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Research Report

Candida albicans adherence on acrylic resin plates immersed in black tea steeping

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ABSTRACT

Background: *Black tea or Cournelia sinensis is one of known tea varieties in Indonesia. Actually, black tea is consumed daily as beverage that can function as antiseptics and fungicides. Black tea containing antibacterial and fungicide properties can reduce the number of Candida albicans (C. albicans) colony attachment on the surface of acrylic resin plates.* ***Purpose:*** *This study was done to determine the effective concentration of black tea steeping used as the immersion material of acrylic resin plates towards the number of Candida albicans colonies.* ***Methods:*** *In this study, acrylic resin plates with the size of 10 × 20 × 1 mm were immersed in black tea steeping with the following concentrations, 3.33%, 6.66%, and 13.33%, for one hour. The growth of C. albicans colonies were then detected by counting the number of colonies growing on Sabouraud's dextrose medium. Data was analyzed by using both One-Way ANOVA with the significance level of 5%, and Tuckey's multiple comparison tests (Tuckey's HSD test).* ***Results:*** *There were significant differences in the number of C. albicans colonies in acrylic resin plates immersed in black tea with the concentration of 3.33%, 6.66%, and 13.33%.* ***Conclusion:*** *It can be concluded that the higher concentration of black tea (13.33%) used to immerse acrylic resin plates, the greater the decreased number of C. albicans colonies.*

Key words: *Black tea, Candida albicans, acrylic resin plate*

ABSTRAK

Latar belakang: *Teh hitam atau Cournelia sinensis adalah salah satu macam teh yang dikenal di Indonesia.* ***Teh hitam seringkali*** *dipakai sebagai minuman sehari- hari dan berkhasiat sebagai antiseptik maupun fungisid. Teh hitam yang mengandung sifat antibakteri dan fungisid dapat menurunkan jumlah koloni Candida albicans (C. albicans) yang melekat pada permukaan lempeng resin akrilik.* ***Tujuan:*** *Untuk mengetahui konsentrasi efektif seduhan teh hitam sebagai bahan perendam lempeng resin akrilik terhadap jumlah koloni C. albicans.* ***Metode:*** *Pada penelitian ini lempeng resin akrilik ukuran 10 × 20 × 1 mm direndam dalam seduhan teh hitam konsentrasi 3,33%, 6,66%, 13,33% selama 4 jam. Pertumbuhan jumlah koloni C. albicans dilakukan dengan cara menghitung jumlah koloni yang tumbuh pada media Sabouraud's dextrose.* ***Analisis data menggunakan uji anova satu arah dengan taraf kemaknaan 5%,*** *uji pembanding ganda tuckey HSD.* ***Hasil:*** *Terdapat perbedaan yang bermakna jumlah koloni C. Albicans yang melekat pada lempeng resin akrilik yang direndam dalam seduhan teh hitam dengan konsentrasi 3,33%, 6,66% dan 13,33%.* ***Kesimpulan:*** *Konsentrasi 13,33% atau makin pekat seduhan teh hitam yang digunakan untuk merendam lempeng resin akrilik menyebabkan penurunan jumlah koloni C. albicans.*

Kata kunci: *Teh hitam, Candida albicans, lempeng resin akrilik*

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INTRODUCTION

Denture base material commonly used is polymethyl methacrylate resin. The properties of the heat cured acrylic resin are non-toxic, easy manipulated, non-irritating, undissolved in oral liquid though soluble in water, full of aesthetics, look-alike gingiva color, stable, repairable, has small dimensional changes, relatively cheaper, and commonly used.[1]

