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Effects Of Extract Endophytic Fungi *Aspergillus* Sp. In Denture Adhesives Of Acrylic Resins Related To The Surface Roughness And *Candida Albicans* Virulence

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ABSTRACT

Antifungal treatment is currently a serious problem because resistance and attachment of *C. albicans* often occur and can have an effect on the acrylic resin surface. One of them is adding antifungal concentration from the extract of endophyte fungi *Aspergillus* sp. contains chemical compounds that can inhibit *C. albicans*. The study aimed was to effect of extract of endophyte fungi *Aspergillus* sp. in denture adhesives of acrylic resins related to the surface roughness and *C. albicans* virulence. The research method was to extract the endophytic fungi *Aspergillus* sp. bio-tolerance test and measure the surface roughness of acrylic resin. Denture adhesive formulation and added *Aspergillus* sp. with concentrations of 3.125%, 6.25%, 12% and 25%. The research sample used hot polymerized acrylic resin, namely the denture adhesive treatment group with a predetermined concentration, the control group using denture adhesive product X and, denture adhesive added nystatin, each group suspended *C. albicans* for 24, 48 and 72 hours. Analysis of differences in bio-tolerance activity and surface roughness of acrylic resin using ANOVA. Bio-tolerance activity of *Aspergillus* sp. against *C. albicans* was the best at concentrations of 12.5% and 25%, the strongest occurred at 24 hours of incubation. The surface roughness test of acrylic resin at a concentration of 3.125% has a good ability it is better to prevent the formation of surface roughness, but there is no difference in the surface roughness of acrylic resin concerning concentration and incubation time. Endophytic fungi extract *Aspergillus* sp. in denture adhesives can inhibit the development and interaction of *C. albicans*. has a surface roughness value of acrylic resin is better than synthetic antifungal.

Keywords: Extract *Aspergillus* sp, Denture adhesive, Bio-tolerance, *C. albicans*, Surface roughness

1. INTRODUCTION

Some of the complaints that are often felt by denture users, in the form of discomfort, looseness and, pain when wearing dentures because the dentures do not get good support or foundation from the alveolar bone

(Basker and Davenport, 2002; Zarb et al., 2013).The number of wearers of loose and uncomfortable dentures reaches 70-85%, caused by some of the supporting tissues experiencing resorption (Basker and Davenport, 2002). Resorption of the alveolar ridge with a flat ridge condition requires surgical therapy,

sometimes the patient refuses to undergo surgery, to reduce retention and stabilization of removable denture wear (Essam, 2010). Loss of retention and stabilization can also occur in elderly patients, namely: decreased neuromuscular control, decreased bite strength, drug-induced xerostomia/radiotherapy and, systemic disease (Zarb et al., 2013; Duqum et al., 2012; Thalib and Purnama, 2011). Several methods have been developed to improve the retention and stabilization of removable dentures, consists of, the use of denture adhesive (DA), relining, rebasing and, placement of implants to support or maintain dentures (Zarb et al., 2013).

Based research by Maia, for several adhesives, showed that DA materials that did not have an antifungal component tended to increase the amount of *C. albicans*, while adhesives that had an antifungal component showed a decrease in the amount of *C. albicans* (Maia et al., 2010). Research by Rajanam and Manoj mentions that the effect of DA is different because it uses different antimicrobials. The development of DA has been carried out by many researchers, especially by making innovations to replace antimicrobials with different antifungals (Rajaram and Manoj, 2017). The researchers used synthetic antifungal agents and natural ingredients that in general can inhibit and suppress the formation of *C. albicans* biofilms, especially minimizing its colonization (Cartagana et al., 2016; Almeida et al., 2017; Da Silva et al., 2008).

The antifungals in DA, such as propyl hydroxybenzoate, hexachlorophene, sodium tetraborate, sodium borate and, ethanol, can cause damage to the surface of the hot polymerized acrylic resin. Several studies on DA using antifungals, such as azoles and nystatin, can increase the surface roughness of acrylic resins and increase *C. albicans* attachment (Rao et al., 2015). The results of the research of Srividya, stated that the higher the surface roughness value of the denture base, the more microorganisms accumulated, which can cause denture users to experience denture stomatitis (Srividya et al., 2013). Research related to the potential of endophytic fungi in mangrove plants growing in West Sumatera has been carried out (Rival et al., 2018). From this research, endophytic fungi derived from *R. mucronata* were proven to be able to inhibit the growth of pathogenic bacteria and fungi. Based on the results of molecular identification, the isolate was identical to *Aspergillus* sp. The results of Rajeswari's study showed that the bioactive metabolites of the endophytic fungus *Aspergillus* sp. could be a promising source as an antifungal agent (Rajeswari et al., 2016).

