



THE EFFECT OF VARIOUS CONCENTRATIONS OF LIQUID SMOKE WITH TIME DIFFERENCES ON THE VIABILITY OF *CANDIDA ALBICANS*

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ABSTRACT

Background: Dentures that are rarely cleaned will trigger the growth of *Candida albicans*, which is a fungus known to be a major cause of opportunistic infections of the oral cavity. Liquid smoke is a chemical produced by distillation of smoke from combustion, containing phenol, carbonyl and carboxylic acids that are effective in killing fungi. **Objective:** To determine the effect of various concentrations of liquid smoke with different periods of time on the viability of *Candida albicans*. **Methods:** This was an experimental laboratory research with post-test only control group design. *Candida albicans* colonies as samples divided to 6 different treatment concentrations of liquid smoke and different periods of time (5 and 15 minutes), with 5 duplications. The growth of *Candida Albicans* colonies were assessed by MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration). **Results:** The Kruskal-Wallis test on the MIC showed that there are significant differences ($p < 0.001$) in the experimental groups, while the test on the MBC also showed the same result ($p < 0.001$). The Mann-Whitney test stated that there was a significant difference between liquid smoke concentrations of 12.5% with positive control. The lowest concentration that could inhibit the growth of *Candida albicans* is 12.5%. At 24 hours incubation with 5 and 15 minute soaking time, the MBC of *Candida albicans* was 100% with 15 minute soaking time. **Conclusions:** Liquid smoke affected the growth of *Candida albicans*, characterized by MIC at a concentration of 12.5% and MBC at a concentration of 100% with a 15-minute soaking time.

Keywords: Liquid smoke, oral candidiasis, *Candida albicans*, Minimum Inhibitory Concentration, Minimum Bactericidal Concentration.

INTRODUCTION

Oral and dental health is one of the most important and fundamental parts of general body health. If the quality of oral and dental health is bad, it can have an adverse effect on the individual. Dental and oral health can also decrease along with the aging process such as cell degeneration, decreased the thickness of the oral mucosa, and systemic changes that have a direct effect on oral and tissues and physiological processes which leads to various changes in the periodontal.¹ Some conditions that often occur in the elderly oral cavity are periodontitis, gum disease, dry mouth/xerostomia, and tooth loss.²

Tooth loss is a process in which the tooth comes loose and fall out by extraction due to caries, periodontal disease, and trauma. Tooth loss causes a decrease in masticatory function, affect the oral cavity and general health, and affect someone's quality of life. Ideally, a replacement for the tooth must be replaced by anticipating various problems that might occur. In Indonesia, the percentage of denture users reaches 4.5 %.^{3,4}

Dentures are teeth that are used to replace missing teeth and restore dental function in terms of

beauty, sound, mastication, and swallowing. This denture is made of acrylic resin or a combination of acrylic-metal resin. The advantage of acrylic resin is that it is lightweight, inexpensive, having the same color as the color of gingiva, easy to make, and easy to do. The properties of the acrylic resin are stable, non-irritating, non-toxic, and can be easily manipulated. However, acrylic resins also have disadvantages of having micropores in order to facilitate food waste and bacteria entering to them.³ Dentures used continuously and rarely cleaned cause a buildup of food waste which predisposes to plaque attached to the surrounding teeth, dentures, antagonistic teeth, and denture bases that cover the mucosa. Therefore, it can trigger fungal growth. Other factors such as lack of saliva, low pH, anaerobic environment and low level of oxygen will slightly increase trauma or mechanical irritation of the oral mucosa under dentures which leads to low epithelial defense against fungal invasion such as *Candida albicans*.⁵

Candida albicans is a pathogenic fungal species from the *Deuteromycota* group. This fungus species is a cause of opportunistic infections called candidiasis and can be found on the skin, mucosa,



and organs in humans. Some characteristics of this species are egg-shaped (ovoid) or spherical with the diameter of 3-5 μm , able to produce pseudohyphae. *Candida albicans* has two types of morphology namely hyphae and yeast. This fungus is able to attach to the host cell and colonize.^{6,7}

Although it is a normal commensal flora in the oral cavity, gastrointestinal system, and vagina, in certain circumstances, *C. albicans* is capable of causing damage (or disease) that leads to oral, vaginal, systemic, or skin candidiasis. In pathogenic conditions, *C. albicans* acts as a variety of infections in the mucosa or systemic as well as in immunocompetent and immunocompromised individuals.^{8,9}

Liquid smoke is a mixture of solution and colloidal dispersion from wood smoke vapor obtained from the results of pyrolysis with a temperature of 400°C. Some stages of making liquid smoke are pyrolysis, condensation, and redistillation. The quality, composition, and components contained in liquid smoke are influenced by the type of raw material used. The main components in liquid smoke consist of acids, phenol derivatives, and carbonyl. These chemical elements can act as flavorers, color-forming, antibacterial, antifungal, and antioxidants.^{10,11} Based on this description, a study was conducted which aimed to determine the effect of liquid smoke on *Candida albicans* as one of the medical alternatives.