Wearing of dentures will make the mucosa covered with removable denture for a long time so that it can block either the mucosal surface or tooth surface from being cleaned by the tongue and saliva. As a result, it can cause the formation of denture plaque.[2,3] Microbial plaque formed on the denture surface facing the mucosal surface is considered as a factor stimulating the pathogenesis of the denture stomatitis. It even has been reported that 65% of users suffer from denture stomatitis.[4] Food accumulation and dental plaque will increase the frequency and density of *C. albicans*. In other words, denture stomatitis caused by the dentures is related to the proliferation of *C. albicans* on the plaque attaching to the dentures. The infection of *C. albicans* is significantly reported as the cause of denture stomatitis.[2] The prevention of denture stomatitis is essential, including the elimination of *C. albicans*. Nevertheless, the infection of *C. albicans* can be prevented by cleaning and removing dentures at night.[5]

The cleaning of the dentures can be conducted in two ways, mechanical and chemical. The mechanical cleaning can be conducted with a toothbrush or ultrasonic equipment, while the chemical cleaning can be conducted by immersing the dentures in the cleaning solution.[4]

Actually, tea can be used as the immersion material of acrylic resin plate since tea is considered as antiseptics. One kind of tea known in Indonesia is *Camellia sinensis* or black tea.[6] Black tea is often used as a beverage since it is cheap, easily obtained, and considered as disinfectants. These disinfectant properties are generated by the content of fluoride, polyphenols, catechins and other oxidated derivatives.[7] However, the optimal level of black tea steeping is determined by the optimal proportion between the amount of tea and water used for steeping the tea. Generally, the standard proportion of black tea steeping is 20 grams of black tea and 300 ml of water equivalent to the concentration of 6.66%.[8]

For those reasons, it becomes essential to know the concentration of the steeping black tea that can effectively be used to reduce the number of *C. albicans* colonies. The study is then aimed to determine the effective concentration of black tea steeping used as the immersion material of acrylic resin plates towards the number of *C. albicans* colonies.

MATERIALS AND METHODS

This study is considered as a laboratory experiment with factorial design. The materials used are heat cured acrylic resin, black tea as immersion solution, and the suspension of *C. albicans.* The sample used, moreover, is a box-shaped acrylic resin with the size of 10 × 20 × 1 mm. And, the location of the study is in Microbiology Laboratory and Materials Department of Dentistry, Faculty of Dentistry.

To make the suspension, *C. albicans* was put in 5 ml of Sabouraud's broth medium, and was incubated for 48 hours at 37° C. One oose of the suspension was taken and put into 5 ml of Sabouraud's broth, and it was then incubated for 48 hours at 37° C in order to be used to contaminate with acrylic resin plates (10 × 10 × 1 mm). Sterile saliva was obtained from a person without any stimulation, and it was then disentrifuged for 20 minutes at 2000 rpm.[9] Finally, the saliva supernatan was put into 5 cc syringe of injection, and it was then filtered with 0.2 µm millipore unit of filter mounted on the syringe needle.[10]

The attachment of C. *albicans* on acrylic resin plates was measured through the following procedures. Acrylic resin plates were immersed in water for 48 hours in order to reduce the residual monomer.[11] After that, the acrylic resin plates were sterilled by using autoclave at 121° C for 18 minutes.[12] Then, the acrylic resin plates were immersed in sterile saliva for 1 hour at the room temperature to form pellicle. The acrylic resin plates were rinsed twice with phosphate buffered saline solution.[9] They were inserted into the test tube containing the suspension of *C. albicans*, and then were incubated again for 24 hours at 37° C. After that, the acrylic resin plates were inserted into the test tube, each of which contained with black tea solution with the concentration of 10 gr/300 ml (3.33%), 20 gr/300 ml (6.66%) and 40 gram/300 ml (13.33%) for 1 hour, and with sterile distilled water as control. Furthermore, the acrylic resin plates immersed in black tea were rinsed twice with PBS and then were inserted into 10 ml of Sabouraud's broth medium, and were vortexed for 30 seconds to release *C. albicans* attaching to the plate. The suspension of *C. albicans* in Sabouraud's dextrose was taken for about 1 ml for spreading, and then was incubated for 48 hours at 37° C.[10] Finally, the number of *C. albicans* colonies was measured in cfu/ml.