The combination of DA with natural antifungals can be an effective alternative to inhibit *C. albicans* biofilm formation, in addition to increasing denture retention. The development of new compounds begins with a bio-tolerance examination, which is stress tolerance to temperature or pH. Immunotolerance to determine the ability of the extract to inhibit the growth of pathogenic cells and obtain the right pH to kill *C. albicans* (Kumar

and Pandey, 2013). Bio-tolerance activity of *Aspergillus* sp. as information to assess the effectiveness of extracts to prevent the attachment and development of *C. albicans* to acrylic resins

2. METHODS AND MATERIAL

The test plates were made of hot polymerized acrylic resin (Acron, GC Japan) with a total of 48 plates. Using the main model made of metal with a size of 10 x 10 x 1 mm (Silva et al., 2016). The manufacture of the plate follows the manufacturer's instructions. The surface of the plate is polished using sand paper of sizes 200, 300 and, 400. Denture Adhesive Material, all formulation compositions were weighed then stirred with a vacuum mixer for 20 minutes. The formula for the denture adhesive used for the test was 20 g after adding the concentration of *Aspergillus* sp. 3.125%, 6.25%, 12.5% and, 25%.

Bio-tolerance activity test of *Aspergillus* sp. against *Candida albicans*, a total of 50 ml of *Aspergillus* sp. of each concentration was put in a glass beaker and added to it 5 ml of *C. albicans* in a Mueller-Hinton Broth (MHB) ratio 1: 10, before incubation, the two mixtures were measured for initial pH (0 hours). Then they were incubated at 37°C for 6 hours, 12 hours, 24 hours, 48 hours, and 72 hours in an anaerobic atmosphere. Every time specified, pH measurements were taken with a pH meter. Changes in pH variation from 0 hours measurement with the specified time become an indicator that *C. albicans* has can to tolerate acidic environments and adapt to pH (McHugh et al., 2004).

The results of the pH measurement of the test material were then measured for acid tolerance activity by *C. albicans* against *Aspergillus* sp. using the spectrophotometer principle. Measurement of acid tolerance activity was carried out based on a predetermined time according to the pH measurement and against it in the shaker at 500 rpm. A series of 96-well triple microplates were coated with 50 µl MHB for 15 min and aspirated. Bio-tolerance activity was measured with an Elisa Reader at a wavelength of 595 nm.

Denture Adhesive Material on acrylic resin after that, it was incubated in 10 ml of critical saliva in PMSF (10:1) pH 6.5 for 30 minutes. Each acrylic resin sample, 300 µl of 1.5x10⁸ CFU/ml *C. albicans* solution was given (Ibraheem and Hammad, 2019). After 15 minutes, the test material endophytic fungi were added based on the concentration (3.125%, 6.25%, 12.5%, 25%) and 2 mg diluted product X solution and nystatin in 10 ml PBS pH 7. The adaptation process for the formation of *C. albicans* biofilm on the acrylic resin surface used incubation times of 24 hours, 48 hours, and 72 hours. The Acrylic resin which has been coated with biofilm together with endophytic fungi was prepared to observe the effect of endophytic fungi on the formation of *C. albicans* biofilm.

Examination of the acrylic resin surface after preparation of *C. albicans* and the test material with an

acrylic resin surface area scanned with AFM (Atomic Force Microscopy) which is $10 \times 10 \mu\text{m}$.

