ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES Knowledge to Innovation

Vol 7, Suppl 2, 2014

ISSN - 0974-2441

Research Article

ANTIBACTERIAL ACTIVITY OF HYDROLYZED VIRGIN COCONUT OIL

JANSEN SILALAHI*, YADE METRI PERMATA, EFFENDY DE LUX PUTRA

Faculty of PharmacyUniversity of Sumatra Utara, MedanIndonesia 20155

Email: jansen@usu.ac.id

Received: 24January 2014, Revised and Accepted: 20February 2014

ABSTRACT

Objective:The aim of this study was to examine the influence of the partial hydrolysis of virgin coconut oil (VCO) on it's antibacterial activity.

Methods:The VCO used in this study was the productof UD SinarNias. Hydrolysis was carried out by enzyme and sodium hydroxide. Enzymatic hydrolysis using lipozyme was conducted in four different incubation time namely, 3 hours, 6 hours, 9 hours and 12 hours. Alkaline hydrolysis preformed with 25%, 50% and 75% NaOH calculated from the saponification valueof coconut oil. Acidified hydrolyzed VCO was extracted with n-hexane. Recovered hydrolyzed products were mixed with water (5 g in water to make 10 ml) to form water in oil emulsion (w/o). Antibacterial activity test was conducted against bacteria *Pseudomonasaeruginosa* (ATCC 25619), *Staphylococcusaureus* (ATCC 29737), *Staphylococcus epidermidis* (ATCC 12228) and *Propionibacterium acnes* (ATCC 6918) by diffusion agar method using the paper disc of 6 mm in diameter. Antibacterial activity of hydrolyzed VCO was compared with tetracycline and ampicillin.

Results: Un-hydrolyzed VCO did not show antibacterial activity but hydrolyzed oil did. The longer the incubation time and the higher the amount of NaOH used in the hydrolysis increased antibacterial activity. VCO hydrolyzed by enzyme was more effective than those hydrolyzed by sodium hydroxide. Hydrolyzed VCO were more effective against *Pseudomonas aeruginosa* than other bacteria.

Conclusions: Un-hydrolyzed VCO did not inhibit bacterial growth, while VCO after hydrolysis was found to have antibacterial activity. Hydrolyzed VCO by enzyme is more active asantibacterial than VCOhydrolyzed by NaOH. Tetracyclin and ampicillin were more active than those of hydrolyzed VCO.

Keywords:VCO, MCT, MCFA, lauric acid, monolaurin,partial hydrolysis, antibacterial

INTRODUCTION

Two kinds of oils can be obtained from coconut tree (*Cocosnucifera*) they are coconut oil (copra oil) and virgin coconut oil (VCO). Coconut oil is extracted from copra by heating process, while VCO is from coconut milk prepared from fresh and mature coconut meat of coconut fruit and processed at low temperature. Coconut oil and VCO are different from most of the other common oils which are usually composed of long chain fatty acids, while coconut oil is composed of short and medium chain fatty acids, and therefore classified as medium chain triglyceride (MCT). Coconut oil has been used in health promotion and also in ailments prevention and medication [1,2]. The quality of VCO is determined by medium chain fatty acid (MCFA) content, especially lauric acid which is influenced by variety and oil extraction process [3].

Antibiotic resistance is a consequence of the evolutionary adaptation of bacteria due to the indiscriminate use of antibiotics. In addition, a high cost and adverse effects are generally associated with synthetic antibiotics. The emergence of antibiotic resistance in microorganisms becomes a threat among medical community. There is a continuous need to discover new antimicrobial compounds with novel mechanisms of action for new infection diseases. Therefore, researchers are turning their attention to antimicrobial of plant origin. Antibacterial activity of ethanolic extract of citrus leaves on Escherichia coli and Pseudomonas aeruginosa was studied and found to be active [4]. Essential oils of some selected plants was evaluated for antibacterial activity on methicillin resistant staphylococcus aureus, and found that essential oils of Clove and Cinnamon to be more active against tested bacteria [5]. Evaluation of antimicrobial activity of Pithecellobiumdulce pod pulp was conducted and found to be potential bactericidal and fungicidal [6]. Potential of medication of coconut oil and coconut products was discovered by Jon Kabara in the year of 1970s, who found that coconut oil has antibacterial, antiviral and antifungal activities exerted by free MCFAs and mainly by their monoglycerides molecule, especially monolaurin[7,8].

