

## The effect of effervescent *acanthus ilicifolius* L. Extract on antifungal properties and flexural strength acrylic

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*Candida albicans*;  
effervescent *Acanthus ilicifolius* L extract; flexural strength

### ABSTRACT

**Background:** *Acanthus ilicifolius* L. has antifungal potential and makes it possible to be a candidate for denture cleanser. This study aims to evaluate the effect of effervescent *Acanthus ilicifolius* L. extract on antifungal properties and flexural strength acrylic after immersion for three duration times.

**Method:** Extracts were made by maceration method with ethanol. For the antifungal test, effervescent *Acanthus ilicifolius* L extract was prepared by combining the *Acanthus ilicifolius* L extract 4% and sodium perborate with two ratios, 1:7 and 1:14. The microdilution method was carried out. Effervescent *Acanthus ilicifolius* L extract 4% with ratio 1:7 was prepared for the flexural strength test. The fifty-four heat-cured acrylic resin (20x10x2.5mm) were fabricated and divided into 3 groups. Group 1 was immersed in aquadest (control); Group 2 was immersed in sodium perborate; Group 3 was immersed in effervescent *Acanthus ilicifolius* L. extract. Each group was immersed for 15 min, 8, and 56 hours (n=6). The flexural strength test was performed using Universal Testing Machine.

**Result:** Effervescent *Acanthus ilicifolius* L. extract exhibited antifungal activity which increased in an effervescent *Acanthus ilicifolius* L extract-dependent manner significantly (0.57 to 0.50 OD). There were no significant differences between sodium perborate, effervescent\_A11 (1:7) and effervescent\_A12 (1:14). The flexural strength was found to be within the range of 66.1 - 68.3 MPa. There were no significant differences between groups (p < 0.05).

**Conclusion:** Effervescent *Acanthus ilicifolius* L extract with the ratios 1:7 had a good antifungal effect and appropriate flexural strength after immersion for 15 min, 8, and 56 hours.

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## INTRODUCTION

Denture care is one of the primary patient treatments that suffering from tooth loss. Most denture users are classified to ( $\geq 65$ )-year-old ages above. Physiologically, the elderly patient's motor skills usually decrease, so that patient's ability to clean their dentures could be a troublesome activity for them. In addition, their denture could not also be optimally hygienic. It easily gets dirtier either, as the upper denture becomes rougher than caused by plaque. *Candida albicans* are also prone to sticking. Therefore, it is not too good aesthetically. Furthermore, it could also create severe *halitosis*<sup>1</sup>. Denture base cleaning can be done mechanically with a toothbrush or cleaned chemically by immersion to reduce *Candida albicans* colonization and prevent denture stomatitis<sup>2,3</sup>.

The effervescent tablet is the most appropriate option as a denture cleanser. The effervescent tablet is very appropriate for elder patients as their denture cleanser without using any motor skills, such as; brushing teeth. However, sodium perborate is also contained inside the Effervescent tablet which could give additional benefits. Those are the reaction ability to produce alkaline peroxide by water and the oxygen bubble production in order to cleanse *biofilm* and spots at dentures<sup>4</sup>. The effervescent tablet is also able to reach narrow areas that could not be gotten easily by toothbrushing, quickly dissolved in water, cleanse stains and calculus, not scratches the dentures, and anti-bacterial, anti-fungus<sup>5</sup>.

*Candida albicans* is a denture stomatitis fungus caused by 11%-67% prevalence. It is primarily found in complete dentures<sup>6</sup>. The use of herbal as alternative medicine has been developed for some decades because it has uses such as antibacterial, antifungal, and others<sup>7,8</sup>. Phytochemistry screening shows that *Acanthus*

*ilicifolius* has chemical compounds such as; flavonoid, saponin, alkaloid, terpenoid, and tannin compare to other mangrove types. The *Acanthus ilicifolius* contains chemical compound that could effectively decrease the total amount of *Candida albicans* by damaging cell membranes and blocking protein formation at the fungal cell wall<sup>9</sup>.