The result of the measurement of the number of *C. albicans* colonies then was tabulated based on the groups. Afterwards, it would be tested by using One-Way ANOVA at 5% significance level. If there were significant differences, it would continually be tested by using Tuckey's multiple comparison test (Tuckey's HSD test).

RESULTS

The results of the mean value and the standard deviation of the number of *C. albicans* colonies attaching to the acrylic resin plates immersed in the black tea steeping can be seen in Table 1.

Table 1. The mean value and the standard deviation of the number of *C. albicans* colonies attaching to the acrylic resin plates immersed in the black tea steeping (cfu/ml)

Concentration	N	$\overline{X}$	SD
Control	6	623.66	2.50
3.33%	6	204.16	9.17
6.66%	6	142.50	2.25
13.33%	6	106.66	6.50

Note: $\overline{X}$: Mean, SD: Standard Deviation

Table 2. The results of the analysis of Tuckey's multiple comparison test (Tuckey's HSD test) on the number of *C. albicans* attaching to the acrylic resin plates

	3.33%	6.66%	13.33%
3.33%	-	S	S
6.66%	S	-	S
13.33%	S	S	-

Note: S = Significance

Before conducting the parametric tests to determine the significance of the differences, this study needed to conduct normality test using Kolmogorov-Smirnov test in order to with the result $p = 0.6274$ ($p > 0.05$). It means that those three treatment groups and the control group have normal distribution. The comparative test of those three treatment groups and the control group then was also conducted by One-Way ANOVA test.

From One-Way ANOVA test, it is known that there was significant difference among those treatment groups with $p = 0.000$ ($p < 0.05$). To analyze the differences among those treatment groups further, the data then was tested by using the Tuckey's HSD test. The result of Tuckey's HSD test, finally, indicated that there were significant differences between the treatment groups and the control group. In other words, the increasing of the concentration of the black tea will reduce the number of *C. albicans* colonies (Table 2).

DISCUSSION

Based on the result, it is known that the average number of *C. albicans* colonies adherence on the acrylic resin plates immersed in the black tea indicates that the higher concentration of the black tea stepping, 13.33%, makes the number of *C. albicans* colonies decreased. Meanwhile, the lower the concentration is, the higher the number of *C. albicans* colonies significantly is. It is because the black tea steeping used as the immersion material of acrylic resin plates is considered as disinfectant by the content of fluoride, polyphenols, catechins and other oxidated derivates.[7] Polyphenols is actually soluable in water,[13] thus, polyphenols contained in tea leaves can also be dissolved in water. Nevertheless, the tea containing polyphenols is still influenced by the concentration of the solution. The more concentrated the solution is, the higher the number of polyphenols in the black tea steeping is. Therefore, it becomes more effective to be used to decrease the number of *C. albicans* colonies adherence to the acrylic resin plates. Besides that, polyphenols classified into phenol group widely used as a disinfectant that has a good antimicrobial activity. It is considered as the fast bacteriacide and fungucide. However, this microbial activity can be decreased due to the dilution since the effectiveness of disinfectant materials is influenced by concentration, duration and temperature.[15]

Based on the mechanism of antimicrobial activity, phenols can kill vegetative cells of fungi and bacteria by conducting protein denaturation and by decreasing the surface tension, as a result, the permeability of those fungi and bacteria is increased.[16] This mechanism actually involves the reaction to protein cells through restricting or killing process by damaging the colloidal system, as a consequence, the coagulation and precipitation of protein occur. This protein coagulation of microbial cell then causes both the disruption of metabolism and the changing of the permeability of membrane cells, and also decreases the surface tension which later causes the increasing of the permeability of membrane cells, the absorption of fluid, and the death of microba.[17,18]

Finally, it can be concluded that the increasing of the concentration (13.33%) or the higher the concentration of black tea used to immerse the acrylic resin plates, the lower the number of *C. Albicans* colonies attached to the plates. However, further researches about antimicrobial activity of black tea against other bacteria in the oral cavity are needed.

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