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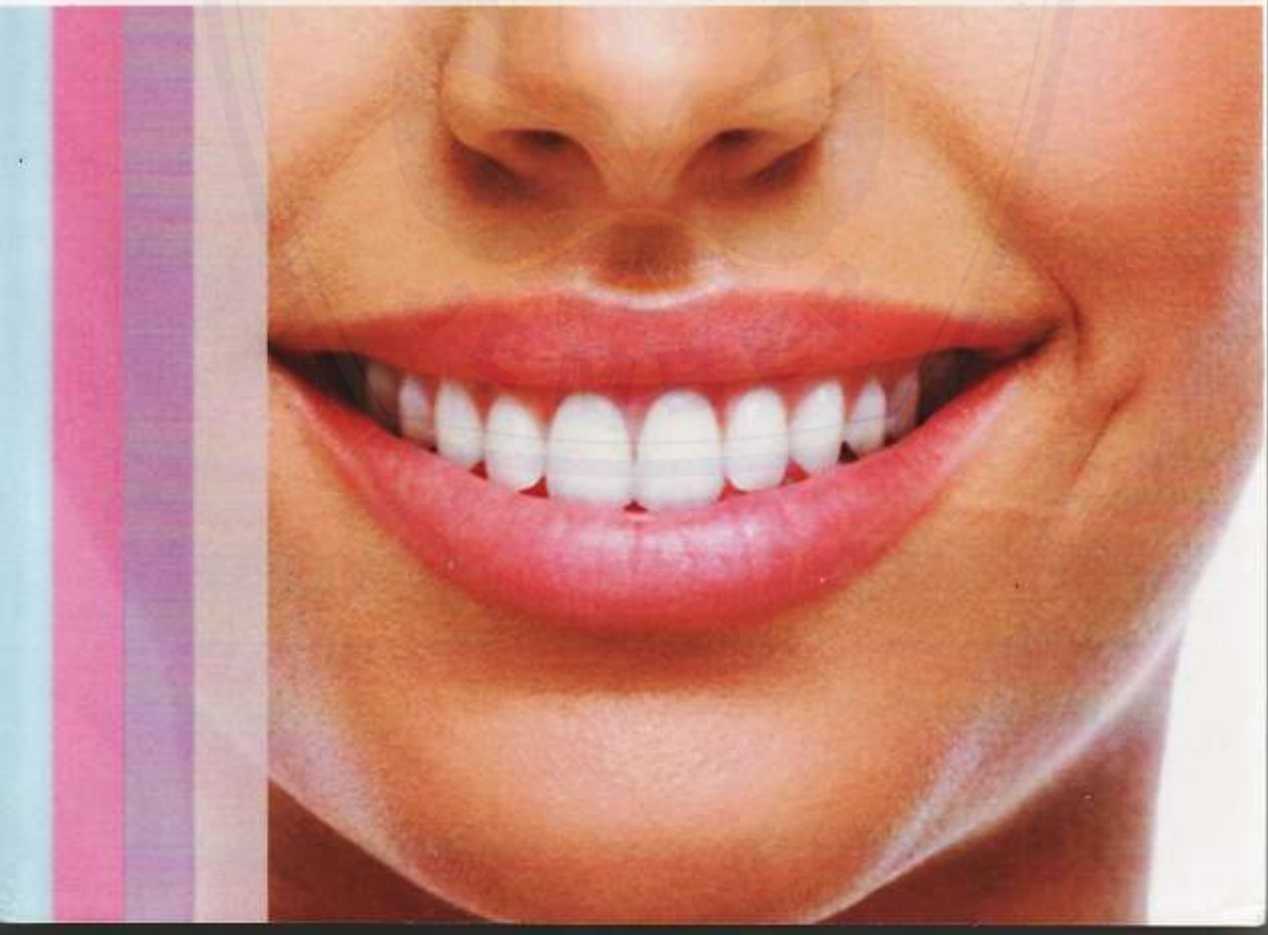