VCO contains high lauric acid content (46-50%) attached to glycerol backbone to form a triglyceride. In the human gastrointestinal tract,triglycerides in VCO can be converted into free fatty acids mainly lauric acid and monolurin which are active as antibacterial, antiprotozoal, and antiviral components. Moreover, MCFAsare easily absorbed into cells and then to mitochondria, increase metabolism, and hence the cells work more efficiently to form new cells and hence substitute damaged cells faster [3,8,9].

Antimicrobial activity is due to free fatty acids of medium chain and their monoglycerides. Triglyceride and diglyceride are not effective as antibacterial. Of the free fatty acids present in coconut oil, lauric acid (C:12:0) is proven to be more active as antibacterial agent compared to caprilic acid (C8:0), capric acid (C10:0), and myristic acid (C14:0). Free fatty acids and their monoglycerides inactivate bacteria by disrupting plasma membrane of lipid bilayer[7,10].

Antibacterial activity of free fatty acid or its monoglyceride has been tested separately [7]. Combination or mixture of free fatty acidsand their monoglycerides generated from coconut oil could be synergistic in bacterial inactivation. To generate monoglyceride from VCO can be done by enzymatic hydrolysis which is effective specifically on sn-1 and sn-3 position. This specific enzyme can be obtained from pancreas, *Thermomyces lanuginose* and *Mucormiehei*[11]. Hydrolysis can also be done by saponification reaction with alcoholic sodium or potassium hydroxide solution. Saponification byNaOH with or above saponification value will hydrolyze all triglycerides completely in to glycerol and free fatty acids as soap [12,13]. However, hydrolysis using NaOH lower than NaOHneeded for total hydrolysis (saponification value) would partially hydrolyze oil into mixture of free fatty acids and their diglyceride or monoglyceride derivatives depending on the amount NaOH used. The aim of this study was to compare the influence of

partial hydrolysis of VCO by enzymeand NaOH on their antimicrobial activity.

MATERIALS AND METHODS

Apparatus used including vortex (Bender,Germany), analytical balance (Sartorius, Japan), hotplate (Heidelberg, Germany), autoclave, oven, spectrophotometer (Shimadzu, Japan), incubator, reflux condenser, water bath, burette, and glass wares. All chemicals were pro analysis grade product of E. Merck (Germany) including potassium and sodium hydroxide, n-hexane, methanol, ethanol, tris-hydroxymethylaminomethane, hydrochloric acid, calcium chloride, anhydrous sodium sulfate, phenolphthalein (1% in ethanol) and Lipozyme TL IM.

VCO used in this study was product of UD SinarNias. Culture media used were Nutrient Agar(NA), Nutrien Broth (NB), and Mueller Hinton Agar (MHA). Bacteria tested were *Pseudomonas aeruginosa* (ATCC 25619), *Staphylococcus aureus* (ATCC 29737), *Staphylococcus epidermidis* (ATCC 12228) and *Propionibacterium acnes* (ATCC 6918). Paper disc used was of Machereynagel with 6 mm in diameter. Antibacterial activity of hydrolyzed VCO was compared with those of tetracycline and ampicillin.

Reagents used were calcium chloride solution of 0.063 M, Tris-HCl buffer solution with the pH of 8, HCl solution of 0.5 N, KOH of 0.5 N, NaOHof 0.5 N, 1% phenolphthalein indicator solution. These solutions prepared according to procedure described in Indonesian Pharmacopeia [14]. Medium used were Nutrient Agar(NA), Nutrien Broth (NB), and Mueller Hinton Agar (MHA). Preparation of these media used as described in Difco Laboratory Manual [15].