Denture cleanser is a material that must meet non-toxic prerequisites, non-irritated material leftover, easily cleaned, stable storage, effective to dissolve organic and inorganic material at denture, and own fungicidal and bactericidal characteristics, and no physical and mechanical change to basic denture<sup>10</sup>. Dental acrylic resin *heat cured* is one of the primary materials that are frequently used as denture base material, despite its poor prone ability to absorb water and porosity causing an effect on denture physical and base material<sup>11</sup>. Some studies reported that the immersion time of the acrylic denture, the contact period between the acrylic denture and the denture cleanser may play an important role in the change in the physical and mechanical denture<sup>12,13</sup>. Porosity in dentures can be an ideal place for food and microorganisms in the mouth cavity including *candida albicans*<sup>14</sup>. It has been reported that a denture base material must have a good-mechanical characteristic such as adequate flexural strength.

Based on these, further research about 4% effervescent extract *Acanthus ilicifolius* as a denture cleanser candidate for antifungal activity testing and *heat cured* acrylic flexural strength is needed.

## Materials and methods *Effervescent Acanthus ilicifolius* extract preparation

The *Acanthus ilicifolius L* extract was made using the maceration technique with 96% ethanol. Liquid extract was evaporated by a *rotary*

evaporator to gain viscosity mass. Furthermore, it is evaporated in a water bath to separate the extract from its solvent. Then, it could gain pure *Acanthus ilicifolius* extract. Finally, the *Acanthus ilicifolius* L extract was 4 % dissolved by effervescent (citric acid, monohydrate tartaric acid, and sodium bicarbonate) in order to form an effervescent powder of *Acanthus ilicifolius* extract.

### Antifungal activity

The antifungal test obeyed the ethical requirements of ethical committee at the Faculty of Dentistry, Universitas Hang Tuah, No. EC/008/KEPK-FKGUHT/VII/2022. For the antifungal test, effervescent *Acanthus ilicifolius* L extract was prepared by combining the *Acanthus ilicifolius* L extract 4% and sodium perborate with two different ratios, 1:7 (Effervescent\_AI1) and 1:14 (Effervescent\_AI2). The microdilution method was carried out. Antifungal activity test was performed by biofilm inhibition test using the microtiter plate method (MTP) (Dhanasekaran, et al, 2014). These results of biofilm inhibition test in the form of optical density values (OD) were read using an ELISA reader (wavelength 509 nm). The biofilm formation inhibition test was calculated using the following formula: (Pratiwi et al, 2015)

$$\% \text{ Inhibition} = \left( 1 - \left( \frac{xODs - xODbs}{xODp} \right) \right) 100\%$$

Information:

Ods: Optical Density (509nm) samples tested

Odb: Optical Density sample blank

Odp: (OD test solvent – OD solvent blank)

### Flexural strength test

Effervescent *Acanthus ilicifolius* L extract 4% with ratio 1:7 was prepared for the flexural strength test. The fifty-four heat-cured acrylic resin

(20x10x2,5 mm) were fabricated and divided into 3 groups. Group 1 was immersed in aquadest (control); Group 2 was immersed in sodium perborate; Group 3 was immersed in effervescent *Acanthous ilicifolius* L. extract (Effervescent\_AI1). Each group was immersed for 15 min, 8 and 56 h. The flexural strength test was performed using *Universal Testing Machine* (AG-SX, Shimadzu Corp., Tokyo, Japan) with crosshead speed 0,5 mm/min, at room temperature. The flexural strength was defined using this equation below:

$$\sigma = \frac{3FL}{2bd^2}$$

Information:

$\sigma$  = Flexural strength (N/mm<sup>2</sup>)

F = Force (N)

L = Length (mm)

b = Width of the specimen (mm)

d = Thickness (mm)

### Statistical analysis

Data from the antifungal activity test and flexural strength test was analyzed using one-way ANOVA and Post Hoc Tukey HSD multiple comparison tests with SPSS software (Version 26.0; SPSS Japan Inc., Tokyo, Japan). The significant difference was accepted at  $p < 0.05$ .

