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Burn Wound Healing Activity of Hydrolyzed Virgin Coconut Oil

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Abstract: Virgin coconut oil (VCO) contains mainly medium chain fatty acids especially lauric acid, easily absorbed, has the potential to accelerate cell metabolism, moisten wound and has anti-inflammatory activity. Partial hydrolysis of VCO would result in free fatty acids, monoglycerides, and diglycerides. Combination of free fatty acids and monoglycerides especially lauric acid and monolaurin are active as antibacterial. The aim of this study was to investigate the effect of healing properties of partially hydrolyzed VCO on burn wound.
VCO used in this study was the product of UD Sinar Nias. VCO was partially hydrolyzed with NaOH solution at the amount of 35% and 70% of the total saponification value. Hydrolysate was acidified with dilute HCl and then extracted with hexane. Hexane extract was evaporated and the evaporated residue was used topically for the treatment of burn induced in rabbits. This study used 10 rabbits (1.5-2 kg) divided in to five treatment groups (negative control, positive control with Bioplacenton®, VCO 0%, 35% hydrolyzed VCO and 70% hydrolyzed VCO). Burn induced by placing a hot metal plate with diameter of 2 cm. Hydrolyzed VCO as tested material was applied topically to the wound every day as much as 0.1 ml. Lesion diameter was measured every day, and the time recorded until the diameter of the healed wound was zero.
The results showed that the healing process was the fastest when treated with hydrolyzed VCO at 70% (12 days), followed by VCO hydrolysis at 35% (15.5 days), VCO 0% (17.3 days), Bioplacenton ® (18.1 days) and the longest time of healing was with untreated/negative control (23.5 days). There was significant difference on the rate of healing of burn wound between each treatment. However, there is no significant difference between the positive control group (Bioplacenton®) and VCO 0% (without hydrolysis). The higher the level of hydrolysis of VCO the more effective the healing process of burn wound.
Keywords: Virgin coconut oil, burn, hydrolysis, healing activity.

Introduction

Burn wound appears as a damaged and injured tissue caused by heating by means of hot material including flame, hot water, chemicals, electric and radiation. Burn wound result in damaged and injured skin, and may affect whole body.[1] Principle of burn wound treatment includes closing the lesion as soon as possible, prevent of infection, reduce pain, prevent mechanical trauma to the skin and the elements within it, as well as to restrict the formation of scar tissue. Healing process of burns divided into three phases, namely the inflammatory phase, proliferation and termination.[2] In the early phase of inflammation, tissue regeneration and scar tissue formation starts with an inflammatory reaction (inflammation).[3] One of the pharmaceutical dosage form used for treatment of burns is Bioplacenton® in the form of gel containing bovine placenta extract and antibiotic neomycin and water.[4]

Virgin coconut oil, (VCO), when used topically, may function as protective barrier on skin and prevent infection, protect skin from free radicals, and moisturizing skin. VCO contains phytosterol that can be beneficial as anti-inflammation.[5] Coconut oil as triglyceride does not have antimicrobial and antiviral activities, but when VCO is partially hydrolyzed, it will generate free fatty acids and monoglycerides. The combination of free fatty acids and monoglycerides are proved to be antibacterial and antiviral agent where as diglyceride is not. Among the fatty acids and monoglycerides, lauric acid and monolaurin (monoglyceride of lauric acid) are

the most active as antibacterial and antiviral through several mechanisms including by liquefying and damaging the lipid layer structure in virus and cell membrane of bacteria.[6,7,8]

The use of virgin coconut oil (without hydrolysis) topically shorten recovery time was comparable to Bioplacenton in the treatment of chemical burns.[5] Un-hydrolyzed coconut oil is active as antibacterial, but partial hydrolyzed one is active. The higher the rate of hydrolysis of coconut oil, the greater the inhibitory activity against pathogens, but less active on probiotic bacteria.[8,9] The purpose of this study was to determine the efficacy of partially hydrolyzed virgin coconut oil on burns wound healing on rabbit as the experimental animals.

