

"Research Article"**The Influence of The Soaking Period of Acrylic Resin in Basil Leaf (*Ocimum Basilicum*) Extract towards The Growth of *Candida Albicans*****Hastoro Pintadi*, Ismi Dea Nurintan**

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* Corresponding author, e-mail: hastoro.pintadi@umy.ac.id**Abstract**

Denture hygiene, which is not taken care of, can lead to denture stomatitis caused by plaque accumulation and bacterial or fungal colonization. Fungal colonization is often found in *Candida albicans*. Denture cleanser that is characterized as nontoxic, antifungal, and antibacterial and can work in a short period is required. Basil leaf contains atsiri oil, flavonoid, saponin, and tannin, which works as antifungal and antibacterial. This study aims to identify the influence of the soaking period of acrylic resin in basil leaf extract on the growth of *Candida albicans*. 15 plates of acrylic resin were divided into 3 groups. Group 1 was soaked into 80% basil leaf extract concentration for 4 hours, group 2 was soaked for 6 hours, and group 3 was soaked for 8 hours. The result of the one-way ANOVA test showed that there was a significant difference $p < 0,05$. The group with 8 hours of soaking period was the most effective in inhibiting the growth of *Candida albicans*.

Keywords: *acrylic resin, candida albicans, basil leaf, Ocimum basilicum***INTRODUCTION**

Tooth loss remains a big problem, especially in developing countries. It can cause disturbances in temporomandibular joint function, mastication disorders, and impact speech function. Various problems that arise as a result of tooth loss can be overcome by using dentures¹.

Heat-cured polymethyl methacrylate acrylic resin is a frequently used denture base material. This material is used as a base for dentures due to its non-toxic and non-irritating tendency, does not dissolve in oral fluids, has good aesthetics, is easy to manipulate, is easy to repair, and has small dimensional changes².

Acrylic resin dentures, like natural teeth, can become a place for plaque accumulation and the formation of stains and calculus. Less maintained denture cleanliness can lead to denture stomatitis. Denture stomatitis is a common event in denture users, where there are reddish lesions under the

denture³.

Approximately 70% of individuals with clinical signs of denture stomatitis show fungal growth caused by fungal colonization of the oral mucosa combined with bacterial colonization. The fungal colony that most often causes denture stomatitis is *Candida albicans*⁴.

Denture cleaning must be done to reduce fungal colonies and prevent denture stomatitis. It can be done by brushing the denture every day and soaking it in a denture cleanser. The ideal denture cleanser must be non-toxic, reduce biofilm activity, antifungal, antibacterial, work in a short time of ≤ 8 hours, and be compatible with the base and denture materials used, and the price is affordable for the public³.

Currently, many denture cleansers are circulating in Indonesia. However, the price of these denture cleansers is very high for lower-class and remote communities due to import materials or high distribution costs to reach the region. Therefore, the

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Indonesian government is promoting the use of traditional medicines derived from plants as alternative ingredients, one of which is the basil leaf plant (*Ocimum basilicum*), which has antifungal power⁵.

Basil leaves contain essential oils, flavonoids, saponins, and tannins. The essential oils contained in basil are eugenol, cineol, methyl chavicol, protein, calcium, phosphorus, iron, sulfur, vitamin A, and vitamin C. Basil seeds contain chemicals, namely saponins, flavonoids, and polyphenols⁶. In the medical field, basil leaves contain properties useful for treating various diseases such as flatulence and fever, improving breast milk, canker sores, and eliminating bad breath or halitosis. Various studies have proven that the essential oil content in basil can inhibit the growth of fungi. Basil leaf extract can inhibit the growth of *Candida Albicans* fungus on acrylic resin as a denture base⁵.

Based on what is described above, this study aims to identify the effect of a long period of soaking process for acrylic resin in basil leaf extract (*Ocimum basilicum*) on the growth of *Candida albicans*.

MATERIAL AND METHODE

This research design is laboratory experimental with an in vitro dilution method, carrying out actions on research subjects and then analyzing the effects arising from the actions. A total of 15 acrylic resin plates, each 10mm in diameter and 2mm in thickness were divided into 3 groups. Previously, all sterilized acrylic resin plates were soaked in artificial saliva to help attach *Candida albicans*, then soaked in *Candida albicans* suspension and incubated for 24 hours. After that, each group was soaked in basil leaf extract with a concentration of 80% for 4 hours, 6 hours, and 8 hours.

Furthermore, each acrylic resin was transferred into 10ml distilled water to be shaken with a vortex mixer for 1 minute and then spread onto agar media. The agar media was incubated for 48 hours at 37°C, and the research results were obtained.

RESULT

This research aims to identify the effect of the period soaking process for basil leaf extract (*Ocimum basilicum*) on the growth of *Candida albicans* on acrylic resin. Results demonstrated the number of *Candida albicans* colonies on acrylic resin discs after soaking basil leaf extract (*Ocimum basilicum*) at 80% concentration for 4 hours, 6 hours, and 8 hours.

Table 1. Average treatment results for each group

Treatment	Mean	Standard Deviation
4 jam	6.8000	1.30384
6 jam	4.2000	0.83666
8 jam	3.2000	0.83666

Based on Table 1, it can be seen that in the treatment using basil leaf extract (*Ocimum basilicum*) with a concentration of 80% for 4 hours, 6 hours, and 8 hours, there was a decrease in the average number of *Candida albicans* colonies.

