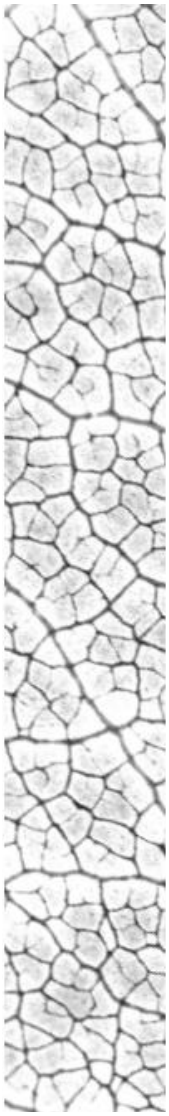

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Antifungal power of chitosan extract from squid pen powder towards Candida albicans

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Abstract
Chitosan is a natural material that can be obtained from fishery waste such as crustacean shells and squid pens. Squid pen is a waste produced from the processing of squid with transparent structures with feather-like shapes attached to the dorsal inside a squid mantle. Chitosan from squid pen has been developed in the medical field because it has biocompatible properties, biodegradable, non-toxic, and also has antifungal activity. Being able to eliminate the Candida albicans fungus which is a predisposing factor for the occurrence of denture stomatitis due to the use of long-term dentures. This study aims to determine the antifungal activity of chitosan extracted from squid pen against Candida albicans. Candida albicans was planted in Saboraud broth liquid media tubes and given Chitosan suspension 1.5% (w / v) with each sample group consisting of 50%, 25%, 12.5%, 6.25%, 3.125%, 1.56%, 0.78% and there are two control groups namely positive and negative controls. All samples were incubated for 24 hours at 37°C Then a visual observation of turbidity on liquid Saboraud broth media was then carried out by planting culture on SDA media with 7 replications, incubated for 2x24 hours at 37 ° Gmd counted the number of living colonies. The results were analyzed using Kruskal-Wallis and Post hoc tukey test. At the concentration of 25% there was no fungal growth in saboraud dextrose agar media. Chitosan extracted from squid pens has antifungal activity against Candida albicans fungi with a minimum concentration of 25%.

Keywords: chitosan, squid pen, antifungal activity, Candida albicans

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INTRODUCTION

Denture stomatitis is a chronic inflammation of the mucous membrane due to the use of dentures for a long time with the condition of the mucosa appear reddish (erythema), sometimes there is pain or burning, and is usually caused by *Candida albicans*. *Candida albicans* is a normal oral microorganism but if the amount exceeds the normal level and denture users have bad *oral hygiene*, *denture stomatitis* might happen (Baskaran, 2015; Moosazadeh, et al. 2016).

Chlorhexidine gluconate 0.2% is an antiseptic mouthwash that has antifungal ingredients (Permatasari, Budiarti, & Apriasari, 2016). Antifungal is a compound that can kill fungi, especially fungi that harm humans (Ariningsih, 2009). However, the side effects from *chlorhexidine* can cause changes in tooth color, increased formation of tartar in teeth, and decreased taste function in the sense of taste (Prasanna, & Lakshmanan, 2016; Hamzeheinejad, & Pal, 2016).

Chitosan is a natural ingredient that can be used as an alternative material because it has biocompatible properties, *biodegradable,* non-toxic, as well as antifungal that can be obtained in fisheries waste such as *crustacean* (hard skinned) and autoclave obtained from squid (De Queiroz Antonino, 2017. Liang, et al. 2015). Autoclave is industrial waste obtained from squid processing which has a transparent structure with a feather-like shape attached to the dorsal part of the squid's mantle (Jeong, et al. 2018). Autoclave which is widely used to be processed into chitosan derived from several types of squid, among others *Loligo chinensis, Nototodarus sloanii, and Dosidicus* gigas (Shavandi, et al. 2015. Cuong, et al. 2016). Chitosan derived from autoclave consists of the main chain amine group and two free hydroxyl groups which functions as an antifungal. The research objective was the chitosan antifungal activity extracted from squid pens against Candida albicans.

