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# The Effectivity of Parsley (*Petroselinum crispum*) Extract on The Growth Inhibition of *Candida Albicans*

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**Abstract**

**Background**: Denture stomatitis is a type of *Candida*-associated infection that mainly affects the palatal mucosa. *Candida albicans* is one of the normal floras that is considered to be the primary etiologic agent in the pathogenesis of denture stomatitis. Denture decontamination is necessary to prevent denture stomatitis. One method of decontamination is by immersing removable dentures in an antifungal solution. Parsley (*Petroselinum crispum*) is a medicinal plant showing antifungal activity. **Objectives**: To determine the effectivity of immersion of acrylic resin Plate in parsley extract on inhibition of growth of *Candida albicans*.

**Methods**: The effectivity of immersion of heat-cured acrylic resin plates in parsley extract on inhibition of *C. albicans* growth was tested using an experimental laboratory study with a post-test-only control group design. Thirty samples were divided into 5 groups as 0.01%, 0.02%, and 0.05% parsley ethanol extract, 0.1% sodium hypochlorite, and sterile aquadest. The number of colony forming units per mL was obtained after calculating the colonies on SDA media, allowing the minimum inhibitory concentration (MIC) obtained according to the formula.

**Results**: 0.01% parsley ethanol extract is MIC with a mean of 7.4 CFU / ml, which inhibited the growth of *C. albicans* by 31.05%. The Kruskal Wallis test ($p<0.001$) results indicate that there is an effectivity of immersion of acrylic resin plate in parsley extract on inhibition of growth of *C. albicans*.

**Conclusions**: The immersion of acrylic resin plates in parsley ethanol extract with a concentration of 0.01%, 0.02%, and 0.04% has effectivity on the inhibition of growth of *C. albicans.*

**Key words:** *Parsley Extract; Candida Albicans; Removable Denture Cleaning Solution; MIC.*
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## INTRODUCTION

Edentulous is the condition of having no teeth that cause trouble with chewing and speech.[1] Data of Riskesdas 2018 showed that in Central Java, 17.35% of edentulous cases happened because of tooth extraction or tooth loss. In Central Java, edentulous occur for 21.93 percent of the population aged 35 to over 65.[2] The use of removable dentures is indicated for patients with no teeth.[3] The function of removable dentures is to replace the lost natural teeth, which the patients can wear and remove themselves.[4]

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The material commonly used as a removable tooth base is heat-cured polymethyl methacrylate (PMMA) resin.[5-6] The use of PMMA as a removable tooth base is advantageous because it is easy to manufacture, relatively inexpensive, stable in the oral environment, has color stability, physical properties, and pleasing aesthetics.[6-7] This type of resin will be formed if the polymerization process is carried out by heating so that a mixture of methyl methacrylate monomer and methacrylate polymer occurs. This process always leaves as much as 0.2% to 0.5% methyl methacrylate because the monomer will not completely react after polymerization is complete.[5-6] Residual monomers can

cause irritation, inflammation, allergic responses to the oral mucosa, angular cheilitis, and denture stomatitis.[5] Denture stomatitis is a type of *Candida*-associated infection that mainly affects the palatal mucosa. This disease is characterized by mucosal inflammation, burning sensation, bleeding, and pain, especially under the upper denture, and changes in the sense of taste.[8–10] The risk factor increased in patients wearing partial dentures to 36.7% and increased to 65% in patients wearing complete dentures.[9] *Candida albicans* is one of the normal flora that is considered to be the primary etiologic agent in the pathogenesis of denture stomatitis.[8] Candidiasis can occur if the hygiene of removable dentures is poor. Therefore, decontamination of removable dentures is essential for the prevention of denture stomatitis.[11–13] Decontamination of dentures can be done by mechanical methods, chemical methods, and a combination of the two methods. The release of chemical solutions can be an option as a chemical method.[3] Sodium hypochlorite (0.10% and 0.22%) is one of the most used chemical solutions as denture cleaners because it is effective in reducing *C. albicans* colonization, is non-abrasive, and can be used directly. However, this solution can cause discoloration on the plate and is corrosive.[13] Another chemical solution that is often used is alkaline peroxide. Soaking the denture in alkaline peroxide triggers a diffusion process that will change the acrylic resin's physical properties, including its hardness.[14] Natural antifungal solutions can be an alternative to removable denture cleaning solutions because of the side effect of chemical-based antifungal solutions as mentioned above.[15]

