ESSENTIAL OIL EXTRACT OF *Citrus Aurantifolia L.* HAS BETTER ANTIBACTERIAL EFFECT THAN SULFUR TOWARDS *Staphylococcus epidermidis*

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ABSTRAK

*Penggunaan sulfur sebagai obat penyakit kulit yang paling umum dikenal di seluruh dunia. Namun efektivitasnya cukup tidak dapat diandalkan beberapa penyakit seperti untuk jerawat vulgaris, penyakit kulit multifaktorial. Salah satu penyebab pertumbuhan jerawat adalah infeksi bakteri, termasuk Propionibacterium acnes dan Staphylococcus epidermidis. Sementara itu, beberapa penelitian menunjukkan bahwa minyak atsiri Citrus aurantifolia L., yang biasanya digunakan untuk memasak, memiliki aktivitas antibakteri. Tujuan dari penelitian ini adalah untuk membandingkan aktivitas antibakteri belerang dan minyak esensial dari Citrus aurantifolia L. terhadap bakteri yang menyebabkan jerawat, Staphylococcus epidermidis. Penelitian ini merupakan penelitian eksperimental laboratorium. Ekstrak minyak esensial dari Citrus aurantifolia L. dibuat dengan Air dan Uap Distillation sementara kami menggunakan sulfur dalam bentuk bubuk. Ekstrak ini diperiksa di Staphylococcus epidermidis. Metode eksperimen adalah seri pengenceran. Hasil dianalisis secara deskriptif dengan Konsentrasi Hambat Minimum (KHM). Pemeriksaan di setiap bakteri diulang 5 kali. Hasil penelitian menunjukkan bahwa ekstrak minyak atsiri Citrus aurantifolia L. memiliki kemampuan untuk menghambat pertumbuhan Staphylococcus epidermidis pada konsentrasi 25%. Sementara kemampuan sulfur untuk menghambat pertumbuhan Staphylococcus epidermidis pada 50% atau tidak ada sama sekali penghambatan (pengamatan visual). Dari hasil pengulangan dalam 5 kali, gram positif lebih stabil daripada gram negatif. Kesimpulannya, ekstrak minyak esensial dari Citrus aurantifolia L. memiliki efek antibakteri baik daripada sulfur terhadap Staphylococcus epidermidis. MIC untuk minyak atsiri Citrus aurantifolia L. pada 25% sedangkan untuk sulfur MIC sebesar 50% **(FMI 2012;48:115-120)**.*

Kata kunci: *antibakteri, Citrus hystrix DC, sulfur, Staphylococcus epidermidis*

ABSTRACT

*The use of sulfur as the cure of most common skin disease is well known around the world. But its effectiveness is quite not reliable some disease such as for acne vulgaris, the multifactorial skin disease. One of the cause for acne growth is bacterial infection, including Propionibacterium acnes and Staphylococcus epidermidis. Meanwhile, some research suggests that essential oil of Citrus aurantifolia L., which is usually used for cooking, have the antibacterial activity. The purpose of this study is to compare the antibacterial activity of sulfur and essential oil of Citrus aurantifolia L. toward the acne causing bacteria, Staphylococcus epidermidis. This research was laboratory experimental study. The essential oil extract of Citrus aurantifolia L. was made with Water and Steam Distillation while we use sulfur in the form of powder. This extract was examined in Staphylococcus epidermidis. Experimental method is serial dilution. Result was analyzed descriptively with Minimum Inhibitory Concentration (MIC). The examination in each bacterium was repeated 5 times. The result showed that essential oil extract of Citrus aurantifolia L. has ability to inhibit the growth of Staphylococcus epidermidis at concentration 25%. While the ability of sulfur to inhibit the growth of Staphylococcus epidermidis on 50% or no inhibition at all (visual observation). From result repetition in 5 times, gram positive is more stable rather than gram negative. In conclusion, the essential oil extract of Citrus aurantifolia L. has better antibacterial effect than sulfur towards Staphylococcus epidermidis. MIC for essential oil of Citrus aurantifolia L. at 25% while for MIC sulfur at 50% **(FMI 2012;48:115-120)**.*

Keywords: *antibacterial, Citrus hystrix DC, sulfur, Staphylococcus epidermidis*

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INTRODUCTION

Acne vulgaris, or acne in general terms, is a skin condition in which the appearance of spots called comedones (blackheads and whiteheads), papules, pustules, and, in some cases, nodules and cysts. Development of acne vulgaris associated with introducing puberty when androgens, testosteron example, the body is released. These hormones can cause sebaceous oversecretion, which resulted in the blockade and a few spots associated with acne vulgaris. In the general population of acne vulgaris teenagers

considered a nuisance, but obtained considerable quantities in early adulthood, around the age of twenty or thirty, with various levels (Marks et al, 1999).

