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ANTIFUNGUS EFFECTIVENESS OF CITRUS AMBLYCARPA PEEL, CITRUS HYSTRIX DC PEEL AND CITRUS AURANTIFOLIA PEEL EXTRACT (Against the Growth of *Candida albicans*)

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ABSTRACT

Background*: Candida albicans is a microorganism that causes many infections in the oral cavity. To treat infections caused by Candida albicans, antifungal therapy is needed. Alternative treatments for fungal infections can utilize traditional medicines, namely by using citrus amblycarpa peel extract, citrus hystrix DC peel, and citrus aurantifolia peel because they contain alkaloids, flavonoids, saponins, and tannins.* ***Objective****: Analyzing the antifungal effects of 100% concentration of citrus amblycarpa peel extract, 100% concentration of citrus hystrix DC peel, and 100% concentration of citrus aurantifolia peel on the growth of Candida albicans.* ***Method****: This study was a true experimental laboratory study with a post test only control group design, consisting of four treatment groups. Minimum Inhibitory Concentration (MIC) and Minimum Killing Concentration (MKC).* ***Result****: Parametric analysis using One Way Anova and Post Hoc Bonferroni KHM showed that there were significant differences in each treatment group and Post Hoc Games Howell test analysis showed that citrus amblycarpa peel extract showed no significant difference to citrus hystrik DC peel, citrus aurantifolia peel extract 100% concentration, while the aquades as a negative control there is a significant difference.* ***Conclusion****: Citrus amblycarpa peel extract, citrus hystrix DC peel extract, citrus aurantifolia peel 100% concentration, and aquades as a negative control had inhibitory effect on Candida albicans. Citrus amblycarpa peel extract concentration of 100% has killing power.*

Keywords : *Candida albicans, Citrus Amblycarpa Peel, Citrus Hystrix DC Peel, Citrus Aurantifolia Peel*

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INTRODUCTION

Maintaining healthy teeth and mouth is necessary to prevent infection or disease in the oral cavity.[1] The microorganism that causes many oral infections is *Candida albicans*.[2] The fungus *Candida albicans* is a normal local microorganism in the oral cavity.[3] *Candida albicans* is a genus found in denture users who are less aware of the health and hygiene of the oral cavity which can cause denture stomatitis.[4] Denture stomatitis is an infection caused by *Candida albicans* causing inflammation of the oral mucosa caused by wearing dentures.[5]

Treatment of infections caused by *Candida albicans* requires antifungal therapy.[2] Alternative treatment for fungal infections can use traditional medicines from natural ingredients or herbal plants which have fewer side effects[2,6] Plants used as medicinal plants are *Citrus amblycarpa*, *Citrus aurantifolia*, and *Citrus hystrix DC* which have many similarities, namely genus, ingredients that can be used as antifungal therapy, and morphology. Similarities in the content of *Citrus amblycarpa*, *Citrus aurantifolia*, and *Citrus hystrix DC* namely alkaloids, flavonoids, saponins, and tannins which can be used as antifungal therapy.[7-9] The content of the *Citrus amblycarpa* peel contains triterpenoid compounds of 899.13 mg/ml, alkaloids of 270.65 mg/ml, saponins of 256.59 mg/ml, flavonoids of 97.58 mg/ml, steroids of 21.81 mg/ml, and tannins of 0.13 mg/ml.[9] The content of *Citrus hystrix DC*

peel is tannin compound 1.8% v/v, steroids 1.5% v/v, terpenoids 1.5% v/v.[10] The content of *Citrus aurantifolia* peel is a flavonoid compound of 23.5 mg/g, 95.6 mg/g of phenol.[11] The *Citrus amblycarpa* peel, *Citrus aurantifolia* peel, and *Citrus hystrix DC* peel exhibit antifungal activity and contain active compounds that are soluble in water.[7-9]