Parsley (*Petroselinum crispum*) is a medicinal and aromatic plant from the Mediterranean that has health benefits.[16–18] This plant contains vitamins, flavonoids, essential components, minerals, phenolic compounds, and other bioactive compounds. Parsley leaves can reduce the risk of gastrointestinal disorders, diabetes, and cardiovascular disease due to their antioxidant, cytoprotective, and antidiabetic activities.[18,19] Other studies have shown that parsley exhibits antibacterial, antifungal, anti-inflammatory, and anticancer activities.[19]

One of the compounds that show antifungal activity and is contained in parsley extract is a flavonoid.[20,21] Flavonoid can break down protein bonds into their primary structure on the cell membrane of *C. albicans* and cause lysis of the fungal cell membrane, allowing the flavonoid can penetrate the nucleus cell. The presence of flavonoid in the cell nucleus can stop the growth of *C. albicans*.[22] Some researchers are interested to develop novel medication because it contains a lot of flavonoids.[21]

From the explanation above, it is necessary to investigate further regarding the effectivity of immersing acrylic resin plates in parsley (*P. crispum*) extract on the growth potential of *C. albicans* as a basis for the development of a removable denture cleaning solution with natural base ingredients.

## MATERIALS AND METHODS

This study was conducted at Universitas Diponegoro Integrated Laboratory and Microbiology Laboratory from March to April 2021 using an experimental laboratory design with a post-test only control group. The material used in this study was parsley (*Petroselinum crispum*) ethanol extract with a concentration of 0.01%, 0.02%, and 0.04%, 0.01% sodium hypochlorite sodium as positive control and sterile aquadest as negative control groups. *C. albicans* were obtained from a culture at the Microbiology Laboratory of Universitas Diponegoro.

*C. albicans* suspension was prepared by culturing the colonies in 10 ml of BHI media and incubated for 8 hours at $37^0$C. Then the suspension of *C. albicans* was diluted by adding sterile distillated water to certain turbidity to 0.5 McFarland standard.

Parsley (*Petroselinum crispum*) taken from Nandisari Plantation was extracted with 96% ethanol solvent (EMSURE®; Merck Millipore, Germany) using the maceration method and diluted with sterile distilled water to obtain concentrations of 0.01%, 0.02%, and 0.04%. Sterile acrylic resin plates were immersed in artificial saliva AFNOR NF S91-141 for 1 hour and rinsed using Phosphate Buffered Saline (Nitra Kimia, Indonesia) 2 times. The acrylic plates were immersed in *C. albicans* suspension with a turbidity of 0.5 McFarland and incubated for 24 hours at 37°C. The McFarland standard was used as comparison of the *C. albicans* suspension by means of a visual turbidity comparison. A total of 30 acrylic resin plates were removed and divided into five treatment groups, namely immersion in 0.01%, 0.02%, and 0.04% parsley ethanol extract and the control group, 0.1% sodium hypochlorite, and aquadest solution, respectively 10 ml. The plates were immersed for 8 hours, allowing the serial dilution was implemented in each test tube to $10^{-3}$. The serial dilution of 0.01 ml was taken and tested on SDA media (Merck Millipore, Germany) and then incubated for 48 hours at 37°C. The number of colony forming units (CFU) per mL was obtained after calculating the colonies on SDA media, allowing the minimum inhibitory concentration (MIC) obtained according to the formula.

$$\text{Fungal Number} = \frac{\text{Colonies number x Dilution factor}}{\text{Calculated solution number}}$$

$$\text{MIC} = 100\% - \frac{\text{Fungal number in control solution x 100\%}}{\text{Fungal number at a certain concentration}}$$

The data obtained were then analyzed by the Saphiro-Wilk test, Kruskal Wallis test followed by the Mann-Whitney test.