Acne vulgaris is usually not considered a significant disease because the effect is not too dangerous. However, psychosocial and emotional effects generated are comparable to the effects of diseases such as arthritis, back pain, diabetes, epilepsy and asthma (Barankin & Dekoven 2002). Based on research conducted on school-age teens, more than 30% of students feel frustrated, embarrassed and tend to act aggressively because the condition (Marks et al 1999). Acne vulgaris has a lot of movement, one of which is a hormone produced Androgens started puberty. This hormone triggers the sebaceous glands hypersecretion. Genetic factors are also believed to be one of the causes of acne vulgaris. Although the disease does not decline, studies showed that children of parents who suffer from acne vulgaris tend to exhibit the same symptoms when they are stepping puberty. Bacteria also one of the important factors for the inflammatory effects caused. The bacteria that cause acne vulgaris include *Propionibacterium acnes*, *Staphylococcus epidermidis*, and *Pityrosporum ovale*. Bacteria have an important role in the formation of whiteheads and scars are often lowering their self-esteem sufferers of acne vulgaris (Rosyad 2009).

Staphylococcus epidermidis is a gram-positive rod bacterium species. These bacteria are normal flora on the skin and easily obtained and cultured. However, these bacteria play a role in nosocomial infections and it is also the cause of acne vulgaris (O'Gara & Humphreys 2001). Sulfur is a topical treatment for acne vulgaris. These substances have the effect of antibacterial and keratolytic effects. Its mechanism of action is primarily through its antibacterial effect. In addition, patients treated with sulfur showed a decrease in fat and free fatty acids as well as desquamation (drying and peeling activity). Nonetheless, ther was no evidence of systemic toxicity due to sulfur in humans (JSJ Pharmaceuticals 2008).

Essential oil or essential oil is commonly called a kind of volatile oil and contain the essential ingredients of a plant produced through steam distillation with water or an aromatic plant. Components of the essential oil is formed from the secondary metabolism of plants and stored with a particular structure. Usually they are isolated so little human intervention is obtained that can change its chemical composition. Citrus oils, produced from the juice of orange peel mechanically also called essential oils. According to ISO, the oil obtained from the dry distillation as cade oil (from stem *Junipherous oxycedrus*) and *Styrax pirogenee* (from Liquidamber spp.) (Burfield 2003).

Lime (*Citrus aurantifolia*) is a plant of the tribe Rutaceae. Lime includes herbaceous plant with many branches and twigs. Woody trunk is tenacious and hard. The surface of the old outer skin is dull. Orange juice is beginning to bear fruit at the age of two and a half years. Orange juice contains a lot of useful chemicals such as geranil acetate, limonene, linalin acetate, fellandren and citral. Based on the research trials, lemon essential oil contains limonene 90.01% which is the highest amount compared to other Rutaceae plants (Sokovic et al 2007). In another test, the components of essential oils can be annoying uncoupler of proton translocation in membrane vesicles and disrupts phosphorylation of ADP (Schelz et al 2010). In a further study, lemon essential oil showed antibacterial effects on *Staphylococcus epidermidis*. However, no studies have shown how the comparison between the antibacterial effect of sulfur commonly used in medicine to treat acne vulgaris and lemon essential oil. The purpose of this study was to compare the antibacterial effects of sulfur and lime essential oils.

MATERIALS AND METHODS

This type of research used in this study was a laboratory experimental design to compare the antibacterial activity of essential oil of lemon peel (Citrus aurantifolia) and the sulfur against *Staphylococcus epidermidis*. The sample used in this research is the essential oil of lemon rind (Citrus aurantifolia) and sulfur tested on *Staphylococcus epidermidis*. This study used a factorial design. The characteristics of the study was a factorial design where there are two or more groups as a control variable or a comparison of the variables studied. The number of repeat 5 times iteration.