Enzymatic Hydrolysis

Fifty (50) g of oil placed in an erlenmeyer of 250 ml to which 50 ml water, 12.5 ml $CaCl_2$of 0.063 M, 25 ml buffer solution Tris-HCl and 500 mg lipozyme were added. This mixture was stirred with magnetic stirrer for 10 minutes to homogenize. Then it was allowed to stand (incubated) for various length of time; 3, 6, 9, and 12 hrs at temperature of 40 ± 0.5^{o}C, and shaken the mixture for 10 minutes in every one hour during incubation. After the hydrolysis was completed, the mixture was transferred into separating funnel, acidified with dilute HCl, extracted with 50 ml n-hexane resulted in two separated layers [13,16]. The upper layer (n-hexane fraction) was separated and called as filtrate I. The bottom layer was extracted again with 50 ml n-hexane and separated as filtrate II. These two filtrates were combined to which then 50 mg anhydrous Na_2SO_4 added and allowed to stand for 15 minutes. It was then evaporated on a water bath to dryness. The recovered hydrolyzed oil was used in the antibacterial experiment after acid value was determined.

Hydrolysis with Sodium hydroxide

Ten (10) g of oil placed in a 250 ml conical flask and 100 ml methanolicNaOHof 0.5 N was added in to it. The flask was attached with reflux condenser and heated. As the ethanol boiled, the flask occasionally shaken till the fat completely saponified (~3 hours). Solution allowed to cool and added 1 ml solution of 1% phenolphthalein indicator, titrated with HClof 0.5 N till the red color disappeared. Saponification value was calculated as the amount in mg of NaOH needed for the saponification [12,13].

$$\text{Saponification Value} = \frac{(\text{ml NaOH} \times \text{N NaOH}) - (\text{ml HCl} \times \text{N HCl}) \times 40 \text{ g/mole}}{10 \text{ g}}$$

Partial hydrolysis of oil was performed with the same procedure as described in saponification procedure but the amount of NaOHused was lowerthan the amount of NaOHused in the total saponification value. Fifty (50) g oil was weighed then added methanolicNaOH with the amount of 25%, 50% and 75% from saponification value, and hydrolysis procedure conducted as already described for 3 hrs. After hydrolysis, then the mixture acidified with dilute HCl in order to convert soap (sodium salt of fatty acids) into free fatty acids. Acidified mixture was then shaken and extracted with 50 ml n-hexane resulted in two separated layers. The upper layer (n-hexane fraction) separated called as fraction I. The bottom layer shaken with 50 ml n-hexane and allowed to stand for a while then hexane fraction was taken as fraction II. The two fractions were combined and dried by adding 50 mg anhydrous Na_2SO_4, allowed to stand for 15 minutes. Dehydrated hexane fraction was then heated on a water bath to evaporate hexane, and dried hydrolyzed oil was then used for acid value determination prior to antimicrobial test.

Acid Value Determination

Acid value determination was carried out for un-hydrolyzed VCO and hydrolyzed oil. Five gram oil was transferred in to 200 ml erlenmeyer, added 25 ml neutralized ethanol of 95%, then heated for ten minutes on a water bath and occasionally shaken. This solution then titrated with KOH of 0.1 N using phenolphthalein indicator solution. Acid value of the oil was calculated [12].

$$\text{Acid value} = \frac{A \times N \times 56.1}{W}$$

Note:

A= the amount of ml KOH for titration

N = normality of KOH solution

W= weight of oil (g)

