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FORMULATION OF NANOEMULSION MOUTHWASH COMBINATION OF LEMONGRASS OIL (*Cymbopogon citratus)* AND KAFFIR LIME OIL (*Citrus hystrix)* AGAINST *Candida albicans* ATCC 10231

FORMULASI MOUTHWASH NANOEMULSI KOMBINASI MINYAK SEREH (*Cymbopogon citratus*) DAN MINYAK JERUK PURUT (*Citrus hystrix*) SEBAGAI ANTIKANDIDIASIS ORAL TERUJI IN VITRO TERHADAP *Candida albanicans* ATCC 10231

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ABSTRACT

Candidiasis is a fungal infection disease that still become health problem in Indonesia, especially oral candidiasis that largerly caused by Candida albicans abnormal growth on oral cavity. Lemongrass oil and kaffir lime oil have been proven to have antifungal activity against Candida albicans. The composition of limonen, α-terpineol, sitronelil acetate, terpineol, β-pinene, on kaffir lime oil and citronelal on lemongrass oil make the combination of both oils have the potential as an alternative theraphy for oral candidiasis. It formulates in the form of nanoemulsion mouthwash. Nanoemulsion is chosen because it has some advantages like clear, stable, and increasing the antifungal activity of lemongrass oil and kaffir lime oil. The study begins with plants determination, essential oils charecterization tests (solubility, refractative index, and specific gravity), and essential oil content analysis by GC-MS. Nanoemulision formulates by water titration method. Optimization of oil mix and VCO do to get a clear and stable nanoemulsion. The best formula is characterized (transmittance, particle size, viscosity, zeta potential, and stability test), then analyze the essential oil chemical compounds in the optimum formula and test the inhibitory effect of the formula by microdilution method. Inhibiton data analysis use One Way ANOVA. Based on the results of the study, 0.4 % oil mix, 3.6 % VCO, 17.3 % tween 80, 8.7 % PEG 400 dan 70 % water is the best formula that can form a nanoemulsion system with an average particle size 21,4 nm, low viscosity, low zeta potential and stable during freez-thaw storage. Nanoemulsion significantly has inhibitory effect more effective than positive control.
Keyword: *nanoemulsion, lemongrass oil, kaffir lime oil, Candida albicans, antifungal*

ABSTRAK

Kandidiasis adalah penyakit infeksi jamur yang masih menjadi masalah kesehatan di Indonesia, kandidiasis terutama oral sebagian besar dikarenakan pertumbuhan abnormal Candida albicans pada rongga mulut. Minyak jeruk purut dan serai telah terbukti memiliki aktivitas antijamur terhadap Candida albicans. Komposisi limonen, α-terpineol, sitronelil assetat, terpineol, β-pinene, pada minyak jeruk purut dan citronelal pada minyak sereh membuat kombinasi keduanya minyak memiliki potensi sebagai terapi alternatif untuk kandidiasis oral. Kedua minyak ini dapat diformulasikan dalam bentuk nanoemulsion obat kumur. Nanoemulsion dipilih karena memiliki beberapa keuntungan seperti yang jelas, stabil, dan meningkatkan aktivitas antijamur minyak sereh dan minyak jeruk purut. Penelitian ini dimulai dengan determinasi tanaman, uji karakterisasi minyak atsiri (kelarutan, indeks bias, dan berat jenis), dan analisis kandungan minyak atsiri dengan GC-MS. Nanoemulision diformulasikan dengan metode titrasi air. Optimalisasi campuran minyak dan VCO dilakukan untuk mendapatkan nanoemulsion yang jernih dan stabil. Formula terbaik dikarakterisasi (ukuran partikel, viskositas, potensi zeta, dan stabilitasnya), dilakukan analisis senyawa kimia minyak atsiril dalam formula optimum dan menguji efek penghambatan formula oleh metode mikrodilusi. Analisis data daya hambat dilakukan dengan One Way ANOVA. Berdasarkan hasil penelitian, 0,4% minyak campuran, 3,6% VCO, 17,3% tween 80, 8,7% PEG 400 dan 70% air merupakan formula terbaik yang dapat membentuk sistem nanoemulsion dengan rata-rata