Materials and Methods

The equipments used in this study included an analytical balance, hotplate, upright cooler, burette, water bath, oven, thermometer, metal plate with diameter of 2 cm, calipers, scissors, razors, cameras and necessary glassware. All chemicals used were pro analysis grade product of E.Merc (Germany) included sodium hydroxide, potassium hydroxide, ethanol, hydrochloric acid, n-hexane, sodium sulfate, potassium biftalat, methyl red, alcohol. The sample used was VCO produced by UD Sinar Nias. Reagents used in including 0.5N HCl, ethanolic NaOH 0.5N, KOH 0.1N, methyl red and phenolphthalein solution as indicator.[10]

Procedure

Hydrolysis of Virgin Coconut Oil

Saponification value was determined using alkaline NaOH. Five (5) grams of oil transferred in to 250 ml round bottom flask, added 50 ml of ethanolic NaOH of 0.5 N, the flask was connected with reflux condenser and heated. As the methanol boiled, the flask occasionally shaken till the fat was completely hydrolyzed (about 3 hours). The solution was allowed to cool and added 1 ml of phenolphthalein indicator solution then titrated with 0.5 N HCl until the pink color disappeared. This procedure was repeated up on the same quantity of ethanolic NaOH without oil sample at the same time and at the same condition as blank determination/test. The amount of NaOH to saponify (total saponification) oil was calculated which is called as saponification value using NaOH.[8]

$$\text{Saponification value (mg NaOH/g)} = (V1 - V2)\ \frac{\text{ml} \times N \times 40}{W}$$

V1= Volume of HCl used in blank test; V2= Volume of HCl used in test; N= normality oh HCl; W= Weight of VCO (g);

Partial hydrolysis of oil was performed as described above but the amount of NaOH used was below (35% and 70%) from that used for total saponification. Fifty (50) gram of oil was weighed then added ethanolic NaOH at the amount of 35% and 70% from total saponification value. After hydrolysis, the mixtrue was acidified with dilute HCl in order to convert soap (sodium salt of fatty acids) in to free fatty acids. Acidified mixtrue then shaken and extracted with 50 ml n-hexane resulted in two saparate layers, and the upper layer, the hexane fraction which then separated as fraction 1. Extraction was repeated on the bottom layer to get the fraction 2. The two fractions were combined and dried by addition of 50 gram of anhydrous Na_2SO_4, allowed to stand for 15 minutes. Dehydrated hexane fraction (hydrolyzed oil) was then dried on water bath to evaporate hexane. The acid value of dried partially hydrolyzed oil was determined.[8]

Determination of Acid Value of Hydrolyzed Virgin Coconut Oil

Acid value was determined either for un-hydrolyzed and partially hydrolyzed oils. Five (5) g oil was weighed and transferred in to an erlenmeyer flask of 200 ml, added 25 ml neutralized ethanol of 95%, then heated for ten minutes on a water bath and occasionally shaken. This solution then titrated with 0.1 N KOH solution using phenolphthalein solution as indicator; titration stopped when the pink color emerged. [11] Acid value was calculated:

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

Note:

A : Total volume of ml KOH used for titration
N : Normality of KOH solution
G : weight of hydrolyzed oil (gram)

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Grouping of Animals

Experimental animals used in this study were ten (10) rabbits (weighing 1.5-2 kg), acclimatized for 5 days in cages and fed them every day. Animals were allowed to free access to water and standard chow diet up to the end of the experimental period and divided into five groups.