The Effectiveness Of White Cambodia Flower Extract (Plumeriaalba L.) As A Denture Cleanser To Decrease The Number Ofcandida Albicans In Soft Liner

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ABSTRACT

INTRODUCTION: Soft liner is a material used to prevent soreness of denture due to excessive bone resorption which sharpens the alveolar ridge. However, soft liner has been proven to be easily contaminated with *Candida albicans*. We need to clean it with denture cleanser to overcome this problem. However, chemical denture cleanser is known could change the physical properties of soft liner. **Objective**: To find out the effectiveness of white cambodia flower extract as a denture cleanser to decrease the number of *C. albicans*in soft liner. **Material and Methods**: Soft liner was soaked in sterile distilled water and white cambodia flower extract in different concentrations (25%, 50%, 75% and 100%). Then, the absorbance value of *C. albicans* was measured using a spectrophotometer to determine the concentration. **Results**: The concentration of C. albicans on soft liner in sterile distilled water and white cambodia flower extract in different concentrations (25%, 50%, 75% and 100%) are 1,576x108 CFU/ml, 1,334x108 CFU/ml, 0,896x108 CFU/ml, 0,464x108 CFU/ml, and a 0,035x108 CFU/ml respectively. **Conclusions**It is shown that theconcentrations of C. albicans decreased with the increasing of the concentrations of white cambodia flower extract. Therefore, white cambodia flower extract is proven to be effective against*C. albicans*.

Keywords: *Candida albicans, denture cleanser, Plumeria alba, soft liner, spectrophotometer*

INTRODUCTION

On the use of denture, it is often obtained that the condition of denture support tissue is not optimal caused by the excessive of bone resorption. In certain cases, the resorption pattern of alveolar ridge causes sharp or knife-edge form.[1] It can cause pain in patients when using denture. Therefore, denture needs to be coated with a soft material such as soft liner to provide comfort during use denture and prevent the continuation of bone resorption.

Soft liner is a material that is used to prevent chronic pain because of using denture because it reduces occlusal forces, also help maintaining the support structure of denture in the oral cavity.[2] Soft liner is used to create a comfortable adhesion between the denture surface and the soft tissues in oral cavity, reduce the trauma of occlusal loaded in patients who experience severe alveolar bone resorption and the area that is in the wound healing process after surgery.[3] However, the previous studies stated that the average number of Candida albicans on acrylic resin plate with using soft liner is higher than without the use of soft liner.[4] In fact, C. albicans is the cause due to the use of denture stomatitis (denture stomatitis) that is most common occurred.[5] Therefore, denture that uses soft denture liner is necessary to keep it clean by soaking it in denture cleaner. However, denture cleaner with chemicals is not recommended for soft liner because it can alter the hardness surface of soft liner itself.[6]

Therefore, we need a natural material instead of chemical-based denture cleanser to reduce the number of C. albicans on a soft liner. The purpose of this study was to determine the effectiveness of white cambodia flower extract as a denture cleanser to decrease the number of C. albicans on the soft liner.

METHODS

This research used laboratories experimental research. This research was done at the Laboratory of Bioscience and Laboratory of Microbiology, Faculty of Dentistry, University of Jember, and the Laboratory of Biology Faculty of Pharmacy, University of Jember. The research was conducted in September-December, 2015. The study began with the manufacture of white cambodia flower extract by maceration method. The white cambodia flower that had been washed and then dried without exposure to the sunlight. Furthermore, white cambodia flower dried was shattered using blender to be soft powder. The extraction is done with the immersion technique (maceration) in 70% methanol solution, closed and stored in the dark room for 3 days and stirred once in a day. Then, the filtrate was taken, evaporated and heated, so that the extract became viscous consistency. Furthermore, the dilution was done and based on respective groups of samples that had been determined. After making the complete extract, it was continued to make 25 samples of rectangular plate soft liner with size 10x10x2 mm, then divided into five groups. Plate soft liner was made using square mold with size 10x10x2 mm that was placed on the glass plate. Then, soft liner stirred accordance to powder and liquid ratio due to the factory provisions until it was homogeneous. Then, the soft liner was placed in mold before undergoing to setting process, then mold and soft liner stacked on glass plate so that the surface became flat and smooth. After setting, glass plate was released and the soft liner plate was removed from the mold, and then washed with water to remove dirt. Then, each sample was immersed in sterile artificial saliva for 1 hour. After that, the sample was rinsed with Phosphate Buffer Saline (PBS) solution, and then it was contaminated by C. albicans suspension for 24 hours. After that, the samples were re-rinsed with PBS solution, and then immersed each sample in sterile