The samples used as many as 25 medium Mueller-Hinton Broth (MHB) liquid (5 treatments / liquid MHB) for each bacteria tested, each treatment trials for 5 times. The sample consists of one (1) application using essential oils with higher levels of 3.125%, one (1) application using essential oils with levels of 6.25%, one (1) application using essential oils with high levels of 12.5%, one (1) applications using essential oils with high levels of 25% and one (1) application using essential oils with higher levels of 50%. In this test made two (2) types of control, namely the positive control (MHB medium density liquid and bacteria with 108 CFU/ml, equivalent to 0.5 Mc Farland turbidity (PML Microbiological, 2001)) and a negative control (MHB liquid media).

In addition, for comparison, we also performed the same steps on the sulfur. 5 The number of treatments that looping 5 times. The samples used by 25 MHB liquid medium (5 treatments/ liquid MHB) for each bacteria tested, each treatment trials for 5 times. The sample consists of one (1) application using the sulfur content of 3.125%, one (1) application using the sulfur content of 6.25%, one (1) application using the sulfur content of 12.5%, one (1) application using sulfur with levels of 25% and one (1) application using the sulfur content of 50%. In this test made two control anyway, which is a positive control and a negative control.

Research for the pre-treatment carried out at the Laboratory of the Faculty of Pharmacy, University of Airlangga, while for stage treatment is done at the Department of Medical Microbiology Faculty of Medicine, University of Airlangga. Research testing the antibacterial activity of essential oil of lemon peel (*Citrus aurantifolia*) on the growth of Escherichia coli and *Staphylococcus epidermidis* was conducted in January 2011. A description of the activity schedule in attachment.

Method for making essential oils used are water and steam distillation. Distillation or steam distillation and the water is done by weighing the lemon rind that has been cleaned and separated from the fruit according to the refining capacity of the tank, then chopped (cut into small pieces) to expand the cross-sectional surface. Put chopped lemon rind over the wire netting that separates it from the boiling water, until only exposed to the vapor. The distillation process carried out for 24 hours. Essential oil obtained was separated from the water by using pumpkin oil separator, and then stored in dark bottles at a temperature of 5°C before they were used to test the antimicrobial activity. To obtain a certain concentration, essential oils mixed with distilled water and Sulfoxides Dimetyl solvent (DMSO) 2% in a certain ratio just prior to use.

Implementation of *Staphylococcus epidermidis* antimicrobial activity test was performed aseptically by the method of dilution in order. In the test tube is made of a series of essential oil content ranging from 50% concentration; 25%; 12.5%; 6.25%; and 3.125% in distilled water and 2% DMSO as a solvent. Of each concentration of 2 ml were taken and put into a test tube that already contains a bacterial culture test in 2 ml of liquid medium with a density of bacteria MHB 108 CFU / ml, equivalent to 0.5 Mc Farland turbidity (cloudiness mixture of barium sulfate and HCl), then incubated at 37ºC for 18-24 hours. Levels of minimal inhibitory (MIC) was determined by looking at the smallest levels that still inhibits the growth of bacteria from each concentration (compared to the negative control, if murky, meaning there are no obstacles, otherwise if a clear means no inhibition of essential oils) obtained measured later recorded and compared with the activity of sulfur.

RESULTS

Dilution test data on essential oil of lemon peel (*Citrus aurantifolia L.*) and the sulfur against *Staphylococcus epidermidis* shown in the table:

Table 1. Results of the dilution test essential oils of lemon rind (*Citrus aurantifolia L.*) and sulfur at various concentrations against *Staphylococcus epidermidis*

Material	Replication	Essential oil (%)					KHM
		50%	25%	12,5%	6,25%	3,125%	
Essential oil	I	J	K	K	K	K	50%
	II	J	J	K	K	K	25%
	III	J	J	K	K	K	25%
	IV	J	J	K	K	K	25%
	V	J	J	K	K	K	25%
Sulfur	I	J	K	K	K	K	50%
	II	K	K	K	K	K	-
	III	K	K	K	K	K	-
	IV	J	K	K	K	K	50%
	V	K	K	K	K	K	-
Positive control				K			
Essential oil negative control				J			
Sulfur negative control				J			

