dentika
DENTAL JOURNAL
p-ISSN: 1693-671X e-ISSN:2615-854X
https://talenta.usu.ac.id/dentika

Disinfection Effect of 10% *Ricinus Communis* Oil on *Candida Albicans* Counts of Heat Polymerized Acrylic Resin

(Pengaruh Desinfeksi Minyak *Ricinus Communis* 10% terhadap Jumlah *Candida Albicans* pada Resin Akrilik Polimerisasi Panas)

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Abstract

Dentures is used to replace tooth loss. The most used denture base material is acrylic resin. The biological properties of acrylic resins give microorganisms the ability to colonize. Certain type of microorganism often found at the dentures base is *Candida albicans*. Denture's disinfection is usually used to reduce *Candida albicans*. One of natural source that now is being widely researched is *Ricinus communis* oil. The purpose of this study was to analyze disinfection effect of *Ricinus communis* oil 10% on *Candida albicans* counts on heat polymerized acrylic resin. This research is an experimental laboratory study with a post-test only design. Samples was heat polymerization acrylic resin plates measuring 10 x 10 x 1 mm were made in the Department of Prosthodontic Faculty of Dentistry, Universitas Sumatera Utara with 10 plates for each for 10% *Ricinus communis* oil group and 0.2% *chlorhexidine gluconate* group (control). The *Candida albicans* colonies count was carried out in the Microbiology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara using colony counter. The data obtained was statistically count with the Kruskal-Wallis Test. The result showed the decrease in *Candida albicans* count with the highest value in the 10% *Ricinus communis* oil group is 9 x 100 CFU/ml and the highest value in the 0.2% *chlorhexidine gluconate* group is 0 x 100 CFU/ml. The Kruskal-Wallis test showed significant results with p value of 0.0001 ($p < 0.05$) indicating there was a disinfection effect of 10% *Ricinus communis* oil and 0.2% *chlorhexidine gluconate* on *Candida albicans* count of heat polymerized acrylic resin. The study concluded that 10% *Ricinus communis* oil effective at decreasing *Candida albicans* count.

Keywords: denture bases, *ricinus communis*, *candida albicans*, heat polymerized, acrylic resin.

Abstrak

Pembuatan gigi tiruan merupakan prosedur perawatan kehilangan gigi dimana bahan basis gigi tiruan yang paling sering digunakan adalah resin akrilik. Sifat biologis resin akrilik memberi kemampuan mikroorganisme untuk berkolonisasi. Jenis mikroorganisme yang sering ditemui pada basis gigitiruan adalah *Candida albicans*. Untuk mengurangi jumlah *Candida albicans* diperlukan desinfeksi gigitiruan. Produk yang sedang banyak diteliti di bidang kesehatan adalah minyak *Ricinus communis* (biji jarak). Tujuan penelitian ini adalah mengetahui pengaruh desinfeksi dengan minyak *Ricinus communis* 10% terhadap jumlah *Candida albicans* pada resin akrilik polimerisasi panas. Penelitian ini adalah penelitian eksperimental laboratoris dengan rancangan *post-test only*. Sampel berupa plat resin akrilik polimerisasi panas berukuran 10x10x1 mm dibuat di Departemen Prostodonsia Fakultas Kedokteran Gigi Universitas Sumatera Utara sebanyak masing-masing 10 plat untuk kelompok minyak *Ricinus communis* 10% dan kelompok *klorheksidin glukonat* 0,2% (kontrol). Perhitungan jumlah koloni *Candida albicans* dilakukan di Laboratorium Mikrobiologi Fakultas Matematika dan Ilmu Pengetahuan Alam Universitas Sumatera Utara dengan menggunakan alat penghitung koloni. Data yang diperoleh diuji secara statistik dengan Uji Kruskal-Wallis. Hasil penelitian menunjukkan penurunan jumlah *Candida albicans* dengan nilai tertinggi pada kelompok minyak *Ricinus communis* 10% adalah 9 x 100 CFU/ml dan nilai tertinggi pada kelompok *klorheksidin glukonat* 0,2% adalah 0 x 100 CFU/ml. Uji Kruskal-Wallis diperoleh hasil signifikan dengan nilai $p = 0{,}0001$ ($p < 0{,}05$) yang menunjukkan ada pengaruh desinfeksi dengan minyak *Ricinus Communis* 10% dan *klorheksidin glukonat* 0,2% terhadap jumlah *Candida albicans* pada resin akrilik polimerisasi panas. Kesimpulan penelitian menunjukkan minyak *Ricinus Communis* 10% efektif untuk menurunkan jumlah *Candida albicans*.