MATERIAL AND METHODS

This research is an experimental research. This study has received a certificate of ethics from the Ethics Committee of the Faculty of Dentistry, Universitas Airlangga No. 165/HERCC.FODM/Vn/2018. This

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research procedure is divided into four steps. The first step, autoclave with an average length of 30.4 cm in the form of a transparent sheet with a feather-like shape found in the dorsal part of the squid was separated from the squid (*Loligo chinensis).* The portion taken from the dorsal portion of the squid was dried in an oven at 50°C for 8 hours. Autoclave of 10.7 grams that has been dried was *ground* use a grinder to produce powder autoclave measuring 60 mesh. Autoclave powder was mixed with 10% NaOH and heated at 60°C with constant stirring at a rotary shaker at 125 rpm for 24 hours to deproteinize. Autoclave powder which has been deproteinized was then put into vacuum filtration to remove any residual water *(leachate). The pH of the* autoclave powder which no longer contains water was neutralized (pH = 7) then lyophilized using *freeze drier* into a powder called chitosan.

In the second step, the chitosan powder obtained was made into a suspension by mixing 1.5 grams of chitosan powder with 100 ml of 1% acetic acid so that a 1.5% chitosan suspension (w / v) could be obtained. Then, the tools used in this study were sterilized deeply with autoclave at 121°C for 30 minutes.

In the third step, test the chitosan antifungal power of autoclave by using 9 concentration groups namely 50%, 25%, 12.5%, 6.25%, 3.125%, 1.56%, 0.78%, as well as the positive and negative control groups that were cultured in Saboraud broth liquid. Then, all concentration and control groups were incubated at 37°C for 1 x 24 hours, and turbidity of the Saboraud broth liquid was observed.

The fourth step, planting the culture in natural media with 7 replications and incubated at 37°C for 2 x 24 hours. Then, counting the fungal colonies that grow on SDA media.

Analysis of research data was done using SPSS statistical test version 16.00 (2007). Data analysis was done using parametric statistical tests of Kruskal-Wallis to find out the difference in the power of chitosan extract from squid pen powder towards *candida albicans*. If significant differences are obtained then testing was done using a test *Multiple Comparison of HSD* (*tukey test*)

RESULTS

This study used 9 concentration groups namely 50%, 25%, 12.5%, 6.25%, 3.125%, 1.56%, 0.78% respectively, as well as the positive and negative control groups. For each treatment, seven replications were performed. From the research conducted, the results were obtained as follows:

In **Figure 1**, the control plate (-), plate 1 (chitosan of 50%), plate 2 (chitosan extract n of 25%) did not get fungal growth, whereas on the control plate (+), plate 3 (chitosan extract of 12.5%) and plate 4 (chitosan extract of 6.25%) obtained fungal growth.

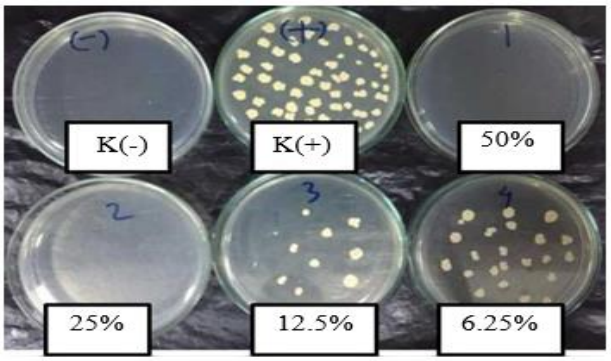

Fig. 1. Single column figure on top of a column

Table 1. Average value and colony standard deviation *Candida albicans*

Treatment Group	N	R	SB	Sig.
1	7	120,7143	3,03942	0.000
2	7	11,1429	1,95180	
3	7	24,5714	.97590	

N = Number of treatments
R = Average research results SB = Standard Deviation
1 = Number of *Candida albicans* colonies in the control group
2 = Number of *Candida albicans* colonies in the 12.5% group
3 = Number *Candida albicans* colonies in the 6.25% group

Table 2. Tukey HSD Test Results

Treatment Group	Control	12.5%	6.25%
Control	-	*	*
12.5%	*	-	*
6.25%	*	*	-

The results of the research that have been obtained are then processed the data to see the average and standard deviation of fungal colony growth of *Candida albicans* in each treatment group.

The Kruskal-Wallis test result in **Table 2** shows the value of p = 0.000 (p <0.05) indicates a difference in *the mean* of fungal colonies from each chitosan autoclave powder concentration group and control group.

The results obtained (p = 0.00 (p <0.05)) showed that there were significant differences in autoclave chitosan extract with a concentration of 12.5%, 6.25% and control group.

DISCUSSION

Candida albicans is the most common fungus found in denture users and is also the cause of *denture stomatitis*[u]. One natural ingredient that is suspected can be used as an alternative to reduce the amount *Candida albicans* in denture users is chitosan. Chitosan is a natural ingredient that can be used as an alternative material because it has biocompatible, *biodegradable*, non-toxic, and antifungal properties (De Queiroz Antonino, et al. 2017). According to a research that has been done on its biocompatibility test with MTT assay, chitosan from autoclave is biocompatible up to 100% concentration (Hemalatha, et al. 2014).