Antibacterial Activity Test

Bacterial inoculum was prepared by suspending bacterial colony in Nutrient Broth Media solution and turbidity was measured at 580 nm to have transmittance of 25% (bacterial concentration is 10^{6}cfu/ml). Antibacterial activity test of VCO and hydrolyzed VCOwas conducted and the results compared with tetracyclineand ampicillin.The volume of 0.1 ml bacterial inoculum was mixed with 15 ml MHA in a petri dish, allowed to stand until the media solidified. Tested material was prepared as an emulsion by mixing VCO and hydrolyzed VCO in water at the same amount of sterile distilled water (5 g oil mixed with water to 10 ml, concentration was 500 mg/ml). Paper disc was then dipped in the emulsion for 15 minutes and then incubated in prepared media at 36 - 37^{o}C for 24 hours. Antibacterial activity was determined by measuring diameter of transparent area around the paper disc (zone of inhibition).The concentration of the tetracyclin and ampicillin tested were prepared in 5 mg/ml, 1 mg/ml and 0.1 mg/ml. The test was conducted in three replicates [14,17].

RESULTS AND DISCUSSION

Acid value of VCO and hydrolyzed VCO

Partial hydrolysis of VCO resulted in the generation of free fatty acids in hydrolyzed VCO, which was measured by acid value. Acid value of VCO and VCO partially hydrolyzed by NaOH and enzyme is presented in Table 1.

Tabel 1: Acid value of hydrolyzed virgin coconut oil

Hydrolysis method	Incubation time and degree of saponification	Acid values (n = 3) (mg KOH/g oil)
Un-hydrolyzed	-	0.74 ± 0.153
Enzymatic	3 hour	72.02 ± 0.517
	6 hour	79.05 ± 3.405
	9 hour	108.08 ± 0.845
	12 hour	150.88 ± 0.818
Alkaline hydrolysis The percentage ofNaOHrelative to saponification value	25 %	68.15 ± 0.483
	50 %	133.87 ± 0.796
	75 %	199.77 ± 2.575

From Table 1 can be seen that the acid value of VCO increased after hydrolysis by enzyme and NaOH. The longer the incubation period in enzymatic hydrolysis and the higher the amount of NaOH used in the hydrolysis the higher the acid value.Acid value exerted by NaOH75%

was higher than that by enzymatic hydrolysis with incubation for 12 h. Enzymatic hydrolysis of a triglyceride molecule resulted in 2 fatty acid molecules and 1 molecule of 2-monoglyceride, while partial hydrolysis by alkaline was difficult to predict [7,13,19].

Zonesof Inhibition by VCO and Partially Hydrolyzed VCO on Tested Bacteria

Typical zonesof inhibition to evaluate the antibacterial activities by measuring diameter of paper disc in agar media of different hydrolyzed products are presented in Fig. 1.

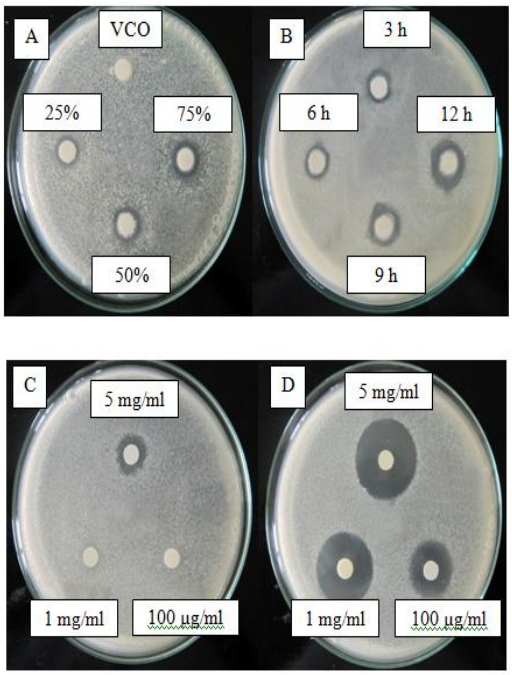

Fig. 1:Antibacterial activities shown by zones of inhibition by VCO, hydrolyzed VCO compared with tetracyclin and ampicillin against *Pseudomonas aeruginosa*

Note: (A) Zone of inhibition by VCO and hydrolyzed VCO by NaOH; (B) zone of inhibition by VCO and hydrolyzed VCO by enzyme; (C) zone inhibition by ampicillin; (D) zone of inhibition by tetracycline.

