

EFFECTIVENESS OF CITRUS LIMON PEEL ESSENTIAL OIL ON CANDIDA ALBICANS : IN VITRO STUDY

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EFFECTIVENESS OF CITRUS LIMON PEEL ESSENTIAL OIL ON CANDIDA ALBICANS : IN VITRO STUDY

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ABSTRACT : *Candida albicans* is a yeast that commensally inhabits the oral cavity might cause opportunistic infections and become pathogen in immunosuppressed condition. More than 90% oral candidiasis caused by *C. albicans* infections. Citrus limon peel has active compound that may inhibits *C. albicans* growth. The purpose of this study is to investigate *in vitro* effectiveness concentration of Citrus limon peel oil on *C. albicans* growth. Citrus limon peel essential oil that has obtained the serial dilution concentrations of 25%, 12.5%, 6.25%, 3.125%, 1.56%, 0.78%, 0.39% and 0.195% and added *Candida albicans* that had been grown in Sabouraud broth media. Furthermore, *C. albicans* grown on Sabouraud Dextrose Agar and colonies were counted. MIC (Minimum Inhibitory Concentration) Citrus limon peel essential oil was 0.78%. MFC (Minimum Fungicidal Concentration) Citrus lemon peel essential oil 1.56%. Citrus limon peel essential oil with concentration 25%, 12.5%, 6.25%, 3.125% and 1.56% had *in vitro* effectiveness inhibiting and cause *C. albicans* death and Citrus limon peel essential oil with concentration 0.78% had *in vitro* effectiveness inhibiting *C. albicans* growth.

Key words : Citrus limon peel, essential oil, *Candida albicans*, antifungal, medicine

INTRODUCTION

Oral candidiasis is an oral disease caused by *Candida* fungi (Glick and Feagan, 2015). Several species in the *Candida* genus are found in humans, including *Candida albicans*, *Candida glabrata*, *Candida krusei*, *Candida dublinensis*, *Kefyr candida*, *Candida guilliermondii*, *Candida lusitanae*, *Candida parapsilosis* and *Candida tropicalis*, but *Candida albicans* is one of the causes for most infections (> 90%) (Samaranayake, 2012). *C. albicans* is a commensal fungus that populate in the oral cavity, digestive tract and vagina (Underhill and Lliev, 2015; Nugraha *et al*, 2018a). Epidemiological studies has been found that *Candida albicans* most superficial and systemic infections (Moran *et al*, 2012).

The existence of predisposing factors that may cause *Candida*, which has commensal to become pathogen (Nugraha *et al*, 2017). The main factor in candidiasis is low immunity, such as in patients with Acquired Immune Deficiency Syndrome (AIDS) for patients undergoing

chemotherapy, patients who use more than long, chronic irritation due to the use of prosthesis (Underhill and Lliev, 2015; Mensana *et al*, 2018; Nugraha *et al*, 2019).

Many antifungal drugs available on the market, the drug side effect might occurred (Icme *et al*, 2014). The use of antifungal drugs made from chemicals such as amphotericin, nystatin, ketoconazole and griseofulvin can lead to resistance (Nugraha *et al*, 2018b). Antifungal drug resistance is defined as stable adaptation or stability, obtained from antifungal drugs, neutrality to the antifungi normally. Antifungal drugs in treating treat candidiasis in the community have very low selective toxicity. So, it is necessary to investigate other source that have more effective antifungal activity (Novianti, 2016). Natural products from plants, animals and minerals have become a treatment of diseases in community. An estimated 80% of people in developing countries still use traditional medicines. The need of herbal medicines are currently popular (Ekor, 2014).

The percentage of Indonesians who use traditional medicine continues to increase over a period of seven

years (2000 - 2006) from 15.2% to 38.30% (Supardi and Leny Susyanty, 2010). In addition, the "back to nature" lifestyle began to be promoted by the people in Indonesia and encouraged researchers to investigate other antifungal alternatives (Arundhina *et al*, 2012).

Lemon peel essential oil (*Citrus limon*) may inhibit growth of *C. albicans* (Prabajati *et al*, 2017). Lemon (*Citrus limon*) has been widely known and consumed by Indonesian. Lemon is one of the sources of vitamin C and antioxidants that are beneficial to human health (Zou *et al*, 2015). Besides, lemon (*Citrus limon*) also contains bioflavonoids, α -terpinene, γ -terpinene, α -pinene, β -pinene and citric acid and volatile oils in the peel such as limonene ($\pm 70\%$) (Swastihayu *et al*, 2015). In addition, lemon (*Citrus limon*) can grow at any time and at any season. The aroma of lemon is perceived as a pleasant aroma that can improve mood (Dosoky and Setzer, 2018). Lemon peel produces the most essential oils compared to other fruits in the same amount. Extract of essential oils in lemon obtained from the skin of the fruit which is a byproduct of the lemon processing industry, makes lemon oil easy obtained (Mahato *et al*, 2017).