Group I : negative control group (no treatment)
Group II : positive control group treated with Bioplacenton®
Group III: test group of rabbits treated with un-hydrolyzed VCO (0%)
Group IV: test group of rabbits treated with hydrolyzed VCO (35%)
Group V: test group of rabbits treated with un-hydrolyzed VCO (70%)

Induction of Burns

Rabbits were cleaned and shaven on the back on the right and left side. Then anaesthetized on the side that has been shaved. Burns on the shaved area was induced by attaching a hot steel plate (2 cm in diameter) that has been heated in boiling water at a temperature of 100^0C for 10 minutes. A hot metal plate was attached to the shaved area on the back of rabbits for 10 seconds and then allowed to stand for 30 minutes prior to the treatment.[12]

Application of VCO on the Burns

Immediately after induction of burns, wound initial diameter was measured and given appropriate treatment groups once each day. Group I left without treatment, group II smeared Bioplacenton® 0.1 ml and group III, IV, V was smeared with un-hydrolyzed VCO of 0.1 ml; group IV with hydrolysis of 35%, and group V with 75% hydrolysis respectively. Topical application on the wound was carried out, and the wound diameter was measured every day until the wound was healed. Burn was declared to be healed if the diameter was zero (burn was disappeared).

Measurement of Burns Diameter

Diameter of induced Burns wound was measured using a caliper. The way of how to measure the diameter of the burn was performed as previously described.[13] Diameter was measured four times as can be seen in Figure 1, then the average from four measurements was calculated.

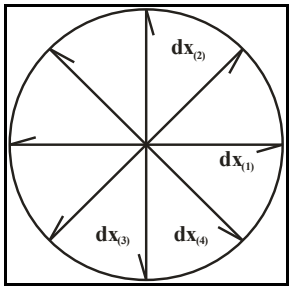

Figure 1. Method of measuring the diameter of burns

Diameter was measured four times as can be seen in Figure 1, then the average from the four measurements was calculated: $dx = \frac{d_1 + d_2 + d_3 + d_4}{4}$

Note: dx is the wound diameter on day x; d1=diameter 1; d2=diameter 2 etc.

Results And Discussion

Acid Value of Hydrolyzed Virgin Coconut Oil

Acid value is expressed as the number of milligrams of KOH used to neutralize the free fatty acids contained in 1 gram of oil or fat.[11] (Ketaren, 2005). Partial hydrolysis of VCO generated free fatty acid then acid value was determined to measure the amount of free fatty acids contained in an oil or fat. Weights and acid value of hydrolyzed oil is shown in Table 1.

Tabel.1 above shows an increase in acid value with the increasing amount of NaOH used in the hydrolysis through the process of saponification. Acid value of oil hydrolyzed with the level of saponification of 70% showed a higer acid value than saponofication at the level of 35%. Saponification is a hydrolysis process, in which free fatty acids will be separated from the oil (triglyceride molecules) in the form of soap (alkaline salts of fatty acids) and glycerols. Complete saponification (total hydrolysis) is achieved if alkaline hydroxyde is used in excess amount above saponification value.[11] But if the amount of the alkali used is lower than saponification value (partial saponification) then not all triglyceride completely saponified (hydrolyzed).

In this study, separation of fatty acids from triglycerides by the presence of alkaline by partial hydrolysis; the result is not easy to measure since hydrolysis may take place on randomly at any position (sn-1,2,3) in triglyceride molecule. The results obtained by partial saponification reaction could be free fatty acids, monoglycerides, diglycerides, or even some amount still remain as triglycerides due to insufficient amount of alkali to completely saponify all triglycerides, and so-called partial hydrolysis.[8] Hydrolysis at the level of 70%, the quantity of the fatty acids as free fatty acids is about 70%, and the rest (30%) is still attached to the glycerol molecule mostly as monoglycerides. Conversely, in the VCO hydrolyzed with saponification at the level of 35% contains only 35% exist as free

fatty acids, and the rest (65%) still attached as glyceride molecules mostly as diglycerides, and small amount could be as monoglycerides and possibly as triacylglycerol or triglycerides.

