using the Levene test. The test obtained a p-value of 0.002, so because it is < 0.05, it means that the data is not homogeneous. With a parametric test, One Way ANOVA, the p result is 0.000, which means the data has a significant difference. The result of the post-hoc using Games-Howell test (Table 3) showed that concentrations of 100%, 50%, 25%, and 12.5% have significant differences with concentrations of 6.25%, 3.125%, 1.56%, and 0.78%.

Table 3 The result of post-hoc using games-howell test

Groups	Control (-)	100%	50%	25%	12,5%	6,25%	3,125%	1,56%	0,78%
Control (-)									
100%	0.003*								
50%	0.003*	1.000							
25%	0.003*	1.000	1.000						
12.5%	0.003*	1.000	1.000	1.000					
6.25%	0.003*	0.013*	0.013*	0.013*	0.013*				
3.125%	0.003*	0.003*	0.003*	0.003*	0.003*	0.000*			
1.56%	0.003*	0.003*	0.003*	0.003*	0.003*	0.001*	0.003*		
0.78%	0.029*	0.002*	0.002*	0.002*	0.002*	0.001*	0.001*	0.001*	

4. Discussion

In this study, the result that obtained from the spectrophotometry test can not be continued to the data analysis because the sambiloto leaf extract was too dark and viscous so it can affect the absorbance value. In the spectrophotometry test, the absorbance value can not determine whether there is an inhibited *Candida albicans*, lysed precipitate, or came from precipitate of leaf extract. The alternative method that can be used is colony counts.

On the colony count test, sambiloto (*Andrographis paniculata Nees*) leaf extract with a concentration of 6.25% can be expressed as MIC (Minimum Inhibitory Concentration) because with this concentration the growth of *Candida albicans* colonies can be suppressed by 91.67% (>90%). Meanwhile, MBC (Minimum Bactericidal Concentration) is determined as the smallest concentration that can kill *Candida albicans* so that no colonies can grow, which is found at a concentration of 12.5%. The result from colony count test was continued by data analysis using SPSS, which showed that all concentrations of sambiloto leaf extract have a significant difference ($p < 0.05$).

The presence of antifungal activity that produced by sambiloto leaf extract is caused by several active ingredients contained in sambiloto such as andrographolides, flavonoids alkaloids, saponins, and tannins. Andrographolides have high antifungal activity which can inhibit the synthesis of ergosterol and interfere the components that form the membrane or cell wall of the fungus. Andrographolides can interfere with ergosterol so that cell membrane permeability is disturbed because Andrographolides can bind to the lipid bilayer [7, 8]. Flavonoids are phenolic compounds, which can interact with cell membrane proteins that resulting in decomposition of cell membranes and penetration of phenolic compounds. Penetration of phenolic compounds result in denaturation of cell membrane proteins [9]. Alkaloids contain nitrogen which will react with amino acids. This reaction causes changes in the composition and structure of amino acids resulting in genetic damage to the DNA chain so the fungal cells will damage [10]. Saponins can reduce the surface tension of the sterol membrane of the fungal cell wall so the permeability of the membrane or cell wall is disrupted and has and impact on disrupting the process of diffusion of substances needed by fungal cell so the cells will swelling [11, 12]. Tannins can cause shrinkage of fungal cell walls by interacting the fungal cells with an absorption process through hydrogen bonds so the fungal cells undergo decomposition and followed by penetration into in fungal cells [13, 14].

5. Conclusion

The conclusion is sambiloto (*Andrographis paniculata Nees*) leaf extract has an inhibitory effect on growth of *Candida albicans* with MIC values at concentration of 6.125% and MBC at the concentration 12.5%.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors have no conflict of interest to declare.

Statement of ethical approval

This research study was approved ethically by Universitas Airlangga Faculty of Dental Medicine Health Research Ethical Clearance Commission, with an ethical clearance letter number: 631/HRECC.FODM/VIII/2022.

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