METHOD

This study was an experimental laboratory with posttest only controls group design. It was carried out at the Microbiology Laboratory of the Faculty of Medicine, Diponegoro University, Semarang, in May 2018.

The sample of this study included the *Candida albicans* colonies obtained from Microbiology Laboratory of the Faculty of Medicine of Diponegoro University, Semarang. The duplication amount was known by Federer's formula and the number of replication was 5 times. The inclusion criteria were *Candida albicans* colonies that grew on Sabouraud Dextrose Broth (SDB) and Sabouraud Dextrose Agar (SDA) media after being exposed to the treatment. Exclusion criteria were colonies of *Candida albicans* which grew on SDB and SDA media with the growth of fungi or other contaminants.

The independent variable in this study was the concentration of liquid smoke and time difference. Meanwhile, the dependent variable in this study was the growth of *Candida albicans* colonies.

In this study, Kruskal Wallis nonparametric test was carried out followed by the Mann Whitney test. H_0 is rejected if the value of significance is <0.05 at the 95% confidence interval. Data processing was done by SPSS for Windows 21.

RESULTS

The data were collected based on experimental tests conducted by researchers at Microbiology Laboratory of the Faculty of Medicine of Diponegoro University. The data obtained were primary data in form of the growth of *Candida albicans* colonies on SDB and SDA media from the treatment and control groups that have met the inclusion and exclusion criteria. From the results, it can be inferred that in the first until fifth repetition, P1-P4 are clear means there are no colonies of *Candida albicans*, while P5 is turbid. This proved that minimum concentration of liquid smoke solutions which is able to inhibit the fungus is 12.5%.

Meanwhile, the 15th minute of the first until the fifth repetition of culture in the P1 tube there was no growth of *Candida albicans* in SDA media. Meanwhile, culturing in the 5th minute of the P1 tube and in the 5th and 15th minute of P2-P5 tube found the colony of *Candida albicans* in SDA media.

The result of the Kruskal-Wallis nonparametric test is $p < 0.05$. Therefore, that $p < 0.05$ showed a significant difference or significant inhibitory concentration which inhibit the growth of *Candida albicans* at least in two groups of liquid smoke concentration. Since the Kruskal-Wallis test showed significant results, the Mann Whitney test was used to determine the differences between treatments.

The Kruskal Wallis nonparametric test results from Table 1 presented a significant difference in the experimental group. In the Mann-Whitney test, the results obtained at P1 (100%) to P4 (12.5%) differences were not significant or did not have any significant difference ($p > 0.05$) to the negative control (formalin). However, it showed a significant difference ($p < 0.05$) for the positive control (SDB) (Table 2). Thus, this showed that that



liquid smoke with a concentration of 12.5% - 100% can inhibit the growth of *Candida albicans* with the same ability as negative controls containing formaldehyde. Therefore, it can be concluded that the Minimum Inhibitory Concentration in this study is P4 with a concentration of 12.5%.

Table 1. The Recapitulation of Kruskal Wallis Test Results on MIC Analysis.

Group	Turbidity				p
	Clear		Turbid		
	n	%	n	%	
P1	5	100	0	0	<0,001*
P2	5	100	0	0	
P3	5	100	0	0	
P4	5	100	0	0	
P5	0	0	5	100	
KS	5	100	0	0	
K+	0	0	5	100	
K-	5	100	0	0	

Description: * Significant

Table 2. The Recapitulation of Mann-Whitney Test Results on MIC Analysis.

Turbidity	P2	P3	P4	P5	KS	K+	K-
P1	1.00 0	1.00 0	1.00 0	0.003 *	1.000	0.003 *	1.000
P2	-	1.00 0	1.00 0	0.003 *	1.000	0.003 *	1.000
P3	-	-	1.00 0	0.003 *	1.000	0.003 *	1.000
P4	-	-	-	0.003 *	1.000	0.003 *	1.000
P5	-	-	-	-	0.003 *	1.000	0.003 *
KS	-	-	-	-	-	0.003 *	1.000
K+	-	-	-	-	-	-	0.003 *

Description: * Significant

Table 3. The Recapitulation of Kruskal Wallis Test Results in the 5th Minute.