3. RESULT AND DISCUSSION

Biotolerance activity of *Aspergillus* sp. as information to assess the effectiveness of extracts to prevent the attachment and development of *C. albicans* to acrylic resins. The results of Lazarin, reported that the interaction between *C. albicans* on acrylic resins is facilitated by salivary proteins, where *C. albicans* uses salivary proteins to colonize the acrylic resin surface which then forms quorum and spreads (Lazarin et al., 2014). This basis becomes a reference for further testing. In the first stage, the biotolerance properties of the *Aspergillus* sp. The purpose of this examination was to determine the tolerance limit given by the test material to the development of *C. albicans* to prevent the fungicidal properties of *Aspergillus* sp. The principle of the test is based on concentration, pH and the interaction between the two. This principle is to obtain the optimal concentration of response and biological adaptation, so that it can be used as a reference for further testing related to the effect of *Aspergillus* sp. against the biological activity of *C. albicans* can be seen in figure 1.

The ability of *C. albicans* tolerance to acid is very important to maintain balance with its environment. This is reinforced by research by O'Driscoll, which states that low pH conditions may have the potential to increase tolerance acid naturally from natural ingredients and to prevent the increase in virulence of the pathogen (O'Driscoll et al., 1996). In this study, *C. albicans* had a significant adaptive acid tolerance after 1 hour in the extract of the endophytic fungus *Aspergillus* sp. (pH 5.5). Susceptibility to acid pH 3.5 depends on the growth phase, where *C. albicans* attempts to adapt by increasing the pH of the solution to pH 5.5 to induce acid tolerance. This indicates that *C. albicans* adapts to synthesize a number of proteins or peptides contained in the test material.

Biotolerance activity of *Aspergillus* sp. there is something to do with the ability of a number of active components such as alkaloids, flavonoids, and terpenoids to act as antifungals (Loizzo et al., 2012; Sandoval et al., 2012). A number of these cellular and molecular activities to maintain a balance of tolerance when interacting with fungal extracts of *Aspergillus* sp. The finding in this study is that the incubation time of 24 hours is a very good pH adaptation response compared to other incubation times. 24 hours is considered the most stable adaptation time to defend against stress responses to the environment is when there is a lack of carbon when entering the resting phase can be seen in figure 2. In addition, the tolerance response by *Aspergillus* sp. may be affected by a number of key proteins involved in responding to oxidative stress such as alkyl hydroperoxide reductase / thioredoxin reductase proteins that increase intracellular concentrations; oxidation-modifying

glycolytic enzymes (D-glyceraldehyde 3-phosphate dehydrogenase, and fructose 6-phosphate aldolase) that increase intracellular concentrations by decreasing ATP production and producing balancing proteins such as chaperone proteins (Steeves., 2011).

Biotolerance activity with pH response of *Aspergillus* sp. against *C. albicans*. At 24 hours incubation, two concentration variables were tangent to each other (6.25% and 50%) and 72 hours incubation time only one concentration had a relationship, the tangent line occurred at 50% concentration. Meanwhile, the incubation time of 12 hours and 48 hours was only close to (6.25% and 75%) and (48 hours: only 50%) see in figure 3. These results indicate that the *Aspergillus* sp. have various tolerance responses. This diversity tends to be influenced by the environment and its ability to suppress the development of *C. albicans*. Shah's research, using Solunecm (Azadirachtin) extract, showed that concentration was the determinant as an antifungal to maintain a balance of stress that was influenced by alkaline pH (Shah et al., 2016).

According to Leclerc, that one of the strategies used by a number of active components of the extract is to opsonize a number of *C. albicans* cells when interactions occur under the influence of pH. The opsonization process is a phyto-response activity of all natural materials in order to provide tolerance to pathogenic cells that potential has something to do with the biological properties of *C. albicans* which is able to survive in low pH conditions. This condition has something to do with the thickness of the cell wall that is able to respond to oxidative stress when exposed to active compounds contained in natural ingredients (Steeves et al., 2011). The assumption from this information is that *Aspergillus* sp. belongs to the synergistic or antagonistic group, where both compounds have adhesion properties, biotolerance, and bioresistance to fungi (Leclerc 2011).

Examination of the surface roughness of acrylic resin as a result of the effect of *C. albicans* activity after the *Aspergillus* sp. The surface roughness reflects the intensity of *C. albicans* after being exposed to a solution of *Aspergillus* sp. In principle, the rougher the surface, the more fungal activity will be, and conversely, the smoother the surface area of the acrylic resin, the stronger the response intensity to *C. albicans* (Almeida et al., 2017).