Figure 1. a) *Citrus amblycarpa* b) *Citrus aurantifolia* c) *Citrus hystrix DC*

Citrus amblycarpa popular in South Kalimantan among Banjar people because the content of kuit lime contains secondary metabolites, namely triterpenoids, flavonoids, steroids, alkaloids, tannins and saponins.[12] *Citrus hystrix DC* often used by the people as a food flavoring.[13] *Citrus hystrix DC* peel can be used as antifungal therapy because it contains saponins.[7] *Citrus aurantifolia* in the peel contain compounds that have effective properties to inhibit growt *Candida albicans.*[14] Unfortunately, the peels of *Citrus amblycarpa*, *Citrus aurantifolia*, and *Citrus hystrix DC* which contain compounds for antifungal therapy are often used as waste in society. Research on the comparison of *Citrus amblycarpa* peel, *Citrus hystrix DC* peel, and *Citrus aurantifolia* peel as antifungal therapy against *Candida albicans* is needed to determine the antifungal effectiveness of *Citrus amblycarpa* peel extract at a concentration of 100%, *Citrus aurantifolia* extract peel at a concentration of 100%, and *Citrus hystrix DC* peel extract at a concentration of 100% on growth. *Candida albicans* with the antifungal test method using the dilution method.

METHODS

The study began with an ethical clearance approved by the Health Research Ethic Commission of the Faculty of Dentistry, Lambung Mangkurat University No. 058/KEPKG-FKGULM/EC/III/2023. The research design used was a true experimental design with *post test only control group design.* The population in this study consisted of four groups to measure minimum inhibitory concentrations (MIC) and minimum killing concentrations (MKC) with 100% concentration of *Citrus amblycarpa* peel, 100% concentration of *Citrus hystrix DC* peel, and 100% concentration of *Citrus aurantifolia* peel on growth *Candida albicans.*

The tools used in the study consisted of tools for culture and antifungal testing consisting of test tubes, sterile loops, erlenmeyer, pipettes and micropipettes, hot plates, label paper, measuring cups, beaker glasses, stirring rods, powder spoons, spirits, autoclaves, masks, handscoons, test tube racks, vortex mixers, yellow tips, magnetic stirrers, anaerobic jars, colony counters, ordinary incubators and Uv-Vis spectrophotometers, as well as tools for making extracts, namely masks, handscoons, shakers, hammer mills, mesh screens, balances analytical, erlenmeyer flask, autoclave, beaker, oven, dropping pipette, heidolph rotary evaporator, waterbath (SMIC)

The research was conducted at Basic Laboratory of the Faculty of Mathematics and Sciences (FMIPA), Lambung Mangkurat University, Biochemistry Laboratory, Faculty of Medicine, Lambung Mangkurat University, Banjarbaru, and Biomedical Laboratory, Faculty of Dentistry, Lambung Mangkurat University, Banjarmasin from February to May 2023.

Determination Test

Citrus amblycarpa, *Citrus aurantifolia*, and *Citrus hystrix DC* were obtained form Astambul subdistrict, Banjar Regency, South Kalimantan. The peel is selected from the old fruit, light green in color and the fruit is relatively large and has a good physical appearance. The deterrmination test was carried out at at FMIPA Laboratory of Lambung Mangkurat University, Banjarbaru.

Extraction of Citrus Peel

The extraction process was carried out at the Biochemistry Laboratory, Faculty of Medicine, Lambung Mangkurat University, Banjarbaru. *Citrus amblycarpa*, *Citrus aurantifolia*, and *Citrus hystrix DC* obtained were selected and weighed as much as 5 kg, then cleaned under running water and cut to separate the flesh and peel of each orange. Then dry the skin at room temperature and put it in the oven at 40-50□C for 12 hours, grind it with a blender and sift it until it forms simplicia powder.

The extract was prepared using the maceration method by inserting each orange peel into an extractor and adding 96% ethanol for 5x24 hours protected from light and stirring during immersion using a shaker and changing the solvent every day.

Filtering in this method is carried out using WH40 filter paper so as to obtain a clear brownish liquid. Then the solution is evaporated with a rotary evaporator at 40°C for 4-6 hours, then heated using a water bath until a thick brownish extract is obtained.