Group	5 minute				p
	Grow		Not Grow		
	n	%	n	%	
P1	5	100	0	0	<0.001*
P2	5	100	0	0	
P3	5	100	0	0	
P4	5	100	0	0	
P5	5	100	0	0	
K+	5	100	0	0	
K-	0	0	5	100	

Description: * Significant

The Kruskal Wallis test results in the MBC analysis in the 5th minute (Table 3) showed a difference ($p < 0.05$) in the experimental group. The results on the Mann-Whitney statistical test in Table 4 show that P1 (100%) to P5 (6.25% differ significantly ($p < 0.05$) with negative controls but have no significant difference ($p > 0.05$) with positive control. This means that liquid smoke concentrations of 6.25% - 100% with 5 minute soaking time did not have the ability to kill *Candida albicans* like negative controls.

Kruskal Wallis test results with the soaking time of 15 minute (Table 5) show the significant difference ($p < 0.05$) on the experimental group. Based on the Mann-Whitney test results presented in Table 6, it is obtained the P1 data (100%) did not differ significantly or did not have any significant difference ($p > 0.05$) on negative controls. However, it differed significantly ($p < 0.05$) on positive controls. Whereas P2 (50%) to P5 (6.25%) had a significant difference on negative controls, but not significantly different from positive controls. This shows that liquid smoke with a concentration of 100% with a soaking time of 15 minute has the same ability as formalin in killing *Candida albicans*. Therefore, it can be concluded that MBC in this study was P1 (100%) with a 15 minute soaking time.

Table 4. The Recapitulation of Kruskal Wallis Test Results in the 5th Minute of MBC Analysis.

5 minute	P2	P3	P4	P5	K+	K-
P1	1.000	1.000	1.000	1.000	1.000	0.003*
P2	-	1.000	1.000	1.000	1.000	0.003*
P3	-	-	1.000	1.000	1.000	0.003*
P4	-	-	-	1.000	1.000	0.003*
P5	-	-	-	-	1.000	0.003*
K+	-	-	-	-	-	0.003*

Description: * Significant

Table 5. The Recapitulation of Kruskal Wallis Test Results in the 15th Minute.

Group	15 minute				p
	Grow		Not Grow		
	n	%	n	%	
P1	0	0	5	100	<0.001*
P2	5	100	0	0	
P3	5	100	0	0	
P4	5	100	0	0	
P5	5	100	0	0	
K+	5	100	0	0	
K-	0	0	5	100	

Description: * Significant



Table 6. The Recapitulation of *Mann-Whitney* Test Results in the 15th Minute of the MBC Analysis.

15 minute	P2	P3	P4	P5	K+	K-
P1	0.003*	0.003*	0.003*	0.003*	0.003*	1.000
P2	–	1.000	1.000	1.000	1.000	0.003*
P3		–	1.000	1.000	1.000	0.003*
P4			–	1.000	1.000	0.003*
P5				–	1.000	0.003*
K+					–	0.003*

Description: * Significant

DISCUSSION

Coconut Shell liquid smoke comes from the pyrolysis process and smoke condensation results from the burning process of coconut shells. Liquid smoke is known to have high polyphenol content. Apart from acting as an antioxidant, polyphenols are also good antimicrobials.¹² The phenol in liquid smoke acts as an antifungal by inhibiting the nucleic synthesis of fungi. This phenol group can also disrupt cell membrane stability and fungal cell metabolism in order to cause disruption of fluid exchange in cells. In addition, the antifungal benefits of phenol can cause damage to the cell wall by shrinking the cell walls. Thus, disrupting cell wall permeability and resulting in inhibited fungal cell growth.¹³ This is consistent with the research conducted by Wedhayanti (2016) on the Effect of Liquid Smoke Concentration as Disinfectant solution to decrease *Candida albicans* in which phenol in liquid smoke with a concentration of 2% has an effect on the decrease in growth of *C. albicans*.

The difference between this study and previous study regarding the concentration of liquid smoke which can inhibit the growth of *C. albicans* lies in the concentration of phenol contained in liquid smoke. In a study conducted by Nadzifun (2015) on the Effectiveness Test of Coconut Shell Liquid Smoke Concentration as a Bioactive Material in Recycled Paper Making, it was found that only 0.2% liquid smoke inhibited fungal growth due to the phenol content in the liquid

smoke which was 5.15%.¹¹ In addition, in a study conducted by Amperawati (2012) on the Inhibition of Coconut Shell Liquid Smoke on Fungal Growth in Copra during Drying Process and the Quality of Oil Produced, it was found that a concentration of 10% with a soaking time of 15-minute could inhibit fungal growth by 0% or not grow at all. That was because the phenol contained in liquid smoke was 12.29%.¹⁴

In this study, the concentration of phenol contained in liquid smoke was only 0.21%. Therefore, it was found that liquid smoke at a concentration of 12.5% could only inhibit the growth of fungus namely *Candida albicans*. Meanwhile, at a concentration of 100% with a 15 minute soaking time, it could kill *Candida albicans*. From the above explanation, it can be concluded that the concentration of phenol contained in liquid smoke affects the growth of *Candida albicans*. In later studies, it can be used in the treatment of dentures as a medium for the growth of *Candida albicans* that causes oral candidiasis.