**Figure 1.** Parsley (*Petroselinum crispum*) taken from Nandisari Plantation

## RESULTS

The plant harvested from Nandisari Plantation identified as *Petroselinum crispum (Mill.) Fuss* by the expert. The research continued with the phytochemical screening of parsley ethanol extract. As shown in table 1, the results of the Lieberman test, tannins, and flavonoids respectively showed red, green, and yellow colors after mixing the reagents for each test.

**Table 1.** Phytochemical screening results of parsley ethanol extract

| Test | Result |
|---|---|
| *Lieberman* | + |
| Tannins | + |
| Flavonoids | + |

The average and standard deviation of 6 groups of *C. albicans* numbers were calculated after soaking acrylic resin plates in 0.01 percent, 0.02 percent, and 0.04 percent parsley extract, sterile aquadest, and 0.1 percent sodium hypochlorite, as shown in Table 2.

**Table 2.** The average and standard deviation of fungal number

| Group | Fungal number average | Standard deviation |
|---|---|---|
| 0.01% Parsley Extract | 7.40 | 0.31 |
| 0.02% Parsley Extract | 3.75 | 0.24 |
| 0.04% Parsley Extract | 1.61 | 0.07 |
| Sterile Aquadest | 10.73 | 0.59 |
| 0.01% Sodium Hypochlorite | 0 | 0.00 |

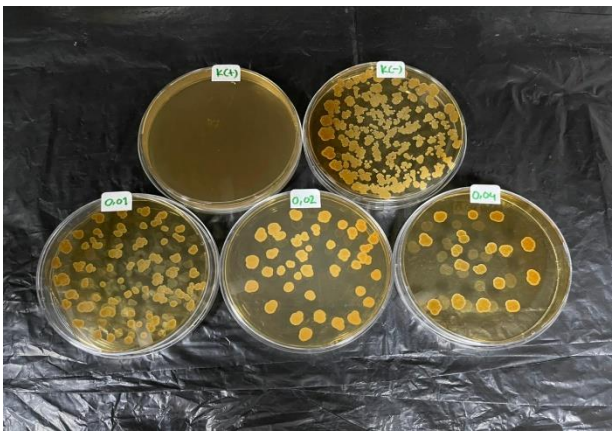

**Figure 2.** *C. albicans* colony count after 48 hours of incubation

In parsley extract groups, it can be seen that 0.04% parsley extract has the least number, namely 1.61 CFU/ml, while 0.01% parsley extract has the highest average number of fungi, namely 7.40 CFU/ml

The average number of fungi for each parsley extract concentration group (*Petroselinum crispum*) obtained was then used in the MIC calculation, which was carried out using a comparison with the sterile aquadest group. The results of the MIC calculation are listed in table 3 using the formula.

**Table 3.** MIC calculation results

| No. | Treatment group | MIC |
|---|---|---|
| 1. | 0.01% Parsley Extract | 31.05% |
| 2. | 0.02% Parsley Extract | 65.06% |
| 3. | 0.04% Parsley Extract | 84.93% |

The results of the Shapiro-Wilk test on fungal numbers data from parsley extract groups of 0.01%, 0.02%, and 0.04% and sterile aquadest showed that the data were normally distributed ($p>0.05$). In comparison, the fungal number of 0.1% sodium hypochlorite group showed that the data were not normally distributed ($p<0.05$). The study continued with the Kruskal Wallis test due to one of the treatment groups showed that the data were not normally distributed.