Tabel.1 Weight and Acid Values of Hydrolyzed Virgin Coconut Oil

No.	Level of Hydrolysis Relative to Saponification Value	Recovered Oil After Hydrolysis	Acid value (mg KOH/g Oil)*
1.	0% (without hydrolysis)	100 g	0.47
2.	35 %	87.01 g	93.52
3.	70%	83.58 g	173.55

Note : *) the values were the average of three replicates

Healing Effects of Coconut Oil Pure Against Burns

Burn Wound Healing Time

Induced burns made on the experimental animals can be classified as the second-degree burns (diameter of 2 cm) shown by the damage reaches the depth of the dermis but there are still remaining healthy epithelial elements. Burns diameter changes were measured until the wound healed declared (diameter wound = 0) for each treatment. Physical profile of the healing process of burns during the experimental period can be seen in Figure 2. Burns diameter changes in all groups presented in Fig.3 and 4.

The changes of burns diameter were measured until the wound recovery declared (diameter wound = 0) for each treatment. From Figure 3 and 4 can be seen that the healing process in the negative control group was found to spend the longest time for healing (23 days) indicated when diameter=0. The healing time in the positive control group (Bioplacenton®) was 18 days which was comparable with that of healing time (17 days) treated with the level of VCO hydrolysis at 0% (un-hydrolyzed).