CONCLUSION

Based on the results of the study, it can be concluded that liquid smoke with certain concentration and immersion time affected the growth of *Candida albicans*. The minimum inhibitory concentration for *C. albicans* was 12.5%. Meanwhile, liquid smoke with a concentration of 100% (0.21 % phenol content) with 15-minute soaking time could kill *Candida albicans*. Further study needs to be done on the effect of liquid smoke immersion on denture resin-acrylic material. It is



important to do further research in order to make liquid smoke can be used as a safe antifungal for the causes of oral candidiasis in specifically for the patient with dentures.

REFERENCES

1. Bianchi CMP de C, Bianchi HA, Tadano T, Depaula CR, Hoffmann-Santos HD, Leite DP, et al. Factors Related to Oral Candidiasis in Elderly Users and Non-users of Removable Dental Prostheses. *Revista do Instituto de Medicina Tropical de Sao Paulo*. 2016;58:6–10.
2. Senjaya A. *Gigi Lansia (Teeth on the Elderly)*. *J Skala Husada Vol*. 2016;13:72–80.
3. Widyaningrum, Sheina. Suhartiningtyas D. *Gambaran Oral Kandidiasis Pada Pengguna Gigi Tiruan Lepas Berbasis Resin Akrilik di Yogyakarta (Overview of Oral Candidiasis in Removable Denture Users Based on Acrylic Resin in Yogyakarta)*. 2016.
4. Rahman F, Saputera D, Adhani R. *Faktor yang Mempengaruhi Permintaan Gigi Tiruan pada Lansia (Tinjauan Terhadap Biaya Perawatan, Kecemasan dan Sarana) (Factors Affecting Demand for Dentures in the Elderly (Review of Treatment Costs, Anxiety and Means))*. *Stomatognathic (J K G Unej)*. 2016;13:5–11.
5. Hamrun N, Mahardhika A. *Jumlah Koloni Candida albicans pada Pemakai Gigi Tiruan Penuh Berdasarkan Lama Pemakaian (Number of Candida albicans Colonies in Full Denture Wearers Based on Usage Time)*. *Faculty of Dentistry, Hasanuddin University, Makassar*. Vol 3, No 3 - June 2014. 2014;1–4.
6. Fridayanti CMA, Priyanto D, Isbandrio B. *Pengaruh Paparan Asap Terhadap Jumlah Candida di Rongga Mulut (Studi Pada Pekerja Pengasapan Ikan di Desa Bandarharjo, Kota Semarang, Jawa Tengah) (The Effect of Smoke Exposure on the Number of Candida in the Oral Cavity (Study of Fish Smokers in Bandarharjo Village, Semarang City, Central Java))*. *J Media Medika Muda*. 2014.
7. Nagao K, Udey MC. *Mushrooming Insights into Skin Dendritic Cell Physiology*. *Immunity*. 2015;42:210–3.
8. Berman J, Sudbery PE. *Candida albicans: A Molecular Revolution Built on Lessons from Budding Yeast*. *Nature Rev Microbiology*. 2002;3:918–30.
9. Jabra-Rizk MA, Kong EF, Tsui C, Nguyen MH, Clancy CJ, Fidel PL, et al. *Candida albicans Pathogenesis: Fitting Within the Host-Microbe Damage Response Framework*. *Infection and Immunity*. 2016;84:2724–39.
10. Ayudiarti DL. *Asap Cair dan Aplikasinya pada Produk Perikanan (Liquid Smoke and its Application to Fishery Products)*. *Balai Besar Riset Pengolahan Produk dan Bioteknologi Kelautan dan Perikanan*. 2010;5:101–8.
11. Nadzifun MK, Musholaeni W, Sasongko P. *Uji Efektivitas Konsentrasi Asap Cair Tempurung Kelapa sebagai Bahan Bioaktif pada Pembuatan Kertas Daur Ulang (Effectiveness Test of Coconut Shell Liquid Smoke Concentration as a Bioactive Material in Recycling Paper Making)*. 2015.
12. Kailaku S, Syakir M, Mulyawanti I, Syah A. *Antimicrobial Activity of Coconut Shell Liquid Smoke*. *IOP Conference Series: Materials Science and Engineering*. 2017;206:012050.
13. Fakhrurrazi, Fanani Hakim R, Keumala CN. *Pengaruh Asam Jawa (Tamarindus indica Linn) terhadap Pertumbuhan Candida albicans (Effect of Tamarind (Tamarindus indica Linn) on the Growth of Candida albicans)*. *J Syiah Kuala Dentistry Society*. 2016;1:96–102.
14. Amperawati S, Darmaji P SU. *The Effect of Coconut Shell Liquid Smoke on the Growth of Fungi during Copra Drying*. *Agritech*. 2012;32:191–8.