Kata kunci: basis gigi tiruan, *ricinus communis*, *candida albicans*, resin akrilik, polimerisasi panas

INTRODUCTION

Denture is an appliance that can replace the function of teeth and the surrounding tissues.[1] Denture base is a part of dentures that contacted with soft tissue. Denture base material can be fabricated from non-metallic materials consisting of thermoset materials such as phenol, volcanoite, formaldehyde and acrylic resin, and thermoplastic materials such as acetone, polycarbonate, and thermoplastic nylon.[2] Denture base type that most frequently used is heat polymerization acrylic resin.[3]

Candida albicans are fungus found in the oral cavity in about 45-65% healthy individuals. The frequency of *Candida* increases in 60-100% of.[4] The growth of *Candida albicans* can be slowed down by the administration of antifungal.[5] Natural properties of acrylic resin give certain microorganisms the ability to colonize on denture base surface of acrylic resin denture. Those commonly found microorganism in denture base is *Candida albicans*.[6]

Dentures disinfection methods can be classified as mechanical, chemical or by combination. Chemical disinfection with chemical solution is proven to be better for denture wearers.[7] *Chlorhexidine* is a commonly used and easy-to-obtain disinfection but has an unpleasant smell and taste, bleaching effects and biological side effects are not yet known.[8]

Vast natural ingredients that are utilized as main source of traditional medications mostly derived from numerous natural resources, including plants which are being employed as a primary source in folkloric medicine.[9] One of the natural products that is commonly being studied in areas of medicine is oil from *Ricinus communis* which also generally recognized as castor oil. It is small plant that has soft wood which grow in region with tropics and warm climate. The tree is discovered to display antimicrobial activity and has been used to treat several ailments.[10]

The purpose of this study was to analyze disinfection effect of 10% *Ricinus communis* oil on *Candida albicans* count of heat polymerized acrylic resin.

MATERIALS AND METHODS

This research was experimental study with posttest only design to analyze *Candida albicans* count oil on heat polymerized acrylic resin after disinfection with 10% *Ricinus communis* and *chlorhexidine gluconate*. The research was conducted in the Department of Prosthodontic, Faculty of Dentistry, and Microbiology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara. The research was conducted from August to October 2020.

The sample in this study was a heat acrylic polymerization resin plate with a size of 10x10x1 mm made following ISO Specification, 2001 (Figure 1). The total of samples in this study was calculated using formulation for simple randomized group experimental research. After the calculation, the total of samples was 8.5 plates. As a consideration if there was any damage of the plate the number of samples was rounded up to 10 plates. The sample consists of 2 groups, the treatment group (10% *Ricinnus communis* oil) and control group (0.2% *chlorhexidine gluconate*).

Suspensions containing *Candida albicans* are made by taking 1-2 inoculating loop pure breeds of *Candida albicans* that have been cultured then mixed with a 0.9% NaCl solution until it in accordance with Mac Farland's standards with a comparison of *Candida albicans* count 1 x 108 CFU/ml. Plate was sterilized using autoclave at 121°C for 1 hour and then contaminated with *Candida albicans* by swabbing. Plate was then immersed in a glass containing 10% *Ricinus communis* oil and 0.2% *chlorhexidine gluconate*. The plate was disinfected for 20 minutes. After that, the plate is rinsed with Phosphatase Buffered Saline twice and inserted into a test tube containing 10 ml of 0.9% NaCl solution then vibrated using a Vortex vibration tool for 30 seconds to release *Candida albicans* attached to the plate. Furthermore, 0.1ml of 0.9% NaCl solution is taken from the test tube using micropipette and then grown on the Plate Count Agar by dripping and spreading the liquid evenly across the surface, then incubated for 24 hours at a temperature of 37°C. *Candida albicans* count was carried out after 24 hours using a colony counter in colony forming units per cubic millimeter (CFU/ml) in 100 ml.

Data is processed using computer programs with IBM SPSS Statistics Version 20. The data analysis consist of univariate and bivariate analysis. The univariate analysis was used to analyze the average and standard deviation of each group. Bivariate analysis used Kruskal-Wallis test to analyze the disinfection effect of 10% *Ricinus communis* oil and 0.2% *chlorhexidine gluconate* on *Candida albicans* count after immersion.

RESULTS

Measurement of *Candida albicans* count was carried out after the plate of heat polymerized acrylic resin was disinfected for 20 minutes with 10% *Ricinus communis* oil and 0.2% *chlorhexidine gluconate*. Ta-

ble 1 showed *Candida albicans* count in 10% *Ricinus communis* oil group with the lowest count was 0 x 100 CFU/ml and the highest count was 9 x 100 CFU/ml. *Candida albicans* count in 0.2% *chlorheksidin gluconate* group with the lowest and highest count was 0 x 100 CFU/ml.