Making essential oils from lemon peel is easiest to do than essential oils from other fruits. Limonene compounds are also available on lemon peel and have the ability to inhibit fungal growth. Essential oils can be extracted from plants using methods such as steam distillation, cold pressing, hydrodistillation, and hydrodiffusion. Of the various methods, the steam distillation method has been widely used mainly for commercial production. Basically, plants are placed in boiling air or heated by steam. The heat will cause the plant cell structure break. Oil, components or essential oils from plants will come out (Tongnuanchan and Benjakul, 2014). The antifungal activity of lemon peel may be from the content contained in it, namely limonene, β -pinene, γ -terpinene (Singh and Singh, 2002). The chemical content of γ -terpinene available in lemon peel has an antifungal effect by inhibiting synthesis ergosterol membrane *Candida albicans* (Icme *et al*, 2014).

The mechanism of action of antifungi that attacks ergosterol and fungal cell walls causes damage to the cell nucleus. The poly-ergosterol complex that occurs can form a pore and through these pores the essential constituents of fungal cells such as K ions, inorganic phosphates, carboxylic acids, amino acids and phosphate esters leak out causing fungal cell death. Inhibition of ergosterol biosynthesis in fungal cells causes irregularity of the fungal cytoplasmic membrane by changing membrane permeability and changing membrane function in the process of transporting essential compounds that

can cause metabolic imbalances that inhibit growth or cause fungal cell death (Ilyas, 2008).

Research by Bertoli *et al* (2012), lemon peel with the main content of limonene can cause the death of *C. albicans* by damaging the integrity of the cell membrane of *C. albicans*. In addition, the β -pinene content can damage the cell membrane of *C. albicans*, causing the cell wall of the *C. albicans* to damage and cause the death of *C. albicans* cells (Bertoli *et al*, 2012).

Based on the description above, it is necessary to investigate the effectiveness of lemon peel essential oil (*Citrus limon*), which has an antifungal effect in inhibiting the growth of *C. albicans* fungal colonies *in vitro*.

MATERIALS AND METHODS

The sample used in this study was *C. albicans* derived from fungal culture stock obtained from oral candidiasis swab results from HIV / AIDS patients treated in the Inpatient Care Unit at Dr. Soetomo then stored in the Microbiology Laboratory, Faculty of Dental Medicine, Universitas Airlangga, Surabaya.

Lemon peel (*C. limon*) essential oil was made with a concentration of 25%, 12.5%, 6.25%, 3.125%, 1.56%, 0.78%, 0.39% and 0.195% with the dilution method. After diluting lemon oil (*C. limon*) essential oil obtained from tubes 1, 2, 3, 4, 5, 6, 7 and tube 8 were 25%, 12.5%, 6.25%, respectively 3.125%, 1.56%, 0.78%, 0.39% and 0.195%. Then, 50 pl inoculums were added in the form of liquid culture with McFarland standard 0.25 in each test tube. Then, two more tubes were prepared, namely 9 and 10 as negative and positive controls. However in 100 tubes, 100 pl inoculum was added which functioned as a negative control, while in tube 9 it was positive control. Furthermore, incubation was carried out for 24 hours at 37°C.

The inoculum that has been incubated is then mixed with osse and planting in Sabouraud Dextrose Agar media to determine the growth of the colonies. Sabouraud Dextrose Agar media is divided into 10 parts consisting of inoculum from tube 1-8 and positive control and negative control. Then, it was incubated again for 48 hours at 37°C. Then planting the results of series thinning on Sabouraud Dextrose Agar media. Cross check by swabbing with osse then replanting the colony.

The results of the inoculum swab on Sabouraud Dextrose Agar were incubated for 48 hours at a temperature of 37°C. Then a sample replication was made at least three times. Then calculate the number of colonies and determine, which concentration is the MIC and MFC. Data analysis was performed using the One-Sample Kolmogorov Test to see its distribution and Levene

Test to see variant homogeneity ($p < 0.05$). Then continued the non-parametric test, namely the Kruskal-Wallis test ($p < 0.05$). After that, it continued with a significance test using the "Mann Whitney Test" ($p < 0.05$).

RESULTS

From Table 1, it can be seen that there is no growth of *Candida albicans* colonies between groups of lemon essential oils with concentrations of 25%, 12.5%, 6.25%, 3.125% and 1.56%. In the treatment group with concentrations of 0.78%, 0.39% and 0.195% showed a growth of *C. albicans* colonies. The negative control group also showed the growth of *C. albicans* colonies, while the positive control group did not show any growth of the colony at all. This showed an increase in the average growth of *C. albicans* colonies on the concentration of lemon essential oils that decreased. This showed that the smaller the concentration of essential oils of lemon peel (Citrus limon), the greater the average growth of *C. albicans* colonies.

Significant values of normality and test results homogeneity in the research group (Table 1). In Table 2, it can be observed that there was a significant difference for the concentration group comparison of 0.78% ($P < 0.05$) except between the concentration group 0.39%, 0.195% and negative control values 0.05, which means

Table 1 : Growth of *Candida albicans* colonies in 8 treatment and control groups.