distilled water and white cambodia flower extract in different concentrations corresponding to treatment groups for 30 minutes. In group I, the sample was stored in sterile distilled water (control group). In group II, III, IV, and V, the samples immersed in white cambodia flower extracts with respectived concentrations of 25%, 50%, 75% and 100%. Then, each sample was rinsed with PBS solution, then put in 2 ml of Sabouraud's Dextrose Broth (SDB) and vibrated with Thermolyne for 30 seconds. After that, the reading of C. albicans absorbance value was done in spectrophotometer to determine concentration.

RESULT

Table 1. Results of the concentration calculation of C. albicans in the Sabouraud's Dextrose Broth (SDB) media

Group	Average
Group I	1,576x10^{8} CFU/ml
Group II	1,334x10^{8} CFU/ml
Group III	0,896x10^{8} CFU/ml
Group IV	0,464x10^{8} CFU/ml
Group V	0,035x10^{8} CFU/ml

Information:

1. Group I: Soft liner plate soaked in sterile distilled water (negative control) scored the concentration of C. albicans 1,575x108 CFU / ml on sample 1, 1,599x108 CFU / ml on the sample 2, 1,653x108 CFU / ml on the sample 3, 1,461 x108 CFU / ml on sample 4, and 1,590x108 CFU / ml in sample 5.
2. Group II: Soft liner plate soaked in white cambodia flower extract 25% gain value 1,320x108 concentration of C. albicans CFU / ml on sample 1, 1,347x108 CFU / ml on sample 2, 1,404x108 CFU / ml on sample 3, 1,278 x108 CFU / ml on sample 4, and 1,320x108 CFU / ml on sample 5.
3. Group III: Soft liner plate soaked in white cambodia flower extract 50% gain value 0,867x108 concentration of C. albicans CFU / ml on sample 1, 0,894x108 CFU / ml on sample 2, 0,990x108 CFU / ml on sample 3, 0.846 x108 CFU / ml on sample 4, and 0,882x108 CFU / ml on sample 5.
4. Group IV: Soft liner plate soaked in white cambodia flower extract 75% gain value 0,555x108 concentration of C. albicans CFU / ml on sample 1, 0,513x108 CFU / ml on sample 2, 0,486x108 CFU / ml on sample 3, 0.411 x108 CFU / ml on sample 4, and 0,354x108 CFU / ml on sample 5.
5. Group V: Soft liner plate soaked in white cambodia flower extract 100% gain value 0,030x108 concentration of C. albicans CFU / ml on sample 1, 0,024x108 CFU / ml on sample 2, 0,045x108 CFU / ml on sample 3, 0,054 x108 CFU / ml on sample 4, and 0,024x108 CFU / ml on sample 5.

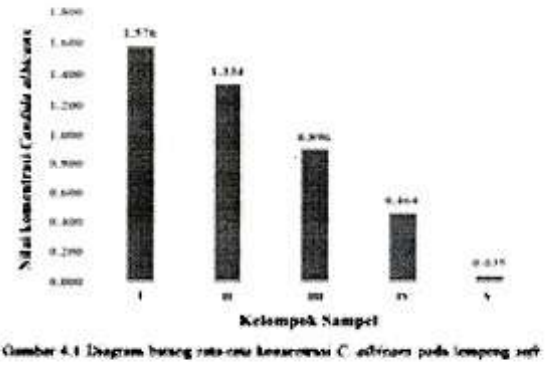

Figure 1. Diagram rod average concentration of C. albicans on soft liner plate after soaking in distilled water, 0.2% chlorhexidine gluconate and white cambodia flower extract (n x 108 CFU / ml)

The research data was tested by normality test using the Kolmogorov-Smirnov test and tested its homogenity using Levene test, it was obtained the distributed normal data and homogeneous data. Furthermore, the data was tested the different test used One-Way ANOVA test followed Least Significant Difference (LSD) to determine differences in the data on each research group with 95% confidence level ($p = 0.05$).