Table 2 showed the average value and standard deviation in 10% *Ricinus communis* oil group was 2.50 ±3.659 and 0.2% *chlorhexidine gluconate* group was 0 ± 0. Normality test were conducted on *Candida albicans* count to determine the normal distribution of data. The data showed an abnormal distribution because there was no variation in *Candida albicans* count of 0.2% *chlorhexidine gluconate* group, so to determine the disinfection effect of heat polymerization acrylic resin on *Candida albicans* count was further analyzed using nonparametric tests (Kruskal-Wallis test). Based on the results, it was known there was significant difference with the p value of 0.0001 ($p < 0.05$) indicating that there was disinfection effect of 10% *Ricinus communis* oil and 0.2% *chlorheksidin gluconate* on *Candida albicans* count of heat polymerized acrylic resin (Table 2).

DISCUSSION

Acrylic resin is the most frequently used denture base material because it has several advantages such as aesthetic quality, good colour stability, non-irritating, non-toxic, relatively cheap, low residual monomer, small porosity, as well as easy processing, manufacture and repair.[3] However, the biological properties of acrylic resins can make denture base absorb fluids and correspond to the ability of certain microorganisms to colonize the surface of denture, for example *Candida albicans*, especially in patients with poor oral hygiene.[6]

In addition, the denture wearing can decrease the flow of oxygen and saliva to the soft tissues underneath the denture base, causing the oral cavity environment to become acidic and anaerobic which favour the excessive growth of *Candida albicans*.[4] *Candida albicans* is a fungus commonly discovered in the human's oral cavity. *Candida albicans* induced dentures stomatitis is a quite common inflammatory process.[11] Among denture wearers, the prevalence of denture stomatitis can be near 70%, and wearing dentures at night greatly increases the odds to experience fungal infection.[12]

There are several methods of disinfection that can be used to eliminate biofilms.[13] One of them is a chemical method. Disinfection with chemical solutions proved better for denture wearer.[14] The advantages of chemical methods are the full accessibility of chemical solutions on all denture surfaces, minimal damage, minimal abrasion, and easy methods for daily use.[13] Overnight *chlorhexidine* and chlorine-based soaks have demonstrated efficacy, but the American College of Prosthodontists does not recommend soaking dentures in sodium hypochlorite more than 10 minutes. *Chlorhexidine* can also be utilized but may discolour the denture. Delayed hypersensitivity reactions *chlorhexidine* mouthwashes have been reported, but such reactions are rare.[12]

Majority of plants have medical property such as antimicrobial so it can be used for alternative disinfection material.[8] *Ricinus communis* is a medicinal plant that has been used as therapeutic agent for 4000 years and used as an herbal medicine for treating many different diseases, disorders, and many infections. Some scholars believe *Ricinus communis* came from Tropical Africa.[9] This plant is mainly grown in Africa, South America, and India. In Indonesia, this plant is commonly planted in Java and Madura.[15]

There are numerous uses of *Ricinus communis* plant for medicinal purposes which utilizes every part of the plant including roots, seeds, bark, leaves, flower, fruit, and stem. *Ricinus communis* also identified as castor oil plant due to the large quantity of oil extracted from the seeds. Castor oil is of great application in various perspectives which is use as an herbal medicine and as a conventional therapy for various ailments. The various biological activities of *Ricinus communis* is due to the presence of a varied degree of bioactive phytochemicals, which are steroids, terpenoids, saponins, alkaloids, flavonoids, and glycosides. Presently, the properties of *Ricinus communis* mentioned including antimicrobial and antifungal.[9] In dentistry, compounds made from *Ricinus communis* have been used, because of their biocompatibility and anti-inflammatory activity, as well as their bactericide and fungicide action.[7] Furthermore, the roots are used for various purposes such as a powerful purgative, for a toothache, and this can be possible when the roots are administered in the form of decoction and paste.[9] *Ricinus communis* oil has been tested as an irrigation solution for endodontic treatment and has demonstrated biocompatibility to periapical tissue, as well as antimicrobial, bactericide, and anti-inflammatory activity.[8] A study evaluated a 3.3% castor oil detergent and showed antimicrobial activity against anaerobes and streptococci in pulp necrosis. A castor oil-based solution has been used for denture hygiene because this oil has potent detergent and antimicrobial action, colourless and has no unpleasant odor.[7]