## Results

### Antifungal activity

The evaluation of antifungal activity confirmed that the effervescent *Acanthus ilicifolius* extract 4% showed statistically significant antifungal efficacy to reduce the growth of candida albicans. There was no significant difference between 1:7 (wt%) and 1:14 (wt%), but this growth inhibitory

effect of effervescent *Acanthus ilicifolius* extract 4% may be increased in a dose-dependent manner

This study showed the lowest OD scores for the aquadest group, followed by effervescent\_AI1, effervescent\_AI2, and the highest to the sodium group (Figure 1). The test result Shapiro-Wilk normality illustrated normal through distributed data ( $p > 0.05$ ) and Levene homogeneity test to represent its data ( $p > 0.05$ ). Then, the ANOVA one-way differential test was done by post hoc LSD. There was significant difference between aquadest and all of the groups. But, there was no significant difference between groups effervescent\_AI1 and sodium perborate ( $p = 0.007$ ), effervescent\_AI1 and effervescent\_AI2 ( $p = 0.071$ )

#### Flexural strength

The mechanical properties mean value of groups 1, 2, and 3 were assessed with flexural strength shown in Figure 2. Group 1 was immersed in aquadest (control); Group 2 was immersed in sodium perborate; Group 3 was immersed in effervescent *Acanthous ilicifolius L.* extract (Effervescent\_AI1). Each group were immersed for 15 min, 8, and 56 hours. Figure 2 shows the highest three-point bending strength found in Group 1, in which the heat cure acrylic plates were immersed in aquadest for 15 minutes. The lowest three-point bending strength found in Group 2 that the heat cure acrylic plates were immersed in sodium perborate for 56 h. Figure 3 showed that based on the *Post Hoc Tukey* HSD test shows there were no significant differences in flexural strength between groups ( $p > 0.05$ ) after immersion for 15 min, 8 and 56 hours.

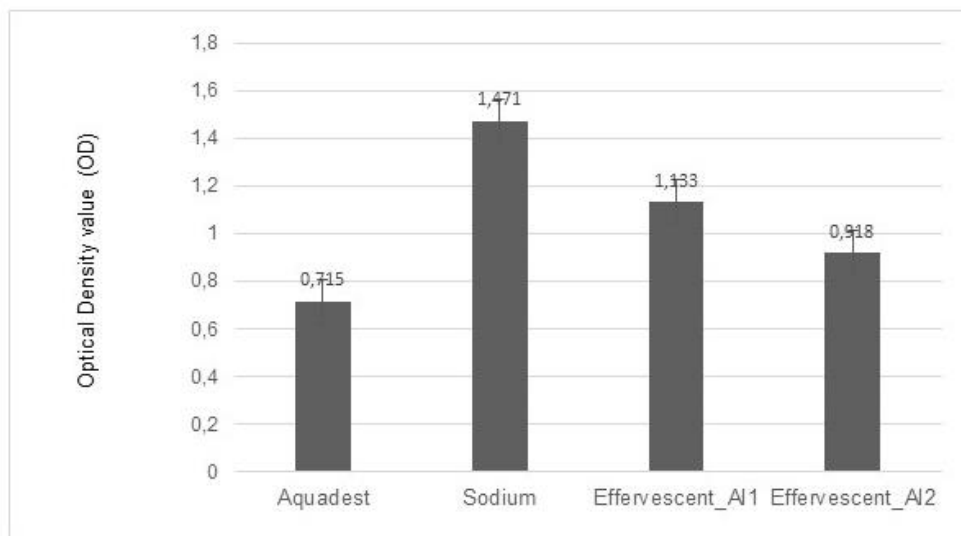


Figure 1. The mean of optical density values (OD) antifungal effervescent *Acanthus ilicifolius* extract.