LSD test results showed that there were significant differences in each group treated with significance value of 0.000 ($p < 0.05$). It showed that the higher concentration of white cambodia flower extract could make the concentration value of C. albicans became lower.

DISCUSSION

Based on research that had been done, the C. albicans concentration appeared to have the highest number in the first group or the control group. In group I, the soft liner plates were soaked in sterile distilled water. It was because the sterile distilled water used to soak the control group did not have antimicrobial and antifungal effects. In group II, the soft liner plate was soaked in white cambodia flower extract with concentration of 25%. In this group, C. albicans had lower concentration when it compared with group I. It showed that white cambodia flower extract could reduce the number of C. albicans, although the C. albicans concentrations did not much decrease from the first group due to the low concentration of the extract. In group III, the soft liner plate was soaked in white cambodia flower extract with concentration of 50%. In this group, the C. albicans concentration decreased when compared to group II. It caused by the increase extract concentration, so that the antifungal chemical composition in white cambodia flower extract also increased. In group IV, soft liner plate was soaked in white cambodia flower extract with concentration of 75%. In this group, the C. albicans concentration was also decreased when compared to group III. It occurred because of the presence of increasing concentrations on white cambodia flower extract. In group V, the soft liner plate was soaked in white cambodia flower extract with 100%

concentration. The average value of C. albicans concentration was the lowest number when it compared to other groups. It showed that the concentration of white cambodia flower extract was higher and it made the antifungal effect was also higher, so that the C. albicans concentration would be lower on soft liner plate.

The lower value of C. albicans concentration on soft liner plates can exist because white cambodia flower extract has active compounds that can inhibit the attachment of C. albicans on the soft liner plate such as tannins, flavonoids, saponins and alkaloids. Thus, the higher concentration of white cambodia flower extract can provide the higher active compound contained therein.

The white cambodia flower extract is effective against decrease in the number of C. albicans because it has four chemical compounds that has antifungal effect, such as tannins, flavonoids, saponins and alkaloids. It is supported by Sibi's research which proves that white cambodia flower extract (P. alba) contains four chemical compounds such antifungals.[7] Research by Sibi also proves that white cambodia flower extract is effective for inhibiting the fungi growth, where the fungi that used in the study was Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Aspergillus terreus, Penicillium digitatum, and Rhizopus arrhizus. These fungis is pathogenic on citrus sinensis fruit. Chemical compounds that are contained in white cambodia flower extracts work with interfering the permeability of fungal cells, damage the fungal cell's protein structure, lower the surface tension of fungal cells and disrupt peptidoglycan components of fungal cell.

Tannins is a secondary metabolic compound in plant and it is one phenol group. Tannins have antimicrobial effect because it has astringent compounds. Astringent compounds in tannins could be expected to interfere the cell wall activity and C. albicans cell membrane.[8] Its mechanism is occurred when tannin attaches to the C. albicans cell wall, further morphologic change cell wall becomes thicker so that there is a change in the space between the cell wall and plasma membrane permeability commonly referred as interference. Permeability disorders can cause cell activity disrupted so that the cells become brittle and eventually die.[9]

Flavonoid compounds can damage the fungal cell by penetrating into the cell membrane, it causes the coagulation of protein (enzyme) in the cell membrane and it affects the protein structure becomes damaged. The instability in the cell wall and cell membrane of fungal cause selective permeability function, activate transport function, and control the composition of proteins from the yeast cells to be disrupted, which can make result a loss of form and lyse cell.[10] Meanwhile, saponin can work as an antifungal by lowering the surface tension of the fungal cell wall that disrupt cell permeability.[11]