Few studies have been conducted regarding the efficacy of castor oil as complete denture cleansers. In

relation to adverse effects, previous studies evaluated 2% solution and showed no significant changes in surface roughness, hardness and colour of artificial teeth and acrylic resin. Clinical trials also showed that 2% castor oil immersion solution was effective in denture biofilm removal, compared with an alkaline peroxide solution, but its antimicrobial action was not evaluated. However, this treatment did not significantly reduce the CFU of *Candida* spp.[7] The results of this study in 10% *Ricinus communis* oil group showed a decrease in *Candida albicans* count. Another study reported the effectiveness of 10% *Ricinus communis* oil detergent on the irrigation of root canals. Moreover, the literature has not shown adverse effects in a 10% concentration.[7] The antifungal activity of *Ricinus communis* comes from various parts of this plant such as root, leaf, and stems. *Ricinus communis* is known to be active against fungi in both methanolic and aqueous extracts form. The research conducted to study the antifungal activity of *Ricinus communis* extract against various fungal species showed maximum antifungal activity against *Candida albicans*.[9] According to Pisani et al., the action of detergent in *Ricinus communis* is associated with the cell walls damage of microorganisms which makes the cytoplasm lose its components and causes cell death.[8] The results of in vitro study by Salle et al. in 2015 showed that immersion of dentures in 10% *Ricinus communis* oil for 20 minutes can eliminate microorganisms, such as *Escherichia coli*, *Candida albicans* and *Candida glabrata*.[7] In addition, research by Kavita in 2018 that compared the effectiveness of 10% *Ricinus communis* oil and sodium hypochlorite on *Candida albicans* count of acrylic resin denture base showed that 10% *Ricinus communis* oil effectively decreased *Candida albicans* count.[16] The above-mentioned studies confirm the antifungal activity of R. communis extract, and it may be a good source for identifying a new drug candidate for inhibiting the fungi.[9]

The results of this study showed that there was no growth of *Candida albicans* in 0.2% *chlorhexidine gluconate* group. *Chlorhexidine* is a chemical antiseptic broad-spectrum activity that can eliminate several types of microorganisms, including *Candida albicans*.[17] *Chlorhexidine* will bond with fungal cell membrane lead to a change in integrity of the fungal cell wall consisting of lipids causing lipophilic chlorophenol ring to enter the wall of fungal cell and causing leakage of intracellular component so that fungal cell membrane will damage.[18] *Chlorhexidine* is widely used as several purpose such as disinfection of dentures, mouthwash solution, and oral candidiasis treatment. *Chlorhexidine* is not marketed specifically as a denture disinfectant, but from various research results showed that *chlorhexidine* effecttively prevents and treat infections caused by dentures.[17] According to Chetan et al in 2011 the use of 0.2% *chlorhexidine* mouthwash as a disinfectant of dentures used for 30 minutes, can reduce 9% *Candida albicans* count in full denture wearer.[18]

The conclusion of this study showed that 10% *Ricinus communis* oil was effective for lowering the *Candida albicans* count. Ten percent *Ricinus communis* oil has the similar effect as 0.2% *chlorhexidine gluconate* and almost had the same disinfection effect as *chlorhexidine* which is a gold standard disinfectant. It is concluded that 10% *Ricinus communis* oil can be used as a denture disinfectant.

Acknowledgements

This research was funded by the Research Institute, Universitas Sumatera Utara, through the TALENTA Program, Universitas Sumatera Utara 2020 (contract number: 347/UN5.2.3.1/PPM/SPP-TALENTA USU/2020). We would like to thank Department of Prosthodontic, Faculty of Dentistry, and Microbiology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara that has great contribution to this research.

TABLES

Table 1. Average *Candida albicans* count on heat polymerized acrylic resin after disinfection using 10% *Ricinus communis* oil and 0.2% *chlorhexidine gluconate*.

Immersion	Total Plate	Average *Candida albicans* Count (x 100 CFU/ml)
10% *Ricinus communis* Oil	10	2.5
0.2% *Chlorhexidine gluconate*	10	0

Table 2. Disinfection effect of 10% *Ricinus communis* oil and 0.2% *chlorheksidin gluconate* on *Candida albicans* count of heat polymerized acrylic resin.

Group	*Candida albicans*		p
	N	$\bar{x} \pm SD$	
10% *Ricinus communis* Oil	10	2.50 ± 3.659	0.0001*
0.2% *Chlorhexidine gluconate*	10	0	

Note: *Significant difference

FIGURES

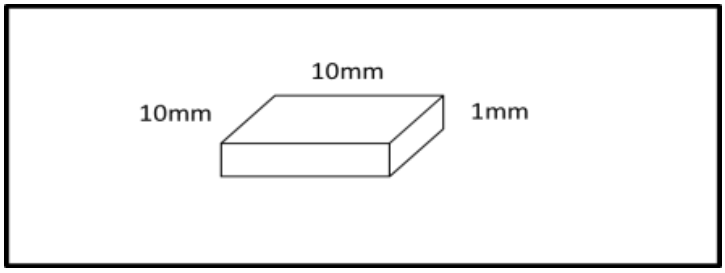

Figure 1. Hot polymerized acrylic resin plate.

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