The Kruskal Wallis test analysis results in table 4 show that the five treatment groups in this study got p-values < 0.001 ($p < 0.05$). Thus, it can be said that there is a significant difference in the fungal number.

**Table 4.** Kruskall Wallis test result

| | Treatment group | Mean Rank | *p*-value |
|---|---|---|---|
| *C. albicans* number | 0.01% Parsley Extract | 21.50 | <0.001 |
| | 0.02% Parsley Extract | 15.50 | |
| | 0.04% Parsley Extract | 9.50 | |
| | Sterile Aquadest | 27.50 | |
| | 0.1% Sodium Hypochlorite | 3.50 | |

The analysis continued using the Mann-Whitney test, which give an insight that all groups had differences in each group with a significance as shown in table 5.

## DISCUSSION

This study obtained the average number of *C. albicans* fungi in the immersion of heat-cured acrylic resin plates in parsley extract concentrations of 0.01%, 0.02%, and 0.04%, 0.1% sodium hypochlorite, and sterile distilled water during 8 hours. The MIC was calculated using a comparison with the sterile aquadest group.[24] Parsley extract at concentrations of 0.01%, 0.02% and 0.04% respectively showed MIC of 31.05%, 65.06% and 84.93%.

**Table 5.** Mann Whitney test result

| | 0.01% Parsley Extract | 0.02% Parsley Extract | 0.04% Parsley Extract | Sterile Aquadest | 0.1% Sodium Hypochlorite |
|---|---|---|---|---|---|
| **0.01% Parsley Extract** | | 0.004 | 0.004 | 0.004 | 0.002 |
| **0.02% Parsley Extract** | 0.004 | | 0.002 | 0.004 | 0.002 |
| **0.04% Parsley Extract** | 0.004 | 0.002 | | 0.004 | 0.002 |
| **Sterile Aquadest** | 0.004 | 0.004 | 0.004 | | 0.002 |
| **0.1% Sodium Hypochlorite** | 0.002 | 0.002 | 0.002 | 0.002 | |

According to the data analysis the data showed a significant difference between positive control values, negative control, 0.01%, 0.02%, and 0.04% parsley extract (*P. crispum*). These results are in accordance with the research hypothesis that there is an effectivity of immersing acrylic resin plates in parsley extract on the growth inhibition of *C. albicans*.

In the 0.1% sodium hypochlorite group, no *C. albicans* colonies were found on SDA media because this solution effectively killed *C. albicans*. At the same time, sterile aquadest could not inhibit the growth of *C. albicans* to obtain the highest average number of fungi in the group among all groups.[13] The 0.01% parsley extract group showed the highest average fungal number of 7.4 CFU/ml, while the 0.04% parsley extract group showed the lowest average fungal number, namely 1.61 CFU/ml. The less the number of molds formed, the higher the antifungal activity of a substance.[25] Immersion of acrylic resin plates in the 0.01%, 0.02%, and 0.04% ethanol extract of parsley (*P. crispum*) showed that the average number of fungi *C. albicans* were lower in numbers compared to the sterile aquadest group. This was caused by antifungal activity in parsley (*Petroselinum crispum*) extract.[20,21,26] In the parsley (*P. crispum*) ethanol extract group, the number of fungi decreased with each increase in the concentration of parsley (*P. crispum*) ethanol extract. The greater the concentration of parsley extract, the greater the ability to inhibit fungal growth.[26] This can occur following the basic chemical theory, which states that the greater the concentration of an antimicrobial substance, the faster microbial cells will be stunted and killed.[27]

From the MIC calculation, it can be seen that the three concentrations of parsley ethanol extract (0.01%, 0.02%, and 0.04%) inhibit *C. albicans* growth on acrylic resin plates and are fungistatic. According to Washington's opinion, if the MIC is less than 99.9%, then the solution is fungistatic.[24] Although the three concentrations of parsley extract (*P. crispum*) can inhibit *C. albicans*, not all concentrations are effective as inhibitors of *C. albicans* on an acrylic plate. The ability of an antifungal agent can be said to be effective if it has a MIC of 80% or more when compared to the control.[24] It can be concluded that 0.04% parsley ethanol extract effectively inhibits the growth of *C. albicans* on acrylic resin plates because it has a MIC of more than 80%, which is equal to 84.93%. However, in the concentration group of 0.01% and 0.02%, it could not effectively inhibit the growth of *C. albicans* on acrylic resin plates because the MICs were only 31.05% 65.05%, respectively.