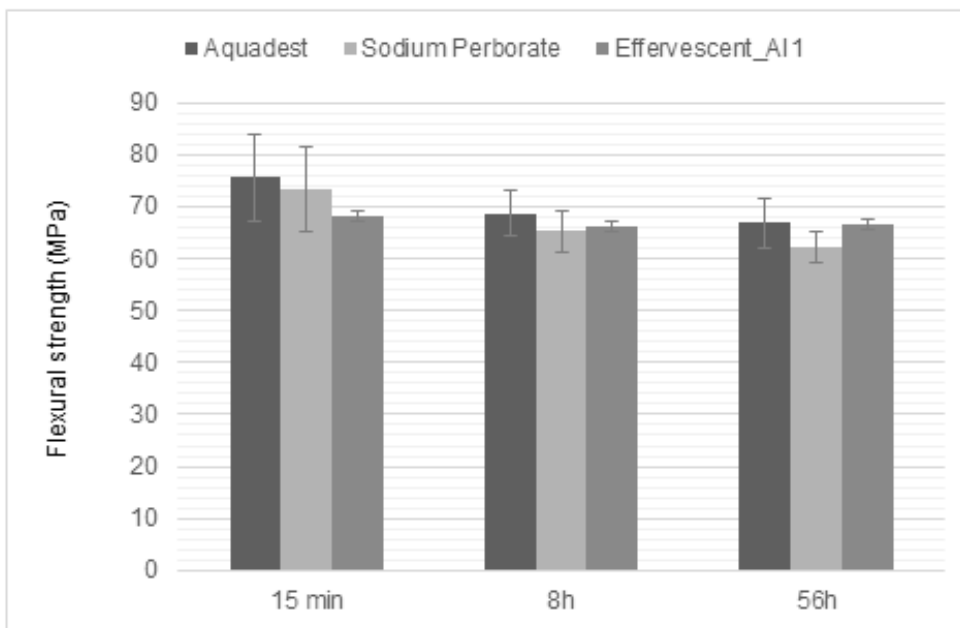


Figure 2. Flexural strength of heat cured acrylic resin plates after immersion.

Groups	Immersion duration time	Aquadest (Group 1)			Sodium Perborate (Group 2)			Effervescent_AI1 (Group 3)		
		15 minutes	8 hours	56 hours	15 minutes	8 hours	56 hours	15 minutes	8 hours	56 hours
Aquadest (Group 1)	15 minutes		.543	.252	.999	.95	.09	.47	.151	.192
	8 hours			1.000	.921	.986	.620	1.000	.997	.999
	56 hours				.668	1.000	.895	1.000	1.000	1.000
Sodium Perborate (Group 2)	15 minutes					.366	.056	.880	.498	.575
	8 hours						.991	.994	1.000	1.000
	56 hours							.692	.966	.941
Effervescent_AI1 (Group 3)	15 minutes								.999	1.000
	8 hours									1.000
	56 hours									

Figure 3. Post hoc table of flexural strength of acrylic after immersion for 15 min, 8 and 56 hours.

**DISCUSSION**

We developed the denture cleanser using biological source materials, but since it has been recommended that the application of effervescent provides many advantages for elderly people. Those are cleanser to dentures without any motor skills ability or assistance, reach narrow areas that could not reached easily by tooth brushing, quickly dissolved to water, cleanse stains and calculus, not

scratches the dentures and anti-bacterial, anti-fungus<sup>5</sup>. Therefore, in this study, we focused on effervescent *Acanthus ilicifolius* extract 4%, derived from a biological source, as an antimicrobial component.

*Acanthus ilicifolius* L. is known as daruju or jeruju. It could be found along the creek, lagoon, swampland, and mangrove around beaches<sup>15,16</sup>.

Traditionally, this plant has been used for dyspepsia, paralysis, asthma, headache, rheumatism, and dermatitis<sup>17</sup>. Govindasamy *et al.* (2013) reported the *Acanthus* stated that *ilicifolius* has a long chain of alcohol, saponin, tannin, flavonoid, alkaloid, steroid, glycoside, sterol, phenol, catechol, fatty acid, and lignan. Saponin, flavonoid, glycoside lignan, and tannin showed high antioxidant and antimicroba activities<sup>18</sup>. *A. ilicifolius* L. leaves extract showed promising activity against *Candida albicans*. Andriani *et al.* (2020) confirmed that *A. ilicifolius* L. leaves extract 8% showed an effect on antifungal activity against *Candida albicans*<sup>19</sup>.

This study successfully demonstrated that the effervescent *Acanthus ilicifolius* extract 4%, has a significant antifungal efficacy against *C. albicans* (Fig.1). The specimens including 1:7 or 1:14 (wt%) of effervescent *Acanthus ilicifolius* extract 4%, exhibited statistically significant antifungal activity to reduce the growth of *C. albicans*. This growth inhibitory effect of effervescent *Acanthus ilicifolius* extract 4%, increased in a dose-dependent manner. These findings indicated that effervescent *Acanthus ilicifolius* extract 4%, inhibited *Candida* growth and subsequent biofilm formation on the material surfaces.