Antifungal Test

Antifungal activity test was carried out by liquid methods at Biomedical Laboratory, Faculty of Dentistry, Lambung Mangkurat University, Banjarmasin. The *Candida albicans* suspension that had been cultured on SDA media was taken mixed with 30 ml of BHI-B solution, diluted using sterile aquades until the turbidity standard was comparable to the McFarland standard of 0.5. 1 ml of *Candida albicans* suspension was put into each test tube group which was filled with 100% concentration of *Citrus amblycarpa* peel extract, 100% concentration of *Citrus aurantifolia* peel extract, 100% concentration of *Citrus hystrix DC* peel extract, and given aquades as a negative control.

The difference in absorbance results before and after each extract and control (-) was observed using a Uv-Vis spectrophotometer with a wavelength of 450 nm to obtain the MIC value. Determination of the MKC value was carried out manually using a colony counter to count the number of colonies on agar media.

RESULTS

Research on the antifungus effectiveness of *Citrus amblycarpa* peel, *Citrus hystrix DC* peel and *Citrus aurantifolia* peel extract against the growth of *Candida albicans* had been carried out. This research was conducted using the dilution method to determine the MIC and MKC of *Citrus amblycarpa* peel, *Citrus hystrix DC* peel and *Citrus aurantifolia* peel extract against *Candida albicans*. The MIC value was obtained from measurements using a Uv-Vis spectrophotometer by looking at the difference in absorbance values before and after incubation. The MKC value was obtained from measurements using a colony counter by looking at the number of colonies.

Table 1. Average Value (Mean) and Standard Deviation MIC of 100% concentration of *Citrus amblycarpa* peel extract, 100% concentration of *Citrus hystrix DC* peel extract, 100% concentration of *Citrus aurantifolia* peel extract against *Candida albicans*

Groups	MIC	Standard Deviation (10^3/uL)	Explanation
***Citrus amblycarpa* peel**	-1,404	0,003	**Down**
***Citrus hystrix DC* peel**	-1,486	0,006	**Down**
***Citrus aurantifolia* peel**	-1,620	0,007	**Down**
C (-)	0,676	0,013	**Up**
Total	**-0,958**	**0,967**	

In the table above it can be seen that there was no growth of *Candida albicans* in the *Citrus amblycarpa* peel extract, *Citrus hystrix DC* peel extract, *Citrus aurantifolia* peel extract, while aquadest as a negative control had *Candida albicans* growth. The *Citrus amblycarpa* peel extract decreased the average absorbance value to -1,404, the *Citrus hystrix DC* peel extract decreased the average absorbance value to -1,486, the *Citrus aurantifolia* peel extract decreased the average absorbance value to -1,620, while aquades increased the average absorbance value of 0,676.

Table 2. Average Value (Mean) and Standard Deviation MKC of 100% concentration of *Citrus amblycarpa* peel extract, 100% concentration of *Citrus hystrix DC* peel extract, 100% concentration of *Citrus aurantifolia* peel extract against *Candida albicans*.

Groups	MKC	Standard Deviation Scoring (10^3/uL)
***Citrus amblycarpa* peel**	0,000	0,000
***Citrus hystrix DC* peel**	3,000	3,406
***Citrus aurantifolia* peel**	12,500	15,630
C (-)	921,333	64,612
Total	234,208	406,458

Citrus amblycarpa peel extract showed no growth of *Candida albicans* with an average value of 0,000, *Citrus hystrix DC* peel extract had growth of *Candida albicans* with an average value of 3,00, *Citrus aurantifolia* peel extract had growth of *Candida albicans* with an average value of 12,500, and aquades as a negative control still has *Candida albicans* growth with an average value of 921,333. .