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ukuran partikel 21,4 nm, viskositas rendah, potensi zeta yang rendah dan stabil selama penyimpanan freez-thaw. Nanoemulsi secara signifikan memiliki efek penghambatan lebih efektif daripada kontrol positif.
Key word: *nanoemulsi, minyak sereh, minyak jeruk purut, Candida albicans, antijamur*

INTRODUCTION

Candidiasis is one of many fungus infection diseases. The most common candidiasis occured in human society is the oral candidiasis (Siregar, 2002). Oral candidiasis is an oportunistic infection in oral cavity mostly due to the abnormal growth of *Candida albicans* (Dalleau *et al*., 2008). In Indonesia, the prevalence of oral candidiasis is quite high as happened in Medan, Aceh, and Central Java with each case number 66.1 %, 41 %, 85.4 % (Harahap, 2000; Jamil, 2014 Kusuma, 2014). Oral candidiasis occurrence is also triggered by the weakened of immune system because of chemotherapy or the usage of wide spectrum antibiotics for long period of time (Dignani *et al*., 2009). If it is not well treated, it can cause the discomfort such as pale, burnt sensation, and dried mouth (Greenberg, 2003). Oral candidiasis can be therepied with mouthwash which contains the anticandida activity.

Lemongrass and kaffir lime are the original Indonesian plant that often used as ingredient of herbs. Lemongrass oil and kaffir lime oil have been proven contain the antifungal activity against *Candida abicans* (Ruckmani, 2013; Prasart, 2014).

This activity is shown with the availability of limonene compound, α-terpineol, terpineol, β-pinen, and acetate sitronelil within the kaffir lime oil and sitronelal within lemongrass oil (Pinto *et al*., 2014; Omran *et al*., 2011; Singh *et al*., 2012; Taweechaisupapong *et al*., 2012).

Lemongrass oil has higher inhibition activity against *C. albicans* rather than kaffir lime oil but lemongrass oil produces unpleasant scent and less freshing than kaffir lime oil scent (Taweechaisupapong *et al*., 2012). The combination of the two type oils will balance the scent so they will give fresh and pleasant sensation when it consumed.

Nanoemulsion preparation offers unique advantages such as clearer, stable, and increasing efficacy of the oil combination. Lemongrass oil and kaffir lime oil have water insoluble characteristic. Formulating these oils into emulison is the way to use them as mouthwash. The ordinary emulsion has unpleasent form because they had a larger particle sizes. Beside, nanoemulsion, that has a small particle, has ability to be stabil consistanly then the ordinary emulison (Sharma *et al*., 2010). Nanoemulsion also has a low turbidity. It's pleasent to be used as mouthwash because it looked like water. Making them into nanoemulsion is a solution to get a clear, stabil, and pleasant mouthwash for people. Some studies also have shown that nanoemulsion can increase the antimicrobial activity of these oils (Mason *et al.*, 2006). Therefore, theoil combination will be better formulated in nanoemulsion mouthwash matter with particle size 20-200 nm (Serano *et al.*, 2014).

Nanoemulsion that had been made consists of mixture of oil phase, surfactant, co-surfactant, and water with certain composition. Lemongrass oil and kaffir lime oil need carrier oil to be able to disperse and form the nanoemulsion. Combination oil will be formulated with some additional substance such as VCO as carrier oil, Tween 80 as surfactant, and PEG 400 as cosurfactant. The composition of VCO, Tween 80, and PEG 400 had been proven to produce nanoemulsion system (Widyaningrum, 2015; K arima, 2015). Nanoemulsion formula from the combination of kaffir lime oil and lemongrass oil is expected to develop as the safe and pleasant oral candidiasis therapy for people.