The results in Figure 4 show the average surface roughness of acrylic resin after interaction with *C. albicans* and *Aspergillus* sp. at a concentration of 3.125% still has a better ability to prevent the formation of surface roughness of acrylic resin due to the influence of *C. albicans* activity. Meanwhile, the nystatin group and product X had a higher roughness value. Each group has a surface roughness value that varies.

The average in each group can be seen that the nystatin group and the increase in the surface roughness value of acrylic resin, because the content of these two

materials has acidic properties and is associated with a low pH value. This is in accordance with Darwish's research, that at lower pH conditions, it can increase the concentration of methyl methacrylate monomer released from acrylic resin where under acidic conditions the polymer surface can soften with loss of ionic structure (Darwish and Nassani, 2016). Another study was that acrylic resin had a good response to *C. albicans* which was assessed based on the biocompatibility of acrylic resin after interaction with *C. albicans*. The use of antifungal agents is an option to increase the resistance of acrylic resin to environmental changes that are influenced by the biological activity of *C. albicans* (Acosta-Torres et al., 2014).

Surface roughness was reported to have a close relationship with the intensity of fungal adhesion, where higher amounts of *C. albicans* were found on the rough surface area compared to the smooth surface after polishing (Maza et al., 2002). Lazarin, revealed that an increase in the surface roughness of acrylic resin can facilitate the retention of microorganisms (Lazarin et al., 2014). However, based on the incubation time in the table from the results of this study, it was shown that there was no significant difference in the surface roughness of acrylic resin after interaction with *C. albicans* and exposure to endophytic fungal extract ($p > 0.05 = 0.32$). Meanwhile, based on the concentration according to the results of the analysis, there was also no significant difference ($p > 0.05 = 0.093$). This means that changes in the surface of the acrylic resin after adaptation to *C. albicans* and the test material were not affected by the concentration of endophytic fungi and incubation time.

The mechanism of protection provided by *Aspergillus sp.* against the activity of *C. albicans* indirectly prevented some *C. albicans* cell surface proteins to increase adhesion. In addition, it is possible that a number of antifungal active components possessed by the extract of *Aspergillus sp.* such as Hexadecanoic acid, 7-pentadecyne, -9-methylene, 4-isopropyl-1,6-dimethyl-1,2,3,4,4A, 7-xahydronaphthalene, 3-hydroxybutan-2-one can prevent cell surface hydrophobicity activity. *C. albicans* (Okmes et al., 2019). Water absorption properties of hot polymerized acrylic resin, depending on the degree of hydrophobicity and porosity of a material (Power and Sakaguci, 2012). Clinically, the surface roughness value accepted in dentistry is less than $0.2 \mu\text{m}$ (Al-Kheraif, 2014). Based on this, the concentration close to the value of $0.2 \mu\text{m}$ is at a concentration of 3.125%. The roughness value exceeds the standard value because water absorption can occur due to molecular diffusion.

4. FIGURES AND TABLES

Bio-tolerance test was carried out to determine the bio-tolerance activity of the extract on the development of *C. albicans*. The strength of this bio-tolerance is basic information is to assess the effectiveness of the

material the test when used as an anti-*C. albicans* material, besides that it also assesses the stability of the test material as well as to measure the optimal time and concentration that can be used as a reference in the antifungal test (figure 1)

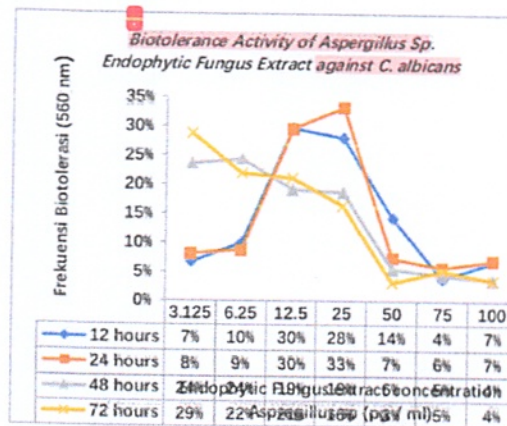


Figure 1 Diagram of the bio-tolerance activity of *Aspergillus sp.*

Figure 1 shows the bio-tolerance activity of the fungal extract of *Aspergillus sp.* against *C. albicans*. The best bio-tolerance activity was at concentrations of 12.5% and 25% with 12 and 24 hours respectively. The activity of bio-tolerance at all concentrations was strongest at 24 hours of incubation compared to other incubation times.