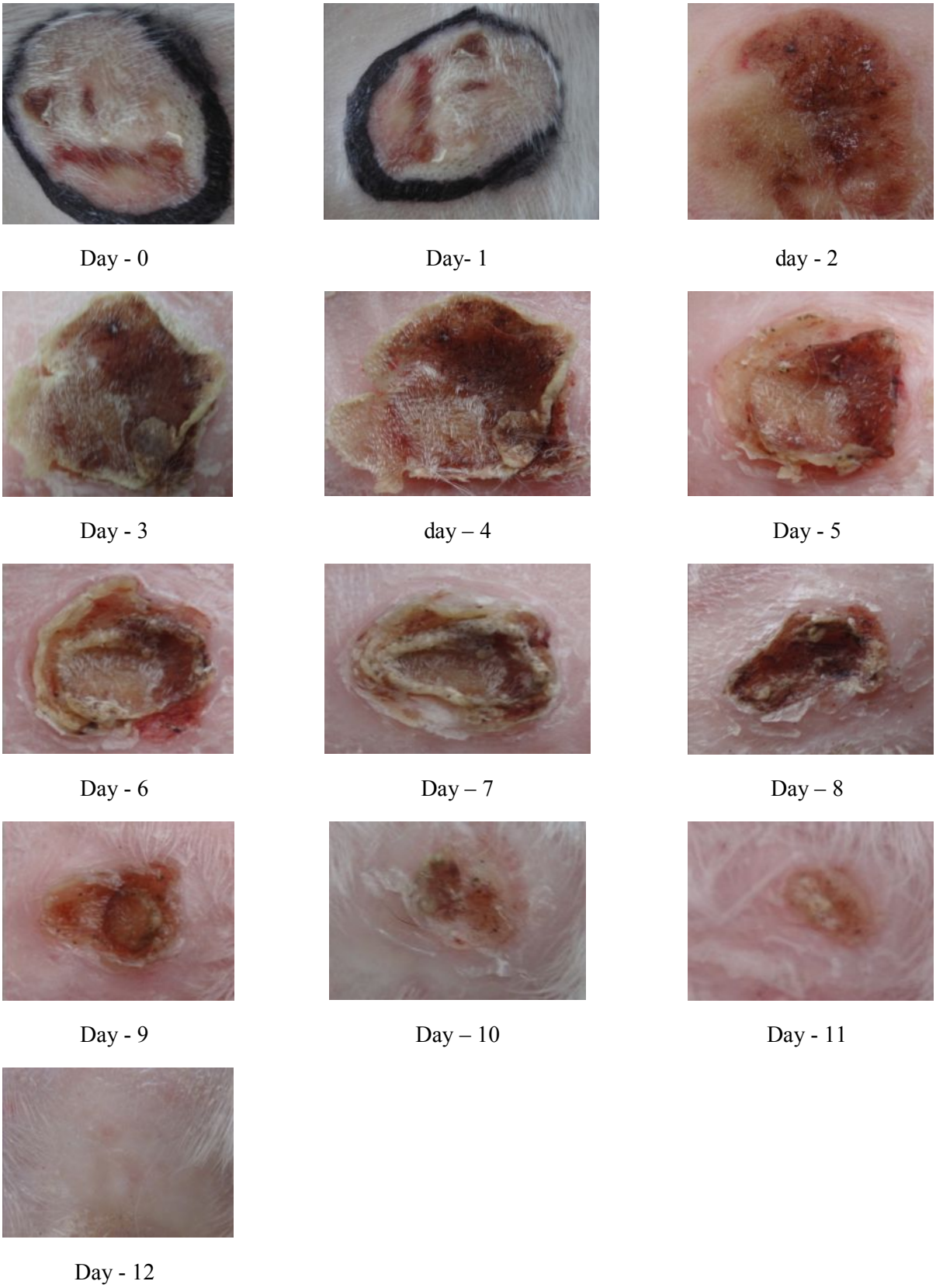

Figure 2. Physical Profile of Burn Healing Process in Group V

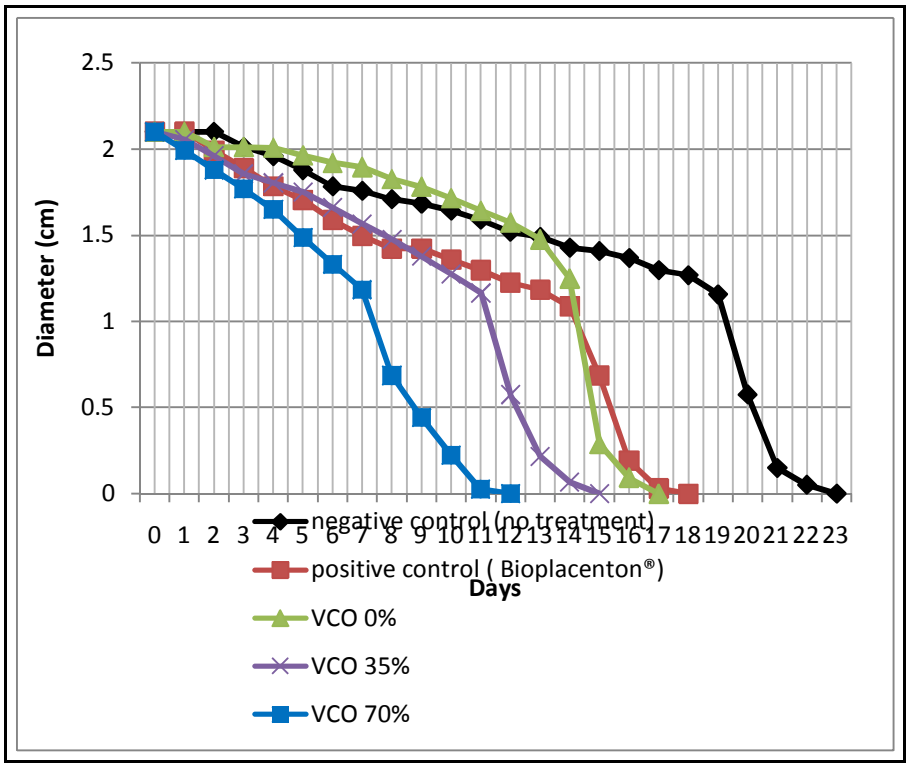

Fig 3. Duration of healing by hydrolyzed VCO based on the diameter of burn wound on rabbit