Zones of Inhibition of VCO hydrolyzed by enzyme and alkaline (NaOH) are presented in Table 2 and Table 3.Bacterial inactivation by enzymatic hydrolysis for 12 hours and that by alkaline hydrolysis (75%) were compared with those of tetracycline and ampicillin (Table 4).

Table 2: Antibacterial activityof VCO hydrolyzed by enzyme

Tested bacteria	Antibacterial activity of Hydrolyzed VCO by enzyme at different incubation time shown by zones of inhibition (mm)			
	3 hours	6 hours	9 hours	12 hours
P. aeruginosa	11.23±0.115	11.30±0.100	12.60±0.278[a]	13.43±0.208[a]
S. aureus	10.10±0.278	10.10±0.350	10.55±0.150	11.28±0.362[a]
S. epidermidis	9.03±0.076	9.68±0.161	10.65±0.477[a]	10.65±0.328[a]
P.acnes	9.45±0.050	9.57±0.153	10.13±0.681	10.08±0.465

Note[a)]Zonesof inhibition is significantly difference (P<0.05) compared with hydrolyzed by enzyme for 3 hours of incubation.

Table 3: Antibacterial activityof VCO hydrolyzed by NaOH

Tested bacteria	Antibacterial activity of hydrolyzed VCO byNaOHat different percentage relative to total saponification value shown by zonesof inhibition (mm)		
	25%	50%	75%
P. aeruginosa	9.87±0.881	10.18±1.056	11.35±1.039
S. aureus	9.20±0.409	9.03±0.029	10.00±0.229[b]
S. epidermidis	8.78±0.569	9.53±0.161	11.20±0.397[b]
P.acnes	9.18±0.808	9.83±0.382	10.53±0.161[b]

Note:[b)]Zone of inhibition is significantly difference (P<0.05) compared with hydrolyzed by NaOH at 25% of saponification value.Negative antibacterial activity indicated by 6 mm in diameter.

Table 4: Bacterial inhibition of hydrolyzed VCO and antibiotic tetracycline and ampicillin against tested bacteria

Tested bacteria	Zones of Inhibition (mm)			
	Hydrolyzed VCO (500 mg/ml)		Antibiotic (mg/ml)	
	Enzymatic (12 hours)	Saponification (75%)	Tetracyclin (0.1)	Ampicillin (5)
P. aeruginosa	13.43±0.208	11.35±1.039	15.90±0.391	-
S. aureus	11.28±0.362	10.00±0.229	9.15±0.265	11.45±0.522
S. epidermidis	10.65±0.328	11.20±0.397	20.95±0.229	10.80±0.290
P.acnes	10.08±0.465	10.53±0.161	14.15±0.312	24.25±0.360

Note:(-)zone of inhibition is zero (if diameter is 6 mm)

Hydrolysis of VCO, either by enzyme or NaOH induced antibacterial activity, but enzymatic hydrolysis was more inductive than by alkaline hydrolysis. Enzymatic hydrolysis resulted in the formation of a mixture containing free fatty acids, monolaurin, and triglycerides depending on the incubation time. The composition ofoil after alkaline hydrolysis (partial hydrolysis) would be composed of free fatty acids, monoglycerides, diglyceridesand/or un-hydrolyzed triglycerides depending on the amount of NaOH used. The most potential antibacterial activity of MCFA exerted by free fatty acid and monoglycerides which may inactivate bacteriaby disrupting microbial plasma membraneof lipid bilayer. Of the many saturated fatty acids, lauric acid (C:12) shown to be the most active as antibacterial compared to caprilic(C8:0), carpric(C10:0), and myristicacid (C14:0) [7,10,18].