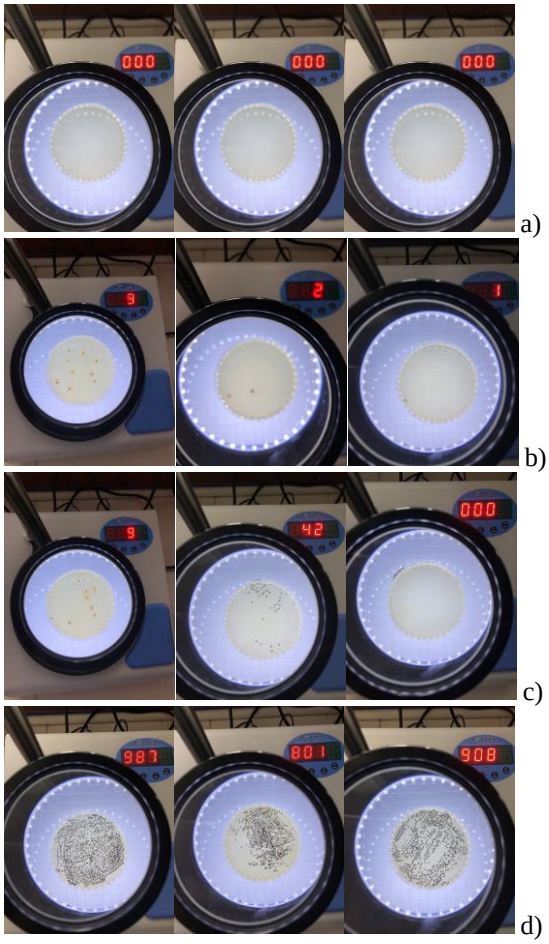

Figure 2. Result of MKC Measurements a) *Citrus amblycarpa*, b) *Citrus hystrix DC*, c) *Citrus aurantifolia*, d) Aquades

Table 3. Results for *Post Hoc Bonferroni* Test of 100% concentration of *Citrus amblycarpa* peel extract, 100% concentration of *Citrus hystrix DC* peel extract, 100% concentration of *Citrus aurantifolia* peel extract against *Candida albicans*

	***Citrus amblycarpa* peel**	***Citrus hystrix DC* peel**	***Citrus aurantifolia* peel**	**C (-)**
***Citrus amblycarpa* peel**		0,000*	0,000*	0,000*
***Citrus hystrix DC* peel**			0,000*	0,000*
***Citrus aurantifolia* peel**				0,000*

The results of the homogeneity and normality tests in the study showed that the data was homogeneous and normally distributed, then the One Way Anova test was carried out. The results of the One Way Anova test showed a sig p value <0.05, which means that the hypothesis is accepted, that is, there is a significant difference in the average MIC of the treatment groups. The Bonferroni Post Hoc test in the table above shows that all treatment groups MIC test between treatment group *Citrus amblycarpa* peel extract concentration 100%, *Citrus hystrix DC* peel exract concentration 100%, *Citrus aurantifolia* peel concentration 100%, and and aquades as a negative control for *Candida albicans* indicating there is a significant difference p <0.05.

Table 4. Results for *Post Hoc Games Howell* Test of 100% concentration of *Citrus amblycarpa* peel extract, 100% concentration of *Citrus hystrix DC* peel extract, 100% concentration of *Citrus aurantifolia* peel extract against *Candida albicans*

	***Citrus amblycarpa* peel**	***Citrus hystrix DC* peel**	***Citrus aurantifolia* peel**	**C (-)**
***Citrus amblycarpa* peel**		0,253	0,314	0,000*
***Citrus hystrix DC* peel**			0,518	0,000*
***Citrus aurantifolia* peel**				0,000*

The results of the One Way Anova test analysis showed a significance value of 0.000 (p <0.05), which means that the hypothesis was accepted, namely that there was a significant difference in the average teaching and

learning groups in the treatment group. The *Citrus amblycarpa* peel extract treatment group did not have a significant difference to the *Citrus hystrix DC* peel with a sig value of 0.253>0.05, *Citrus amblycarpa* peel extract did not have a significant difference to *Citrus aurantifolia* peel extract with a sig value of 0.314<0.05, *Citrus amblycarpa* peel had significant different to aquades with a sig value of 0.000<0.05. *Citrus hystrix DC* peel extract did not have significant difference to *Citrus aurantifolia* peel with a significant value of 0.518<0.05, *Citrus hystrix DC* peel extract had a significant difference to *aquades* with a sig value of 0.000<0.05. *Citrus aurantifolia* peel extract had a significant difference to *aquades* with a significant value of 0.000<0.05.