METHODOLOGY

Preparation of Lemongrass Oil and Kaffir Lime Oil

Lemongrass oil and kaffir lime oil are from CV. Orizho Indonesia, Mantup Baru, Bantul, Yogyakarta. Lemongrass oil and kaffir oil are made through steam distillation method. Lemongrass and kaffir lime are raw materials needed to produce the essential oil. From lemongrass plant, the stalk and the leaves are the used parts, and from the kaffir lime, the leaves are the used part. Those plants are from Kinahrejo, Cangkringan, Yogyakarta. The determination of samples was done to prove the authenticity of used plants species in Pharmaceutical Biology, Faculty of Pharmacy UGM.

Spesification Test of Lemongrass Oil and Kaffir Lime Oil

Spesification test of lemongrass oil, kaffir lime oil, and VCO is tested in Laboratorium Penelitian dan Pengujian Terpadu (LPPT-Integrated Laboratory of Research and Examination), Universitas Gadjah Mada, Yogyakarta. The spesification test included refractive index test, solubility, and density from each oil.

Analysis the Content of Lemon Grass oil and Kaffir Lime Oil

Essential oil contents is analysed by Gas Chromatography Mass Spectrometer *QP 2010 S Shimadzu*. The used column is Agilent HP-5MS. The column is length 30 m and diameter 0.25 mm. Carrier gas was helium. The column temperature was 70°C. The analysis methode was developed by Organic and Biochemical Laboratory team, Faculty of Mathematics and Natural Sciences, UGM.

Nanoemulsion Mouthwash Formulation of Lemongrass and Kaffir Lime Oil

First, Lemongrass oil and kaffir lime oil are mixed in the composition of 1:1, called combination oils. Then this combination oils is used for formulating process of nanoemulsion. Regarding to the old experiment, the composition, consists of 4% VCO (as carier oil); 17.3% Tween 80; 8.7% PEG 400; 70% water in (b/b), can produce nanoemulsion (Widyaningrum, 2015). So this composition is determined as the base formulation for making nanoemulsion.

Second, screening the proper composition of VCO and combination oils is conducted. The proper composition of VCO and combination oil from 1:9 till 9:1 is formulated with the other base nanoemulsion formula. The mixture of VCO, combination oil, tween 80, and PEG 400 were stirred with magnetic stirrer for 10 minutes, ultrasonicated for 10 minutes. Water with temperature about 70°C is measured and poured slowly into the surfactant mixture and oil phase using spotting pipette. This process was conducted while the magnetic stirrer was working. the mixture particle is reduced through ultasonication again for 10 minute. The optimum formula is selected due to the high and stable transmittance value result from the formula.

Characteristic Evaluation of The Combination Lemongrass Oil and Kaffir Lime Oil Nanoemulsion

Characterisatic evaluation is conducted to ensure the desired nanoemulsion formula.

Transmittance Measurement

Measurement of formula transmittance is performed by using UV/Vis spectrophotometer with wavelength 650 nm (Jain *et al*., 2013). Distilled water is used as the blank. Transmittance value closed to 100% is proven that the mixture has nano size (Widyaningrum, 2015).

The Measurement of Particle Size Distribution

Particle size distribution and average droplet nanoemulsion are measured through the Dynamic Light Scattering (DLS) method and Particle Size Analyzer (PSA) tool Horiba Scientific-100 SZ. Nanoemulsion formula has an average droplet size less than 100 nm (Auburn *et al*., 2004). Measurement is performed in the laboratory of Drug Analysis, Food and Cosmetics (AOMK) Faculty of Science, Islamic University of Indonesia, Yogyakarta.

Viscosity Measurement

Formula viscosity is measured by using viscosimeter Rheosys type cone and plate. The measurement is conducted in Physical Pharmacy Laboratory, Faculty of Pharmacy, Universitas Sanata Dharma Yogyakarta.

Stability Test

Nanoemulsion stability formula is tested through six-cycle freeze thaw method. Samples are kept in temperature -21°C and 25°C for each 24 hours (Vior *et al*., 2011). Every one cycle, the observation do through measuring the sample transmittance with spectrophotometer UV/Vis on the wavelength 650 nm.

Zeta Potential Measurement

Zeta potential is measured through DLS method and PSA (Horiba Scientific SZ-100) tool. The measurement of zeta potential is conducted in AOMK Laboratory, Faculty of Mathematics and Natural Sciences, UII Yogyakarta.