In this study, VCO did not show to have antibacterial activity on tested bacteria, because it contained small amount of free fatty acid and there was no monolaurin present.On the other hand, a study showed that VCO without hydrolysis was effective on *Pseudomonas aeruginosa*and*Staphylococcus aureus*, using glycerin as solvent [20].Bacterial growth inhibition by hydrolyzed VCO was found to be more active against gram negative *Pseudomonas aeruginosa*than gram positive *Staphylococcus aureus*. The inhibition of*Staphylococcus epidermidis*, was found to be higher by VCO hydrolyzed by alkaline than that by enzyme, but antibacterial activity was very low against *Propionibacterium acnes.*

The evaluation of inhibition can be classified into three categories based on the diameter of zones of inhibition; very active (above 11 mm), medium activity (active) (between 6-11 mm), while non-active (6 mm).According to this criterion, un-hydrolyzed VCO was not active as antimicrobial, where as hydrolyzed VCO by enzyme for 12 hours and by alkaline of 75% were very active since the diameter of zones of inhibition were above 11 mm (13.43 mm) and 11.35 mm respectively [21]. The antibacterial activity of synthetic

monolaurinagainst *Staphylococcus aureus*was previously conducted [18] and reported that zone of inhibition was 13 mm (500 mg/ml) was better than hydrolyzed VCO in this study with inhibition zone ranged from 10-11 mm (500 mg/ml) on the same species of bacteria.This difference could be due to lower content of monolaurin in the partially hydrolyzed VCO in the present study.

Bacterial inhibition was more effective on gram negative than gram positive bacteria. It is probably due to the components of hydrolyzed VCO are non-polar molecules, and therefore they easily interact with cell membrane and disrupting lipid layer present in the outer part of cell membrane of gram negative bacteria, while the cell membrane of gram positive bacteria composed of more peptidoglucan layer compared with that in gram negative bacteria.The peptidoglucan layer in gram positive bacteria is rigid and resistant to osmotic lysis [22,23,24].

Pseudomonas aerugiosa is an opportunistic bacteriacausing infection when the immunity system of the host is getting weaker.*Pseudomonas aeruginosa*could survive from host immunity system because this bacteria has lipidpolysacharide as a protectingcomponent [25,26]. It is postulated that the mechanism of how lauric acid and monolaurin may inactivate bacteria is that by dissolving lipid component present in bacterial cell membrane [27]. Lipidpolysacharide present in *Pseudomonas aeruginosa*membrane through which lauric acid and monolaurin may interact and disrupt bacterial cell membrane.

Propionibacterium acnes is a gram positive bacteria, itcan not be inhibited by hydrolyzed VCO. This bacteria may cause skin acnes, a local inflammation on hair follicle resulted from two stages. In the first stage is that the excessive sebaceous secretion accumulates in the hair follicle that is previously blocked by ceratine cells (komedo). On the second stage is the formation of acne, the excessive sebum converted into fatty acid by lipase enzymereleased by skin normal flora *Propionibacterium acnes*, resulting in inflammation on the follicle. Acne medication can be done by reducing sebum productionwith retinoic acidor by lifting off komedoand decreasing fatty acid content or lipid on the skinwith benzoyl peroxide [24,27].

It is still not clear by which mechanism the fatty acids acting as antimicrobial agent. But the main target is cell membrane of bacteria and other mechanisms may involve on the membrane. Retarding growth effect is related to amphiphilic property of fatty acids enabling them to interact with cell membrane generating temporarily or permanent pores of various sizes. With the high concentration, detergent such as free fatty acids being able to dissolve cell membrane and hence releasing or disrupting larger portion.Free fatty acids also influence energy production in cell membrane by disturbing electron transport chain and oxidative phosphorilation [28].Probable other processes are cell lysis, impairing enzyme activities, inactivating macromolecular synthesis, disturbing nutrient absorption or protein DNA denaturation. Monolaurinmay act as antimicrobial agent by this mechanism [28,29].