DISCUSSION

The MIC value of *Citrus aurantifolia* is very effective for inhibiting the growth of *Candida albicans* which can be seen there is a decrease in the absorbance value of -1.620. In line with Tyas research (2019) *Citrus aurantifolia* has strong and very strong inhibition of *Candida albicans*.[2] This is because the content of the most abundant metabolic compounds in *Citrus aurantifolia* peel extract is phenolic compounds, namely 95.6 mg/g.[11] Phenol compounds can be used as antifungals because the H+ ions in phenol can attack the phosphate groups in phospholipids, because of this the phospholipids will decompose and the shape of the cell membrane cannot be maintained which causes the cell membrane to leak and experience inhibition of fungal growth.[15]

The MIC value of *Citrus hystrix DC* peel extract is effective enough to inhibit the growth of *Candida albicans* which can be seen there is a decrease in the absorbance value of -1.486. Supported by Iskandar research (2015) *Citrus hystrix DC* peel has growth inhibition against *Candida albicans*. This is because the most abundant content of metabolic compounds in *Citrus hystrix DC* peel extract is tannin, which is 1.8% v/v. Tannin compounds can enter into lysed cell walls and coagulate the protoplasm of microorganism cells which causes inhibition of the growth of *Candida albicans*.[16]

The MIC value of *Citrus amblycarpa* extract is effective enough to inhibit the growth of *Candida albicans* which can be seen there is a decrease in the absorbance value of -1.404. This is because the content of the most abundant metabolic compounds in *Citrus amblycarpa* peel extract is triterpenoid compounds. Triterpenoid compounds can interfere with cell permeability, this is because in ergosterol in fungal cells, ergosterol is a sterol component that is easy to attack. [18,] The polynen-ergosterol complex can release organic phosphates, amino acids, carboxylic acids, K ions, and phosphate esters so that they can cause the death of the fungus.[17]

The MIC value of distilled water as a negative control experienced an increase in the absorbance value with an average value of 0.676 or did not inhibit the growth of *Candida albicans*. This is supported by the research of Muljono (2016) and Khotimah (2017) that distilled water can be used as a negative control in a study and distilled water is distilled water that has pure properties.[18,19]

The results of the study in the treatment group with 100% concentration of *Citrus amblycarpa* peel extract showed no growth of *Candida albicans*, but 100% concentration of *Citrus hystrix DC* peel extract, 100% concentration of *Citrus aurantifolia* peel extract, and aquades as a negative control still showed Candida growth. albicans, so that the *Citrus amblycarpa* extract showed the presence of MKC. This is due to the content of the most abundant metabolic compounds in the lime peel extract, namely triterpenoid compounds of 899.13 mg/ml.[20] Triterpenoid compounds can kill and inhibit microbial growth by interfering with the formation of cell membranes so that the fungal cell membrane is not formed or not formed.[21] *Citrus hystrix DC* peel extract and *Citrus aurantifolia* peel extract not contain triperpenoid compounds.[21]

Secondary metabolites in *Citrus amblycarpa* peel extract, *Citrus hystrix DC* peel extract, and *Citrus aurantifolia* peel extract have antifungal properties, namely alkaloids, saponins, flavonoids, steroids, triterpenoids, tannins, and phenols.[20,22,23] Alkaloid compounds are nitrogenous bases derived from animals and plants.[24] The mechanism of the antimicrobial alkaloid compounds is to inhibit DNA and RNA polymerases and estarase from *Candida albicans*.[24] Saponin compounds are antifungal because there are glycoside compounds found in plants which increase their permeability by decreasing the tension on the surface of the sterol membrane from the cell wall.[24] The increased permeability causes the more concentrated intracellular fluid to be pulled out of the cell so that the fungus dies.[24] Flavonoid compounds are compounds of the phenol group.[3] Phenol compounds act as antifungals by inhibiting the formation of fungal cell membranes and lysing the fungal cell membranes that have already been formed.[3] Steroid compounds are a class of triterpenoid compounds. The mechanism of microbial inhibition by damaging the microbial cell membrane.[25] The activity of tannin compounds interferes with the living activity of fungal cells by shrinking the fungal cell walls. This causes the growth of fungal cells.[3] In conclusion, this study showed that *Citrus amblycarpa* peel extract at a concentration of 100% had MCI and MKC against *Candida albicans*, *Citrus hystrix DC* peel extract and *Citrus aurantifolia* peel extract at a concentration of 100% had MCI but did not have MKC against *Candida albicans*.

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