In this study using the dilution method with depletion was done to obtain a concentration of 50%, 25%, 12.5%,

6.25%, 3.125%, 1.56%, 0.78%, the dilution method was used to determine the minimum inhibitory concentration and minimum kill concentration (Azrifitria, 2010). Selection of chitosan was done using a concentration of 1.5% (w/v) because according to previous research, chitosan 1.5% has good dispersion ability (Ali, et al. 2013).

Based on the results of phytochemical content tests conducted at the Surabaya research and industry consulting center, extract from autoclave has a chitosan extract of 86.94%, a degree of acetylization of 70.82 °, deacetylization degree of 0.98 °, and a molecular weight of 11.36 kDa.

Through the results of this study it was found that chitosan has antifungal power against *Candida albicans.* Chitosan derived from autoclave consists of the main chain amine group and two free hydroxyl groups which functions as an antifungal. Chitosan is one of the ingredients that is potentially effective as an antifungal. Ionic interactions between positive amine functional groups (NH3+) in chitosan and fungal cell walls of *Candida albicans* which is negatively charged under acidic conditions will result in changes in the permeability of microbial cell membranes and will change the internal osmotic balance. This is widely believed by researchers to be able to inhibit microbial growth. Based on this, it is believed that the mechanism that occurs in chitosan antifungals has the same mechanism as antimicrobials. Unbalanced osmotic pressure will hydrolyze peptidoglycan from fungal cell walls, causing loss of electrolytes, proteins, amino acids, and glucose in fungal cells (Costa, et al. 2014; Hafdani, & Sadeghinia, 2011). The result is chitosan will inhibit *Candida albicans* and cause cell death.

The free hydroxyl group in chitosan is very reactive. This group can interact with important nutrients needed by fungi and will interfere with the absorption of fungal nutrients so that fungal growth will be inhibited (Goy, Morais, & Assis, 2016). The presence of cations from chitosan molecules can increase the bonding strength on the surface of microbial cells, which causes the cell membrane to shrink slowly. Some other possibilities are the polycation of chitosan molecules interacting with the anion component in the microbial cell wall predominantly, resulting in damage to intracellular components due to changes in permeability, thus inhibiting the entry of nutrients into cells (Prashanth, & Tharanathan, 2007).

The parameters that affect the nature of chitosan are molecular weight and degree of deacetylation. The purity of chitosan is determined by the degree of deacetylation, the more acetyl groups can be removed, the higher the value of the degree of deacetylation. The high degree of deacetylation is influenced by the concentration of NaOH (<40%) which serves to break the bonds between hydroxyl groups with nitrogen atoms. The higher the degree of deacetylization shows the more amine groups (NH2) on chitosan molecules, causing the chitosan to be more reactive. This reactive chitosan can interact with fungal cell walls which can inhibit fungal growth (Mursida, Tasir, & Sahriawati, 2018).

Molecular weight is closely related to the solubility of chitosan in acetic acid, solubility will decrease when the molecular weight is large (Wiyarsih, & Priyambodo, 2009). Conversely, when a small molecular weight solubility in chitosan will increase so that many amine groups are protonated (Yien, et al. 2012). This allows the amine group from chitosan to interact with fungal cell walls which can result in the hampered growth of *Candida albicans*.

The solvent used in this research was 1% acetic acid, this is because chitosan has better properties than other weak acids and effectively increases the ability of chitosan to inhibit mold growth (Kim, et al. 2006). However, 1% acetic acid has no effect on mold growth *Candida albicans* because this fungus lives in an environment with a pH of 6.5-7 and a 1.5% chitosan suspension used has a pH of around 6-7 so that 1% acetic acid has no effect on the growth of *Candida albicans.*

Antifungal test results using the dilution method showed that the concentrations of 25% and 50% were the highest concentrations that did not have fungal growth because at this concentration 0% fungal growth was found. At concentrations of 12.5% and 6.25%, fungus growth with different numbers of colonies was found to be low. At a concentration of 12.5% the growth of fungal colonies was obtained with an average of 11.14 fungal colonies, while at a concentration of 6.25% the average number of colonies was 24.57.

CONCLUSION

Chitosan extract from autoclave powder effectively inhibits *Candida albicans* fungus growth. Chitosan extract from autoclave powder at a concentration of 25% can kill *Candida albicans.*

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