Chemical Content Analysis within The Combination Nanoemulsion Formula of Lemongrass and Kaffir Lime Oil

Analysis of the chemical compounds in nanoemulsion formula is performed with Gas Chromatography Mass Spectrophotometer 2010 S Shimadzu QP. The column used is Agilent HP-5Ms with column length 30 m, diameter 0.25 mm. The carrier gas is helium. Column temperature is 70°C. This analysis method is developed by the Laboratory of Organic and Biochemistry, Faculty of Mathematics and Natural Sciences, UGM.

Activity Test of Nanoemulsion Mouthwash Formula as Anticandida

The method used for test the inhibitory activity of this nanoemulsion is microdilution. *C. albicans* suspension is standardized by checking the suspension's turbidity. *C.albicans* is recultured from SDA media to SDB media, incubated for 48 hours. The suspension of the microbe is measured for the absorbance on the 520 nm wave length. The suspension test microbe is diluted till the absorbance value reach 0.38 equal to *C. albicans* as much as 1×10^8 CFU/mL (Dovigo *et al*., 2011).

The mixture of samples, media, and microbes are filled in 96 well flat bottom microplate polysterene up to a total volume of 200 µL. The first group contains 15 µL of nanoemulsion formula combination of lemongrass oil and kaffir lime oil, 180 µL SDB media and 5 µL microbal test as well as the replication. The second group containsed 195 µL single lemongrass oil 0.4%
(v / v) and 5 µL microbal test as well as the replication. The third group contains 195 µL single kaffir lime oil 0.4% (v / v) and 5 µL microbal test as well as the replication. The fourth group contains a combination of oil of 0.4% (v / v) and 5 µL microbal test as well as the replication. The fifth group of ethanol contains 195 µL samples of 0.37% (v / v) and 5 µL microbal test as well as the replication. The sixth group contains positive controls, such as 15 µL of fluconazole 3 mg/mL, 180 mL SDB media and 5 µL culture of *C. albicans* as well as the replication. Positive control, is the group of microbe suspension treated by a certain compound inhibiting the growth of *C. albicans* ATCC 10231. The seventh group contains 200 µL SDB media as media controls. The negative control is 195 µL SDB media and 5 µL test microbe as well as the replication.

Initial OD values are calculated using microplate reader, incubated at a temperature of 37°C for 48 hours. OD values are measured again to determine the percentage of inhibition by the equation Quave, *et al.* (2008) as follows:

$$\%\text{Inhibition} = 1- \left(\frac{(ODt48 - ODt0)}{(OD\ gc\ t48 - OD\ gc\ t0)} \right) \text{ X } 100\%$$

For: ODt48 = Optical Density testing sample after incubation for 48h; ODgc48 = Optical Density negative control after incubation for 48h; ODgc0 = Optical Density negative control before the incubation.

Minimum Bacterial Concentration (MBC) is obtained from the high value MIC measured upon solid media of Saboraud Dextrose Agar (SDA) contained *C. albicans*. The media is incubated in temperature 37°C for 48 hours. MBC is selected due to the smallest concentration that not cause 90% *Candida albicans* growth.

RESULT AND DISCUSSION

Plant Determination and Spesification Test of Lemongrass and Kaffir Lime Oil

The plants are used as the raw material to obtain the lemongrass oil from CV Orizho Indonesia, Mantup Baru, Bantul, Yogyakarta. They are the true *Cymbopogon citratus* and *Citrus hystrix*. Lemongrass oil has a solubility to ethanol. Lemongrass oil has a refractive index of 1.4597 and a specific gravity of 0.9021 g / mL. Lime oil soluble in alcohol. Lime oil has a refractive index of 1.4764 and density of 0.892 g / mL. Essential oils used in this study is similar to standard essential oil from the Indonesian National Standard (Anonymous, 1995).

Combination Nanoemulsion Formula of Lemongrass Oil and Kaffir Oil

Formula 9 is selected as the optimum formula because it has a large and stable transmittance value until the third cycle. Formula 9 has a ratio of the VCO with oil combination 9:1. The existence of VCO in the formula can help to build nanoemulsion containing essential oil (Widyaningrum, 2015). Essential oil consists of terpenes while the VCO is composed of fatty acids (Baser *et al*., 2010; Bawalan *et al*., 2006).