From Table 4 can be seen that antibacterial activity of enzymatic hydrolysis is greater than that of alkaline hydrolysis against P. aeruginosa and S. aureus, but similar toward S. epidermis and P. acnes. Hydrolyzed VCO indicates much lower antibacterial activity compared with tetracyclin and ampicillin at very low concentration. Tetracyclin and ampicillin show different activity against tested bacteria. Tetracycline is most active toward S. epidermis and the lowest on S. aureus. On the other hand ampicillin is active against P. aeruginosa and it is most active against P. acnes. It is reported that monolaurin and lauric acid derived from coconut oil inactivate pathogenic bacteria but not the beneficial microorganismor probiotic. In addition, lauric acid and monolaurin do not develop microbial resistance while the antibiotic would do [30,31].

CONCLUSIONS

Un-hydrolyzedVCO is not active as antimicrobial, but partial hydrolysis will increase antibacterial activity. The longer incubation time in enzymatic hydrolysis and the higher the percentage of NaOH relative to total saponification during alkaline hydrolysis resulted in the more effective in antimicrobial activity of hydrolyzed VCO.Hydrolyzed VCO is more effective against *Pseudomonas aeruginosa* (gram negative) compared to other tested bacteria. Hydrolyzed VCO is not as effective as tetracycline and ampicillin. Ampicillin is not effective against*Pseudomonas aureginosa*. The benefit of VCO used orally as antibacterial is that VCO does not cause any side effectsince it is a common food component which will be hydrolyzed by lipase in the gastrointestinal tract. Antibacterial activity of hydrolyzed VCO is necessary evaluated by *in vivo* experiment in order to determine the effective dosage of VCO.

REFERENCES

1. Conrado SD. Coconut Oil In: Health and Disease: Its And Monolaurin's Potential As Cure For HIV/AIDS. Cocotech Meeting Chennai. 2000; XXXVII.
2. EnigMG.Health and nutritional benefits from coconut oil and its advantages over competing oils. Indian Coconut Journal 2010; September: 9-15.
3. Sari N. The effect of virgin coconut oil (VCO) on the profile of immunohistochemical antioxidant superoxide dismutase (SOD) in the kidney of diabetic rat. Thesis.FakultasKedokteranHewan. InstitutPertanian Bogor 2009
4. Das S, Borah M, Ahmed S. Antibacterial Activity of the Ethanolic Extract of Leaves of Citrus maxima (Burn) Merr. On Escherichia coli and Pseudomonas Aeruginosa. Asian J Pharm Clin Res, 2013; 6 (Suppl 4): 136-139.
5. Kaushik S, Tomar RS, Shrivastava V, Shrivastavav a, Jain SK. Antimicrobial Efficacy of Essential Oils of selected Plants and vaccine Design AgainstmecA Protein of Methicillin Resistant StaphyliococcusAureus. Asian J Pharm Clin Res, 2014; 7(Suppl 1): 52-56.
6. Pradeepa S, Subramanian S, Kaviyarasa V. Evaluation of Antimicrobial Activity of PithecllobiumDulce Pod Pulp Extract. Asian J Pharm Clin Res, 2014:7(Suppl 1): 32-37.
7. Kabara JJ, Swieczkowski DM, Conley AJ, Truant JP. Fatty Acids and Derivatives as Antimicrobial Agents. Antimicrobial Agents Chemotheraphy.1972; 2(1): 23-28.
8. Marina AM, CheManYB, Nazimah SAH, Amin, I. Chemical Properties of Virgin Coconut Oil. J Am Oil Chem Soc. 2009;86: 301-307.
9. Gupta A, Malav A, Singh A, Gupta MK, Khinchi MP, Sharma N, et al. Coconut Oil: The Healthiest Oil on Earth. International Journal of Pharmaceutical Sciences and Research.2010;1 (6): 19-26.
10. Enig MG. Health and Nutritional Benefits from Coconut Oil: An Important Functional Food for the 21st Century. AVOC Lauric Oil Symposium. Ho Chi Min City. Vietnam. 1996: 25 April.
11. Silalahi J. Modification of Fats and Oils. An Indonesian Pharmaceutical Journal. 1999; 7(1): 1-16.
12. Hamilton RJ, Rossel JB. Analysis of Oils and Fats. Elsevier Applied Science. London. 1987;12-15.
13. Boyer, R.F. Modern Experimental Biochemistry. Canada: Addison Wesley Publishing Company. 1986;361-368.
14. Ditjen POM. Farmakope Indonesia. Edisi IV. Jakarta: Departemen Kesehatan RI. 1995; 891-899.
15. Difco Laboratories. Difco Manual of Dehydrated Culture Media and Reagents for Microbiology and Clinical Laboratory Procedures. IX.Edn, Detroit Michigan: Difco Laboratories. 1977;32, 64.
16. Satiawihardja B. Studi Pembuatan Mentega Coklat Tiruan dari Minyak Sawit dengan Proses Interesterifikasi Enzimatik. Jurnal Teknologi Indonesia Pertanian. 2001;10(3):129-138.
17. Ugbogu OC, Onyeagba RA, Chigbu OA. Lauric Acid Content and Inhibitory Effect of Palm Kernel Oil on Two Bacterial Isolated and *Candida albicans*. African Journal of Biotechnology.2006;5(11): 1045-1047.
18. Widiyarti G, HanafiM, Suwarso WP. Study on The Synthesis of Monolaurin as Antibacterial Agent against *Staphylococcus aureus*. Indo. J. Chem. 2009; 9(1): 99-106.
19. Fessenden RJ, Fessenden JS. Kimia Organik. Jilid 2. Edisi III. Jakarta: Penerbit Erlangga. 1989; 408-412.
20. Ginting DP. Preparation and antibacterial activity test of Virgin Coconut Oil cream against *Staphylococcus aureus* ATCC 29737