Essential Oil concentrate	Mean±SD	Kolmogorov Smirnov Test	Levene Test
Control (+)	0		0.000
Control (-)	72.67±3.09	0.766	
Concentration 25%	0		
Concentration 12.5%	0		
Concentration 6.25%	0		
Concentration 3.125%	0		
Concentration 1.56%	0		
Concentration 0.78%	12.67±1.88	0.871	
Concentration 0.39%	25.00±3.56	0.999	
Concentration 0.195%	40.67±4.11	0.886	

Table 2 : The result of the Mean Whitney U test.

	Control (+)	Control (-)	Concentration 0.78%	Concentration 0.39%	Concentration 0.195%
Control (+)		0.037*	0.034*	0.037*	0.037*
Control (-)	0.037*		0.046*	0.05	0.05
Concentration 0.78%	0.034*	0.046*		0.046*	0.046*
Concentration 0.39%	0.046*	0.046*	0.046*		0.05
Concentration 0.195%	0.037*	0.05	0.046*	0.05	

* significant difference ($p < 0.05$).

that there is no significant difference among the three groups. The results of *Candida albicans* culture with lemon peel essential oil (Citrus limon) were obtained on Sabouraud broth media. At concentrations of 25%, 12.5%, 6.25%, 3.125%, 1.56% showed no growth in *Candida albicans*, but at a concentration of 0.78% showed the growth of *Candida albicans* colonies so that a concentration of 0.78% was determined as a MIC (Minimum Inhibitory Concentration), which is the lowest concentration that inhibits growth. At a concentration of 1.56%, no colony growth was observed so that a concentration of 1.56% was determined as MFC (Minimum Fungicidal Concentration), which is the lowest concentration that did not indicate the growth of colonies.

DISCUSSION

Lemon peel essential oil with a concentration of 25%, 12.5%, 6.25%, 3.125%, 1.56%, 0.78%, 0.39%, and 0.195% showed differences in the growth of *C. albicans* colonies among the treatment groups. In groups with concentrations of 25%, 12.5%, 6.25%, 3.125% and 1.56% showed no growth of *C. albicans* at all. In the treatment group 0.78%, 0.39%, and 0.195% showed a growth of *C. albicans* colonies. Based on statistical tests obtained concentration there were significant differences in each treatment. There were a significant difference in the treatment group 0.78%. The absence of growth in the group of lemon peel essential oils with a concentration of 25%, 12.5%, 6.25%, 3.125%, and 1.56% in this study due to active compounds that can be used as antibacterial, antifungal, antiparasitic and antiviral (Bertoli *et al*, 2012). *Citrus limon* essential oil contains volatile components, namely a mixture of monoterpene (limonene) and sesquiterpene hydrocarbons and oxygen derivatives such as aldehydes, ketones, acids, alcohols and esters (Prabajati *et al*, 2017). Limonene has strong antifungal activity against *C. albicans*. This terpenoid group works to inhibit ergosterol synthesis that occurs in the cell membrane so that it can cause increased permeability of substances that exist in *C. albicans* cells, especially on the cell wall (Bertoli *et al*, 2012).

The ability of lemon peel essential oils in inhibiting the growth of *C. albicans* due to the content of limonene

compounds that cause interference with the cell membrane of *C. albicans* so that the cellular components come out. Limonene changes the methylesterification structure of pectin which is a major component of the cell wall of the *C. albicans*. The changes in the structure of pectin are related to changes in adhesion ability, influence of *C. albicans* growth, integrity and permeability of *C. albicans* cell membrane (Queiroga *et al.*, 2013). Limonene which is a monocyclic monoterpene is the main component of lemon essential oil, but antifungal activity is more dependent on the oxygenated monoterpene component. Higher levels of oxygenated monoterpene, the greater the antifungal effect of lemon essential oil (Krzys, 2014).

In addition to limonene there is another content in the lemon peel essential oil which functions as an antifungal, namely citral and linalool. Citral affects the structure of the cell membrane of *C. albicans*, blocking the synthesis causing cell death. In addition, citral also inhibits germination spore proliferation, and cell respiration (Clerya *et al.*, 2014). Linalool is a class of monoterpene which functions as an antifungal. Linalool inhibits the production of enzymes that protect cell membranes (Herman *et al.*, 2015).

From the results of this study, it was found that the higher the concentration of essential oil the less the number of *C. albicans* colonies that can grow. In the statistical test, there was no significant difference in the concentration of 0.39% and 0.195%. At this concentration of lemon peel essential oil did not effective to inhibit or eradicate *C. albicans* colonies. While at concentrations of 25%, 12.5%, 6.25%, 3.125%, 1.56% and 0.78%, there was a significant difference in statistical tests, which means that there is an effectiveness *Citrus limon* in inhibiting and also kill *Candida albicans*. A concentration of 0.78% is determined as a MIC (Minimum Inhibitory Concentration) value and a concentration of 1.56% is determined as MFC (Minimum Fungicidal Concentration).

CONCLUSION

From the results above can be concluded that 0.78% concentration of lemon peel essential oil can inhibit and eliminate the *C. albicans* growth

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