VCO contain the fatty acid constituents of which are mainly lauric acid (Anonymous, 1995). Lauric acid is useful for emulsifying agent in formulation (Rowe *et al*., 2009). Lauric acid can help to turn the surface tension down so the essensial oil can be dispersed to the water medium very well. Tween 80, one of the important part of this formula, has a function as surfactant. Surfactant has hydrophobic and hydrophilic structures that will bridge the gap between oil phase (hydrophobic) and the aqueous phase (hydrophilic) by reducing the surface tension at the interface between the oil and water molecules. Formulation of nanoemulsion need much more energy to lower the surface tension because the water composition in the formula is quite much than the oil phase composition. Thus, nanoemultion also use PEG 400 to be co-surfactant. PEG 400 is a mid chain hydrocarbon that could be placed among the gap of nanoemulsion system through forming hydrogen chain on them. This process will maximize the emulsification process to develop the nanoemulsion (Kurnia, 2015).

Nanoemulsion Formula Evaluation of Transmittance Lemongrass Oil and Kaffir Lime Oil Combination

Maximum transmittance value indicates clarity of certain mixture. The higher transmittance value certain mixture, the smaller particle size would be (Lalwani *et al*., 2013). Based on the measurement, the transmittance value obtained for 95.4% ± 0.4. This number shows that the result formula has a high clarity. Nanoemulsion can obtain high transmittance value because it doesn't disperse light on visible wave length (Graves, 2008). However, high transmittance value only is not enough to prove

Tabel I. Screening Combination Nanoemulsion Formula of Lemongrass Oil and Kaffir Oil

Formula	VCO: Combination of Essesntial Oil (g)	Transmittance (%)	Freeze-thraw (cycle)
1	1:09	2	1
2	2:08	88,5	1
3	3:07	92,1	2
4	4:06	61,4	1
5	5:05	38,8	1
6	6:04	96,9	2
7	7:03	4,8	1
8	8:02	11,2	1
9	9:01	96	3

Table 2. Nanoemulsion Formula Viscosity of Lemongrass Oil and Kaffir Lime Oil Combination

Time (s)	RPM	Shear Stress (Pa)	Shear Rate (1/s)	Viscosity (Pa.s)	Temperature (°C)
50	10	1,468	10,945	0,13413	28,87
100	20	1,642	21,891	0,07501	28,80
150	30	1,734	32,836	0,05281	28,80
200	40	1,792	43,781	0,04093	28,79
250	50	1,596	54,727	0,02916	28,79
300	60	1,436	65,672	0,02187	28,80
350	70	1,547	76,617	0,02019	28,80
400	80	1,535	87,562	0,01753	28,81
450	90	1,413	98,508	0,01434	28,81
500	100	1,484	109,453	0,01356	28,81

that the formula has formed the nanoemulsion (Widyaningrum, 2015). Thus, some characterisation such as particle distribution measurement is performed.

Particle Size Distribution

Emulsion is categorized as nanoemulsion if it has average particle size less than 100 nm (Auburn *et al*, 2004). Where microemulsions average particle size is found to be less than 200 nm (Prajapati *et al.,* 2011). Measurement result shows that average particle size of the gained formula was 21.4 nm with PI (Polydispersity index) value 0.324. PI is the deviation standard between average droplet size that shows the homogeneity of droplet size in samples (Gupta *et al*., 2010). The smaller PI value will cause the more homogenous particle size distribution. Thus the obtained nanoemulsion formula has quite homogenous droplet diameter size. Mixing droplet size has nanometer scale because of surfactant, cosurfactant, and nanoemulsion creation method that have been conducted (Widyaningrum, 2015).