The forth chemical compound that contains antimicrobial and antifungal in white cambodia flower extract is alkaloid. Alkaloid is organic compound that exists in nature and it has alkaline characteristic. Olivia et al. states that the alkaloids are active substances from plants that have function as drugs, and have strong activator of the immune cells that destroy bacteria, viruses, fungal and cancer cells. According Aniszewki, the suspected alkaloid mechanism is interfered with DNA chain components in the cell nucleus.[12,13] Microbial DNA

in the cells is damaged by the alkaloids. If the basic groups contact with the microbes, the basic groups will react with the compound of amino acids that make up the cell walls and DNA for making up the cell nucleus. This reaction causes a change in the composition of DNA chain, resulting DNA damage and ultimately lead to lysis. It makes the cell wall layers are not fully formed, and lead to death in these cells.

The effectiveness of tannin, flavonoid, saponin and alkaloid in inhibiting the growth of C. albicans was supported by Arundhina's research using alamanda leaves extract to inhibit the growth of C. albicans and Pityrosporum ovale.[14] In his research, Arundhina explained that Alamanda leaves contain certain chemical compounds that are antifungal such as tannin, flavonoid, saponin, and alkaloid, the same as white cambodia flower which also contain with all four of these compounds.

These compounds are contained in a solution that also can enter the microporosity gap on the surface of soft liner materials. This is also supported by the opinion of Anusavice which states that a fluid can enter the gap on soft liner coating material microporosity diffusion.[15] Diffusion is the ability of a fluid to enter the cavity provided by the use of passive energy.

The high number of C. albicans in the soft liner surface is supported by Listya's research which states that there are differences in the number of C. albicans with the use and without the use of soft liner on acrylic resin plate.[4] The average number of C. albicans on acrylic resin plate with the use of soft liner is higher than without the use of soft liner. C. albicans colonization on a soft liner is due to the saliva and pellicle that is overlying on soft liner in the oral cavity of patients.[16] In fact, C. albicans is the cause due to the use of denture stomatitis that is the most common occurred.[5] To prevent the increase of C. albicans, we need denture cleanser materials to reduce C. albicans colonization in denture mainly covered by soft liner. However, Yilmaz (2004) states that the chemical denture cleanser can increase the hardness surface and water absorption of soft liner itself. Therefore, the existence of alternative denture cleanser from natural ingredients such as white cambodia flower extract that is expected to reduce the number of C. albicans on soft liner denture, so that it can reduce the risk of denture stomatitis.

Adherence of C. albicans on soft liner is influenced by several factors such as surface roughness and chemical constituents of soft liner.[17] Soft liner is encountered in commercial ethanol content that is ranging between 4-20%, although it finds some materials contained ethyl alcohol of more than 40%.[18] Excess ethyl alcohol on soft liner can evaporate. Ethyl alcohol vapor formed will influence the faster compaction process and formed polymer porous as ethyl alcohol vapor is trapped in the solid polymer.[19]

The surface roughness on soft liner is due to the application of soft liner with the gel form that allows the plate surface shape on both sides on the acrylic resin becomes uneven. It is corroborated by the opinions of Lundin and Emilson which states that the rougher or not the surface, it can make the plaque attached more increase.[19]

The attachment mechanism of C. albicans on soft liner can be caused by attachment of C. albicans on the soft liner surface that occurs through hydrophobic interactions. Hydrophobic interactions occur because of C. albicans has hydrophilic relative characteristic

which requires water or saliva that serves as media for a place to live and breed so that it easily attaches to the soft liner materials, while the soft liner has hydrophobic properties.[19]

CONCLUSIONS AND SUGGESTIONS

Based on the research above, it can be seen that the higher concentration of white cambodia flower extract is on the lower concentration of Candida albicans. So that, it can be concluded that white cambodia flower extract is effective in reducing the number of C. albicans.

For those who will continue this research, it is advisable to find out more about the biocompatibility tests of white cambodia flower extract to the oral tissues. Secondly, it is advisable also to study the effectiveness of white cambodia flower extract using other extraction methods.

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