The antifungal activity of parsley (*P. crispum*) extract is due to biochemical compounds in this plant.[28] Liberal et al. (2020) stated that parsley contains bioactive compounds such as flavonoids which have been shown to have the ability to inhibit fungal growth.[19,29] According to Alcamo, flavonoids are a class of phenolic compounds with fungistatic properties which support the previous statement.[24] Phytochemical screening carried out in this study also showed the presence of flavonoids in the ethanol extract of parsley (*P. crispum*) due to a yellow color change when the extract was mixed with magnesium powder and amyl alcohol.[30]

According to Aboody and Mickymaray (2020), flavonoid compounds can inhibit fungal growth by various mechanisms, namely by damaging the fungal plasma membrane, damaging mitochondria, and inhibiting cell wall formation, cell division, RNA, and protein synthesis in fungi.[31] Flavonoid can also break down protein bonds into their primary structure on the cell membrane of *C. albicans* and cause lysis of the fungal cell membrane, flavonoids can then enter the cell nucleus. The presence of flavonoid in the cell nucleus can stop the growth of *C. albicans*.[22] Other studies have shown that flavonoid compounds in parsley such as apigenin can cause *C. albicans* to experience cell shrinkage.[32,33]

There are other compounds that can inhibit the growth of fungi in parsley (*P. crispum*) extract, namely triterpenoid compounds.[34] Phytochemical screening carried out in this study showed the presence of triterpenoids in the ethanol extract of parsley (*P. crispum*) due to a red color change when mixing the extract with acetic anhydride and concentrated $H_2SO_4$.[30] This compound is a bioactive compound that has an antifungal function. Triterpenoids have a mechanism of decreasing the permeability of cell membranes of microorganisms and can be associated with protein and lipid molecules so that they can have an impact on the physiological functions of cell membrane proteins and enzyme proteins from fungi.[35]

The number of *C. albicans* on SDA media after immersing heat-cured acrylic resin plates in parsley ethanol extract at a concentration of 0.04% showed the lowest number of fungi compared to concentrations of

0.01% and 0.02%, on the other hand the growth inhibition of *C. albicans* in 0.1% sodium hypochlorite showed 1.17 times more effective results than the 0.04% parsley (*P. crispum*) ethanol extract. This is a disadvantage of parsley ethanol extract (*P. crispum*) as a growth inhibitor of *C. albicans* on acrylic resin plates. Hence, further research is needed to increase the effectiveness of parsley (*P. crispum*) ethanol extract in inhibiting the growth of *C. albicans* on acrylic resin plates which can be done by increasing the concentration of the ethanolic extract of parsley (*P. crispum*).

## CONCLUSION

Research on the effectivity of immersing acrylic resin plates in parsley (Pe*troselinum crispum*) extract on the growth inhibition of *C. albicans* concluded that there was an effectivity of immersing acrylic resin plates in parsley (*P. crispum*) extract on the growth inhibition of *C. albicans*. In addition, in this study, 0.04% parsley ethanol extract was the best concentration in inhibiting the growth of *C. albicans* on acrylic resin plates.

This research is an initial study, and more research is needed to establish the concentration of parsley ethanol extract that is most effective in inhibiting the growth of *C. albicans* on acrylic resin plates at concentrations larger than 0.04 percent. Furthermore, it is also necessary to do qualitative phytochemical screening of parsley extract and redetermination of *C. albicans* on the culture results.

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