Table 2. Statistical results of the Shapiro-Wilk normality test

Treatment	Shapiro-wilk Statistics	df	Sig.
4 hours	0.902	5	0.421
6 hours	0.881	5	0.314
8 hours	0.881	5	0.314

Based on the results of the normality test, the 4-hour treatment group showed a significance value of 0.421 ($p > 0.05$), the 6-hour treatment group showed a significance value of

0.314 ($p > 0.05$), and the 8-hour treatment group showed a significance value of 0.314 ($p > 0.05$). It can be concluded that the data is normally distributed with a p -value > 0.05 . The results were continued with the homogeneity test to identify whether the samples were homogeneous.

Table 3. Homogeneity Test

Levene Statistic	df1	df2	Sig.
1.105	2	12	0.363

The results of the homogeneity test revealed that the number of *Candida albicans* colonies on acrylic resin treated at 4 hours, 6 hours, and 8 hours had a probability of 0.363 ($p > 0.05$), indicating the data is homogeneous.

After identifying the normality and homogeneity test results, the ANOVA test was conducted to determine the effect of the length of soaking acrylic resin in 80% basil leaf extract for 4 hours, 6 hours, and 8 hours, as shown in Table 4.

Table 4. One-way-ANOVA calculation result

Variance Source	Sum of Square	Degree of freedom	Square F Mean	Error Chances
Among groups	34.533	2	17.267	16.710
Inner groups	12.400	12	1.033	
Total	46.933	14		0.000

The results of the ANOVA test calculation revealed $p < 0.05$, indicating there was a difference in the amount of *Candida albicans* in acrylic resin soaked in basil leaf extract at a concentration of 80% for 4 hours, 6 hours, and 8 hours. After that, an LSD test was carried out to determine differences in *Candida albicans* growth between groups.

Table 5. Result of LSD Test

Treatment	Mean Difference	Std. Error	Sig.
4 hours and 6 hours	2.60000*	0.64291	0.002
6 hours and 8 hours	1.00000	0.64291	0.146
4 hours and 8 hours	3.60000*	0.64291	0.000

Based on the table above, it can be seen that the 4-hour immersion group and the 6-hour group have a significant difference of $p = 0.002$ ($p < 0.05$). The 6-hour and 8-hour groups did not have a significant difference, $p = 0.146$ ($p > 0.05$), whereas the 4-hour and 8-hour soaking groups had a significant difference of $p = 0.000$ ($p < 0.05$).

DISCUSSION

This study aims to determine whether there is an effect of a long period of soaking process in basil leaf extract (*Ocimum basilicum*) in inhibiting the growth of *Candida albicans* on acrylic resin.

The results of this research revealed an influence of basil leaf extract (*Ocimum basilicum*) on the growth of *Candida albicans*. It was proven by the results of the ANOVA test showing the value 0.000 ($p < 0.005$), indicating that H_0 was rejected or there was a significant difference between the 4-hour, 6-hour, and 8-hour soaking process in the growth of *Candida albicans* on acrylic resin. After that, the LSD test was used to compare which variables were the most significant.

Treatment of immersing acrylic resin discs in basil leaf extract resulted in a decrease in the growth of the number of *Candida albicans* colonies starting from 4 hours of immersion with a mean of 6,800. Meanwhile, in the 6-hour group, the

mean was 4,200, and for the 8-hour group, the mean was 3,200. It revealed the influence of the soaking time for basil leaf extract on the growth of *Candida albicans* colonies, with 8 hours of treatment as the most effective soaking time for the extract. The results align with David Felton's (2011) statement that the ideal denture cleanser is able to work in a short time, namely less than or equal to 8 hours.

According to Pitojo (1996), basil leaves contain essential oils, flavonoids, saponins, and tannins. The essential oils contained in basil are eugenol, cineol, methyl chavicol, protein, calcium, phosphorus, iron, sulfur, vitamin A, and vitamin C.

Essential oils are one of the residual products of metabolic processes in plants, and they are synthesized in plant tissue glands and resin vessels. Essential oils are non-polar compounds. According to several previous studies, non-polar compounds can induce changes in *Candida albicans* membrane permeability through interactions between the active side of the compound and the active side of the cell membrane, especially the cholesterol and ergosterol parts. This interaction results in changes in membrane kinetic energy, leading to changes in permeability and causing the cell membrane to become unstable and hyphal cell death⁷.

The mechanism of essential oils in killing hyphal cells is the same as the mechanism of antifungal drugs, namely by reducing ergosterol synthesis that can affect microtubule function or nucleic acid synthesis and polymerization. Furthermore, there is inhibition of hyphal cell wall synthesis and inhibition of mitosis⁸.

Basil leaf extract also contains flavonoids, which can function as an anti-

fungal agent. The mechanism of action of flavonoids in inhibiting fungal growth is by disrupting the permeability of fungal cell membranes. The hydroxyl group contained in flavonoid compounds causes changes in organic components and nutrient transport, which ultimately results in toxic effects on fungi⁹.

Acrylic resin, which is frequently used as a denture base, will cause self-cleansing, leading to the reduction of the tongue and saliva cleansing and causing a buildup of *Candida albicans* colonies and denture stomatitis.

According to Combe (1992), one of the properties of acrylic resin discs is that they easily absorb water. Therefore, when soaked using basil leaf extract, they will absorb the flavonoid compounds and essential oils contained therein, which will come into direct contact with *Candida albicans* and will reduce the number of attached *Candida albicans* colonies on acrylic resin discs.

A high concentration of basil leaf extract also affects the growth of *Candida albicans* due to the greater levels of the active ingredient. In addition, increasing contact time will increase the antifungal chemical reaction, leading to decreased *Candida albicans*¹⁰.

CONCLUSION

Based on the results, it can be concluded that the soaking period in basil leaf extract affected the growth of *Candida albicans* on acrylic resin, with a soaking time of 8 hours being the most effective time. Further research is needed on the effect of basil leaf extract on the properties of acrylic resin.

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