Viscosity

Viscosity is a very important parameter in the stability and efficiency of the active component release. Viscosity is the friction between adjacent layers of fluid as it moves across each other (Siregar *et al*., 2013). Components of water, oil, and smix in the formula will affect the formed nanoemulsion viscosity. Increasing the amount of water will decrease the viscosity. The increasing of viscosity value of nanoemulsion formula in line with the rising value of shear stress. The relation of viscosity with rate shear (shear velocity of the substance) forming a curve (Figure 1).

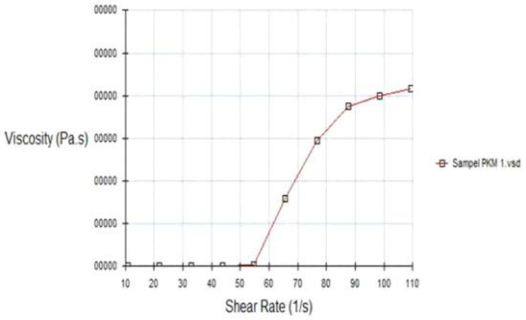

Figure 1. The Curve of Shear Rate vs Viscosity Relation

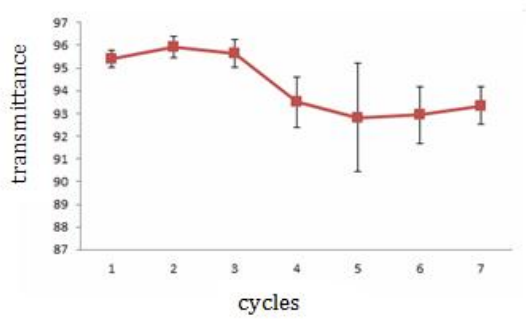

Figure 2. Transmittance Stability Testing Chart

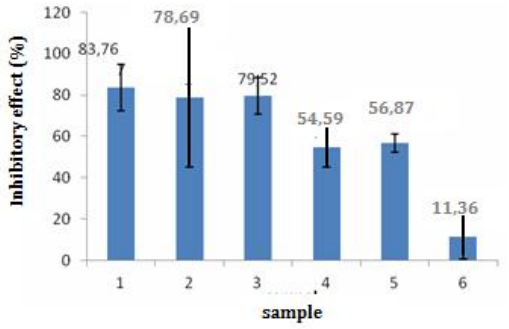

Figure 3. Inhibitory of *Candida albicans* ATCC 10231 Chart 1. Nanoemulsion, 2. Combination Oil, 3. Lemongrass Oil, 4. Kaffir Lime Oil, 5. Ethano, 6. Positive Control

The curve describes the flow type of dilatant with non-Newtonian system. This system is a heterogeneous dispersion one owned by formula nanoemulsion. This system does not follow the rules of Newton's laws I about the flow, but indicates deviation in the administration of relative increase friction (Bird *et al*., 1960).

When compared to the absolute viscosity of water, measured with the same tool and conditions, the nanoemulsion formula has relatively same as fluidity as the water. Nanoemulsion formula will be easily gargled and used as a mouthwash.

Zeta Potential

Zeta potential value associated with the stability of the disperse system shows the level of rejection of the same charge particles adjacent to each other in the system (Sinko, 2006). Zeta potential of 0 to ± 30 mV indicate instability while the zeta potential higher than ± 30 mV indicate stability (ASTM, 1985).

The obtained zeta potentia valuel is -5.6mV. Zeta potential value can be influenced by various factors, such as surfactants type, disperse medium concentration of electrolytes (ionic strength), morphology and particle size, solution pH, and hydration (Simunkova *et al*,. 2009). The surfactant used is tween 80. Tween 80 is one of nonionic surfactants. Tween 80 does not have a charge on a hydrophobic group, so that the surface of the oil phase droplet covered by surfactants tend to be uncharged (Wahyuningsih and Putranti, 2015).

There are two mechanisms of stability, namely electrostatic and steric mechanisms. Electrostatic stability occurs because of repulsion between the droplets that have a surface charge, while the steric stability mechanism occurs because the layer formation on the surface of the droplet. Both of these mechanisms hinder aggregation. (Wahyuningsih and Putranti, 2015). Nonionic surfactants such as tween 80 has the possibility of stabilizing nanoemulsion with steric mechanism. Stability test is accelerated to determine the stability of the nanoemulsion formula.