Notes:
K = Cloudy, J = Clear, positive control = suspension of *Staphylococcus epidermidis* untreated, negative control = Essential oils of lemon rind and without added sulfur bacterial suspension

In a second experiment, the third, fourth and fifth in *Staphylococcus epidermidis* showed that the essential oil of lemon peel (*Citrus aurantifolia L.*) has antibacterial activity against *Staphylococcus epidermidis*. This can be seen from the results of the dilution test to determine the Minimal Inhibitory Concentration (MIC), which shows only the clarity found only in tubes containing a concentration of essential oil of lemon rind (*Citrus aurantifolia L.*) by 50% and 25%; while on the tube that has a concentration of essential oil of lemon peel (*Citrus aurantifolia L.*) of 12.5%; 6.25%; 3.125%; 1.563% obtained turbidity.

Only in the first experiment, the test tube is a tube that shows the clarity test concentrations of essential oil of lemon rind (*Citrus aurantifolia L.*) by 50%; while the test tube showing turbidity is a test tube containing a concentration of essential oil of lemon peel (*Citrus aurantifolia L.*) by 25%; 12.5%; 6.25% and 3.125%. Clarity and turbidity test tube determined by comparison to the control tube with the same concentration.

In the first and fourth experiment we found results that sulfur has antibacterial activity against *Staphylococcus epidermidis*. This can be seen from the results of the dilution test to determine the Minimal Inhibitory Concentration (MIC) that shows the clarity starts from the tube that has a sulfur concentration of 50%; while on the tube that has a sulfur concentration of 25%; 12.5% 6.25% and 3.125% obtained turbidity. Clarity and turbidity test tube determined by comparison to the control tube with the same concentration. Sedangakn in the second experiment, the third and the fifth had no antibacterial activity of sulfur found in bacteria *Staphylococcus epidermidis.*

Positive control in the study aims to prove that the bacterium *Staphylococcus epidermidis* grown in liquid BHI medium after incubation for 24 h at 37°C, whereas the purpose of the negative control is to test whether the essential oil of lemon peel (*Citrus aurantifolia L.*) without bacterial suspension was not contains bacterial contaminants. At planting positive control obtained growth of bacterial colonies, whereas the negative control was not obtained growth of bacterial colonies.

Clarity tube was inferred by comparing the germ tube which had been planted with germ tubes were not planted at the same concentration. In the negative control did not appear to be any change in turbidity. This indicates that the essential oil of lemon peel (*Citrus aurantifolia L.*) had no contamination due to the growth of contaminating bacteria was inhibited.

DISCUSSION

We have conducted antimicrobial activity research of essential oils of lemon rind and the sulfur against *Staphylococcus epidermidis*, can be determined, in accordance with Table 5.1, the essential oil of lemon peel (*Citrus aurantiofolia L.*) has the potential antibacterial better than the sulfur against *Staphylococcus epidermidis*. Ability antibacterial lemon essential oil against *Staphylococcus epidermidis*, in this study, shows relatively consistent results. In experiment II, III, IV and V showed MIC 25% but at the trial I was at a concentration of 50%. This is in contrast with the results obtained in MIC sulfur. MIC sulfur tends to be less consistent. From experiments I and IV results are the same, namely the concentration of 50%, while experiments II, III and V at all levels of inhibition obtained. Levels of Minimal Inhibitory (MIC) of essential oil of lemon peel (*Citrus aurantiofolia L.*) against *Staphylococcus epidermidis* are more consistent and more effective than the MIC sulfur.

Sulfur has a long track record of use in dermatological disorders, among others, the treatment of acne vulgaris, anti-dandruff shampoo and as an antidote for exposure to radioactive substances. Sulfur is also useful in the treatment of wounds with keratin has a calendar notes in the treatment of skin rashes. Topically, sulfur has a keratolytic role through the formation of hydrogen sulfide through a reaction that depends on the direct interaction between sulfur particles and keratinocytes. Topically, sulfur can induce a variety of histologic changes, including hyperkeratosis, acanthosis, and dilatation of the capillaries. In addition, the sulfur baths have a long history in the treatment of psoriasis arthritis pain and infection, and is prescribed for asthma (Parcell 2002). Until now the exact mechanism of sulfur antibacterial effect has not been found. Therefore, the results obtained in its use tend to be inconsistent and vary on individuals. Although the effect is likely to limited and rarely found in topical use, a number of fatalities in the use of sulfur in children (Parcell 2002).