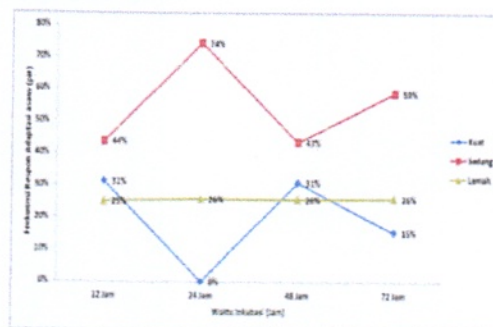


Figure 2. Diagram of the percentage response of pH adaptation with incubation time of *C. albicans*.

The general, *Aspergillus sp.* has a balanced pH adaptation response to the acidogenic and aciduric activity of *C. Albicans* based on the percentage activity obtained at each incubation time. All incubation times showed a moderate scale of 12 hours (44%), 24 hours (74%), 48 hours (43%), and 72 hours (59%). These results indicate that the extract of the test material has a balanced activity against changes in pH. The incubation time of 24 hours is a very good pH adaptation response compared to other incubation times.

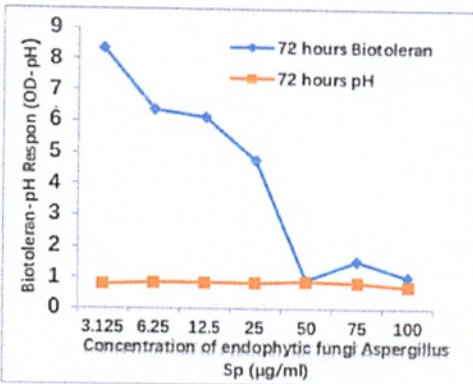
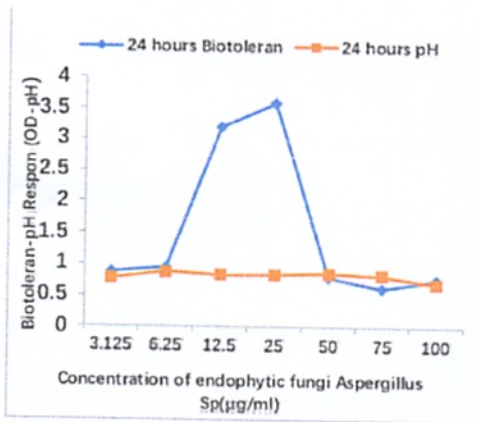


Figure 3 Diagram of the relationship between biotolerance activity and the pH response of *Aspergillus* sp. against *C. albicans*

The incubation time of 24 hours has two concentration variables that are tangent to each other (6.25 and 50%) and the incubation time of 72 hours is only one concentration that has a relationship, the tangent line occurs at a concentration of 50%. Tolerance with changes in pH occurred at a concentration of 50% for 24 hours and 72 hours incubation

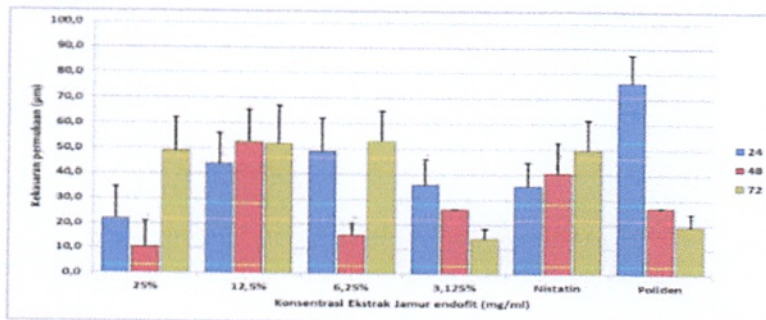


Figure 4. Diagram of extract concentration percentage with acrylic resin surface roughness

The concentration of 3.125% had a better ability to prevent surface roughness from forming compared to other concentrations. So it can be assumed that the concentration in addition to having good absorption capacity also has tolerance to the growth of *C. albicans*. Thus preventing the spread of *C. albicans* on the surface of the acrylic resin and resulting in a decrease in roughness

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