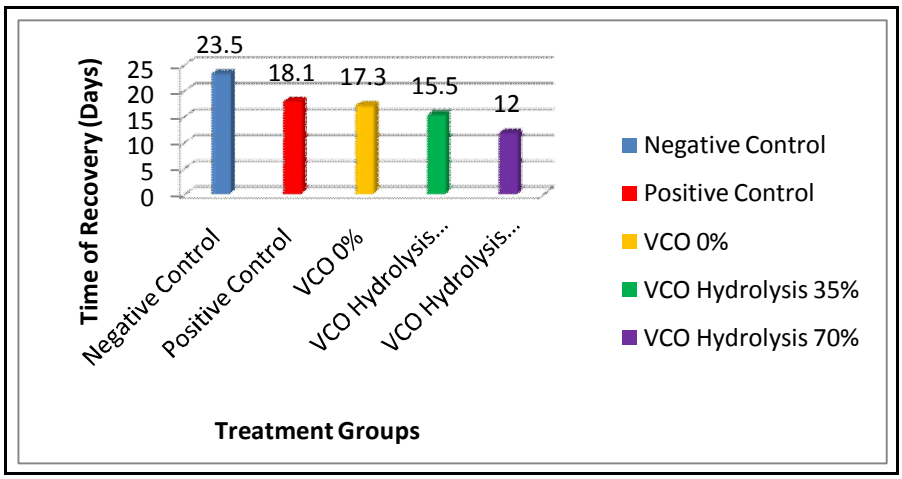

Fig 4. Duration of healing of burn wound treated with hydrolyzed VCO

The healing time of each treatment was different. The negative group shown to be the longest time of healing (23 days), and the positive group treated with Bioplacenton was shorter healing time (18 days) than negative group. Treatment of burn with Bioplacenton (a standard pharmaceutical preparation for burn) was a bit faster one day (17 days) the group treated with VCO without hydrolysis. Healing activity of un-hydrolyzed coconut oil could be due to the anti-inflammatory of the oil,[5] and presence of the free fatty acids as antibacterial agent,[7] as indicated by acid value in Tabel 1. Healing activities of the groups treated with hydrolyzed VCO were significantly better than treated with either by Bioplacenton or VCO without hydrolysis. The healing time in the group treated with hydrolyzed at level of 35% found to be 15 days and treatment with hydrolyzed VCO at level of 70% was 12 days, 3 days faster.

From this experiment can be seen that the healing activity of hydrolysis at the level of 70% was found to be the most active due to the higher content of free fatty acid and monoglycerides. This is also to indicate that the degree of hydrolysis of the VCO influences the acceleration of the healing of burns, because this hydrolyzed VCO contains the mixture of free fatty acids and their monoglycerides acting as antibacterial agent. In this study, the combination of free fatty acids and monoglycerieds is the highest in the hydrolyzed at 70%.[8]

Topically VCO serves as a moisturizer that provides an optimal environment for wound healing. VCO contains phytosterols or plant steroids are potent to reduce inflammation that can stop the bleeding and prevent widespread burns (Wijaya, 2012). VCO contains medium chain fatty acids, especially lauric acid (C:12:0) (Medium Chain Fatty Acid; MCFA) unique properties that is easily absorbed into the cells and increase metabolism. In addition to increasing metabolism, cells will work more efficiently to form new cells and replace damaged cells that will accelerate the healing of the sick.[7,8,14]

Conclusion

Hydrolysis degree of virgin coconut oil at levels of 0%, 35% and 70% shown to have different activity of healing on the burns. The higher the level of VCO hydrolysis the higher the activity on healing burn wound. Burn healing effects between groups un-hydrolyzed VCO (without hydrolysis) and Bioplacenton® was found to be comparable, while the healing effect of hydrolyzed VCO shown to be much faster than Bioplacenton®. Further study need to test the effect of hydrolyzed VCO in pharmaceutical dosage forms such as creams, gels for its healing activity on wound of any type.

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