Essential oils contain a variety of chemical components are different and can be classified into four groups, namely terpene that has to do with isoprene, straight-chain compounds do not contain branched-chain, benzene derivatives, and a variety of other compounds, such as alcohol derivatives (linalool, borneol, cineol, eugenol), aldehydes (benzaldehyde, anisaldehid, citral), ketones (camphor, menthol, piperiton) (Guenther 1987). Lemon essential oil obtained from the meat with the skin fresh. This oil is used as a medicinal ingredient and flavor (fragrance) on food and drinks (Guenther 1987). Rind of lemon (*Citrus aurantiofolia L.*) contains saponins, tannins 1%, steroids, triterpenoids and volatile

oil containing D-limonene, Simena, Sitronela, sitronellal, ß-linalool which acts as an antibacterial.

Antibacterial used in this study is essential oil which is a natural substance that has been known to have antibacterial activity. Even clove essential oil has been used since long in various European hospitals to treat infections with Mycobacterium tuberculosis. Research has been done Mulyani et al (2009), the antibacterial activity of the essential oil from the leaves of lime against *S. epidermidis* is more active than the lemon rind, while the activity of the oil of lime rind more active against E. coli than the leaf oil. Plant essential oil of lemon (Citrus aurantifolia) has potential as an antibacterial against E. coli and *S. epidermidis*. Based on the above genus citrus fruits such as lemon peel contains essential oils that have potential as an antibacterial.

In *S. epidermidis*, oxygenated terpene class of compounds causing polar compounds cause lysis of the cell wall lysing *S. epidermidis*, *S. epidermidis* and eventually will be stunted. The observation of the activity of essential oil of lemon peel (*Citrus aurantiofolia L.*) and the sulfur against *Staphylococcus epidermidis* obtained the smallest concentration of essential oils that is able to inhibit the growth of *Staphylococcus epidermidis* is consistently in the test tube II-IV was 25% but showed MIC 50% in I tube, while the smallest sulfur concentration to inhibit the growth of *Staphylococcus epidermidis* is 50%, but in tube II, III and V inhibition can not be found at all. This means that the essential oil of lemon peel (*Citrus aurantiofolia L.*) in concentrations of 25% can inhibit the growth of *Staphylococcus epidermidis*, while sulfur can inhibit the concentration of 50% after incubation for 24 hours at 37? C.

Various things may make different results in Experiment IV is the presence of bacterial contaminants found in the preliminary test, namely *Bacillus subtilis* mainly on preparations of sulfur, and less inequality mixing essential oils and sulfur in a test tube though it was tested on 2% DMSO. In this study, it can be proved that the rind of lemon (*Citrus aurantiofolia L.*) can be an alternative in handling a wide range of new antibacterial dermatological cases. The concentration of essential oil of lemon required to inhibit *Staphylococcus epidermidis* smaller and less consistent than in sulfur.

Various studies mentioned above, is expected to open the opportunity to find a cure for the treatment of Acne vulgaris. New drugs can be prepared by chemical modification of the active substance contained in the essential oil of lemon rind. The discovery of new drugs proficiency level has a positive purpose, namely to increase the benefit of many plants that are all around us that was originally only used as a spice in the kitchen or food flavoring, such as lemon rind into a seasoning for some Indonesian specialties such as selling traditional *pecel*, *trancam*, *kobbu* and *bika ambon*, so it can be a medicinal plant that has antibacterial properties. This will benefit the health of the community and the world.

CONCLUSION

Essential oils of lemon peel (*Citrus aurantiofolia L.*) has the ability antibacterial against *Staphylococcus epidermidis* better than sulfur. Minimum Inhibitory Concentration (MIC) of essential oil of lemon peel (*Citrus aurantiofolia L.*) against *Staphylococcus epidermidis* tend to be at a level of 25%. While sulfur tend not to exist, despite showing an antibacterial effect on the level of 50%.

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