Stability

Stability test do through six-cycle freeze thaw method by observing the transmittance value on each cycle. The observation result data shows that fluctuation occurred. After that, analysis with Paired T Test is done with trust rate 95% for determining whether the decrease in transmittance in the last cycle of significant value or not.

Transmittance value before and after the stability test did not differ significantly from before and after storage. This proves that the formula is stable during storage at extreme temperatures.

Steric stability mechanism may occurs in the produced nanoemulsion system. In nanoemulsion system of oil in water, contained tween 80 as surfactant, will form a film on the surface of the droplet (Wahyuningsih and Putranti, 2015). The film coating will prevent droplet

merging the dispersing medium. This caused a more stable nanoemulsion formula.

Chemical Compounds Content in Lemongrass Oil, Kaffir Lime Oil, and Nanoemulsion Formula

Based on GC-MC analysis result upon chemical compound in lemongrass oil, samples of lemongrass oil contains alpha-pinene, D-limonene and its isomers, pyrollidine, piperidine, citronellol, geraniol, citronellyl acetate, geranyl acetate, germacrene-D, trans-farnesol, elemol, hedycaryol, alpha-asarone, acid pentadokanoat, acid heksadekanoat, and acid octadekanoat. Geraniol and citronellal are components that had the highest composition. Citronellal is one component of lemongrass oil that proven to have inhibitory activity against *C. albicans*.

The results of GC-MS analysis shows that the lime oil containied 5 types of main components, namely limonene 22.87%, 15.71% α-terpineol, terpineol 14.26%, 9.68% β-pinene and γ- terpinen 5.50%. Limonene compound, α-terpineol, terpineol, β-pinene, sitronelil acetate have the activity as an antifungal against *C. albicans* (Pinto *et al*., 2014; Omran *et al*., 2011; Singh *et al*., 2012; Lima *et al*., 2005).

Nanoemulsion formula has the following composition: beta-pinene, limonene, citronellal, citronellyl acetate, and geranyl acetate. The highest proportion of the compounds are citronellal, limonene, and beta-pinene. These compounds are an active components in lemongrass oil and kaffir lime oil that have inhibitory activity against *C. albicans*. It indicates that the formulation proces doesn't make unstability or cause lost of component.

Antifungal Activity Against *C. albicans* ATCC 10231

The result of OD measurement after 48 hours incubation is converted to inhibitory percentage. Nanoemulsion has antifungal activity as much as 83.76% against *Candida albicans*. This inhibitory of nanoemulsion is seven times higher than the positive control.

Nanoemulsion of lemongrass oil and kaffir lime oil combination has a significant difference. Very small particle size can increase the penetration of lemongrass oil and kaffir lime oil, thus providing a better antifungal effect (Widyaningrum, 2015).

Based on a series of research, it can be said that the nanoemulsion formula of lemongrass oil and kaffir lime oil is more effective in inhibiting the growth of *C. albicans* ATCC 10231 than a single oil or a combination of the oil before it is formulated.

CONCLUSION

In summary, lemon grass oil, kaffir lime oil, Tween 80, PEG 400 and water combination cause nanoemulsion formation. The nanoemulsion formula composition of lemongrass and kaffir lime oil consists of 4 grams of oil phase (0.4 grams oil combination and 3.6 grams VCO), 26 grams smix (17.3 grams tween 80 and 8.7 grams PEG 400), and 70 grams of water. It has average particle size 21.4 nm, means the mixture was nanoemulsion. The high transmittance it has also showed that it has high optical clarity. The tested nanoemulsion showed stability during 6 cycles freeze thaw storage. Nanoemulsion formula has low viscosity, so it will be easily gargled to be applied as a mouthwash.

Nanoemulsion formula of lemongrass oil and kaffir lime oil is significantly more effective in inhibiting the growth of *C. albicans* ATCC 10231 than a single oil or a combination of the oil before it is formulated. So the nanoemulsion formula of lemongrass and kaffir lime oil combination is potent developeved as an alternative therepy of oral candidiasis.

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