and *Pseudomonas aeruginosa* ATCC 25619. Thesis. FakultasFarmasi. Universitas Sumatera Utara. 2008.

21. Nurliana, Sudarwanto M, Sudirman LI, Sanjaya AW. The Prospect of Acehnese Traditional Foods as a Healthy Food: The Initial Detection of Antimicrobial Activity of Pliek u Oil and Plieku Crude Extracts. *Disertasion*. Program studi Sains Veteriner. SekolahPascaSarjanaInstitutPertanian Bogor.2009.
22. Pratiwi S.T. Mikrobiologi Farmasi. Jakarta: Erlangga. 2008;23, 111-117.
23. Waluyo L. MikrobiologiUmum. Cetakankedua. Malang: Penerbit UMM Press. 2005; 191-212.
24. McKane L, Kandel J. Microbiology: Essential and Applications. Edisi II. New York: McGraw-Hills.Inc. 1996;76-84, 645.
25. Volk AW, Margaret FW. MikrobiologiDasar. Edisi V. Jakarta: Erlangga. 1988; 235.
26. Debois AP, Smith VJ. Antibacterial Free Fatty Acids: Activities, Mechanisms of Action and Biotechnological Potential. ApplMicrobiolBiotechnol. 2010;85: p.1629-1642.
27. Swanson JK. Antibiotic Resistance of Propionibacterium acnes in Acne Vulgaris. Dermatology Nursing. 2003; 15(4): 359-362.
28. Delden CV, Iglewski BH. Cell-to-cell Signaling and *Pseudomonas aeruginosa* Infections. Emerging Infectious Diseases. 1998;4(4): 551-560.
29. Skrivanova E, Marounek M, Benda V, Brezina P. Susceptibility of *Eschericia coli*, *Salmonella* sp. and *Clostridium perfringens* to Organic Acids and Monolaurin. VeterinarniMedicina. 2006;51(3): 81-88.
30. Lieberman S, Enig MG, Preuss HG. A Review of Monolaurin and Lauric Acid. Natural Virucidal and Bactericidal Agents. Alternative & Complementary Therapies. 2006; December:310-314.
31. Hasibuan DO, Silalahi J, Sitompul E.Antibacterial activity of the hydrolyzed virgin coconut oil against pathogenic and probiotic bacteria. Paper presented at the "Seminar NasionalFarmasi". FakultasFarmasi USU Medan, 29 September, 2012.