This study showed a lower density optical score (OD) from *aquadest* group compared to the sodium group, effervescent A11 dan A12. This also represented its lower turbidity level compared to another group. The turbidity also showed the total amount of bacteria growth to certain media. It is caused by pure and uncolored *aquadest*. Therefore, it has smaller scores than other groups. The *aquadest* is used as a negative control to extract inhibition towards *Candida albicans* growth, regardless of its inhibition areas<sup>20</sup>.

Sodium perborate effervescent is a common material used in dentistry. This material is very effective to decrease the amount of *Candida albicans*. Some studies reported that effervescent sodium perborate significantly inhibits *Candida albicans* proliferation to all denture resin<sup>21,22</sup>. This research, OD score is gained as the highest from sodium, compared to effervescent A11 dan A12. It represented that sodium inhibited *Candida* growth as the lowest, while compared to Effervescent A11 and A12 were less effective. Sodium perborate effervescent is one of effervescent tablets for denture cleanser that worked chemically by releasing oxygen from a neutral enzyme peroxide solution. This material could decrease the total amount of *Candida albicans* cells eventhough it is ineffective for biofilm.

It is already known that *Acanthus ilicifolius* extract has antifungal and antioxidant in-vitro and became antifungal to mice that suffered from oral candidiasis<sup>19,23,24</sup>. This study confirmed that the effervescent A11 and A12 could inhibit *Candida albicans* better compared to sodium perborate. A11 has less turbidity than A11. A12 also represented more effectively in terms of inhibiting *Candida albicans*. It was caused by polyphenol that could affect *Candida albicans* biofilm activity to acrylic plate. The *Acanthus ilicifolius* chemical compounds are flavonoids, alkaloids, glycosides, polyphenols, tannins, and steroids that have a role to inhibit biofilm *Candida albicans*<sup>19</sup>.

In the clinical application, it is very important to examine the effect of effervescent *Acanthus ilicifolius* extract 4% on the mechanical properties of acrylic resin as a denture base material. Both flexural strengths of all immerse times were found to be within the standard values set by ISO 10139-1. However, the flexural strength of acrylic resin has a tendency to decrease as the

immerse time in effervescent *Acanthus ilicifolius* extract 4% (Fig.2). Flexural strength is the load that occurs on the denture in the mouth during the masticatory process. The longevity of partial dentures depends on the flexural strength of acrylic resin after immersion in denture cleanser<sup>25</sup>. The results of the difference test between groups using the Tukey HSD test showed that there was no significant difference in the value of flexural strength in the overall study results, but there was still a decrease in that group that was immersed with effervescent *Acanthus ilicifolius* extract 4%. Denture base materials have polymer chains that can be separated due to the presence of solvent molecules, thereby will affect the chemical structure and causing a decrease in polymer strength. This statement is supported by the matrix degradation theory, namely that resins immersed in water can enter through a diffusion mechanism into the chemical structure of the resin so that the distance between polymers can increase and cause the strength of the matrix to decrease<sup>26</sup>.

This study confirmed that denture cleansers decreased the flexural strength of denture base resin in comparison with *aquadest* immersion. Some studies were reported that the flexural strength reduction of acrylic resins can occur when exposed to peroxides<sup>27,28</sup>. When the immersion period increased, the effect of denture cleansers on flexural strength can be decreased because the solvent in the denture cleanser will be able to damage the polymer chain even bigger so that it can affect the mechanical strength. In the treatment group, the effervescent *Acanthus ilicifolius* extract 4% can affect the decrease in flexural strength, this was due to the presence of sodium perborate from the effervescent which forms alkaline peroxide compounds when in contact and dissolves in water then causes oxidation of the

tertiary amine accelerator caused by oxygen released so that can attack the polymer chain backbone causing polymer degradation producing the polymer chain becomes weak and the flexural strength of heat cured acrylic decreases<sup>25,29</sup>.

## CONCLUSION

Combination of the *Acanthus ilicifolius* L extract 4% and sodium perborate with the ratios 1:7 had a good antifungal effect and appropriate flexural strength at 15 minutes, 8 hours, and 56 hours of immersion. Effervescent *Acanthus ilicifolius* L extract 4% and sodium perborate with a ratio of 1:7 can be used as a candidate for denture cleanser.

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## CONFLICT OF INTEREST

The author claims there is no conflict of interest

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