

Formulation of Peel-Off Mask Containing Natural Antibacterial: Study on Poly Vinyl Alcohol (PVA) and Virgin Coconut Oil (VCO) Contents

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**Formulation of Peel-Off Mask Containing Natural Antibacterial: Study on Poly Vinyl Alcohol (PVA) and Virgin Coconut Oil (VCO) Contents**Dewi M. Hariyadi^{1*}, Isnaeni Isnaeni¹, Sisunandar Sudarma², Ni-Made K. Shandra¹, Noorma Rosita¹¹Department of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Surabaya-Indonesia²Department of Biology, Universitas Muhammadiyah Purwokerto, Indonesia

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ABSTRACT

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Some natural antibacterials have been investigated widely. Virgin Coconut Oil is one of natural resources which has been known to have antibacterial activity. Lauric acid as main component of VCO has been observed to have antibacterial activity for acne treatment. VCO and the extract have been tested previously for antibacterial activity. This research aims to prepare peel-off mask of VCO kopyor and investigate the effect of PVA and VCO concentration on the physical characteristics. VCO 20% and 30% were formulated containing Poly Vinyl Alcohol (PVA) in various concentrations (8% and 10%). The emulgel peel-off mask was then evaluated for viscosity, pH, drying time, spreadability, particle size and distribution, zeta potential, antibacterial activity, and also stability thermal cycling evaluation at the temperature $40\pm 2^\circ\text{C}$ and $4^\circ\text{C}\pm 2^\circ\text{C}$ in 3 cycles. The selected optimum formula was formula 1 containing 20% of VCO and 8% of PVA. This formula has a pH that is suitable with skin pH 4.5-6.5, had no significant difference in zeta potential, good spreadability, and provided antibacterial activity that had no significance different from other formulas that contain more concentration of VCO and PVA. In Vitro characteristics of peel-off VCO mask demonstrated potential therefore it is recommended for further *in vivo* study for acne diseases.

Keywords: Natural Antibacterial, PVA, VCO, Peel-off Mask, Acne Diseases.

Introduction

Pimple or *acne vulgaris* is an inflammatory condition on the skin, where there is a blockage in the sebaceous glands and hair (polysebaceous follicles).¹ The condition of the blocked pilosebaceous follicle and sebum build up occurs, resulting in *Propionibacterium acnes* bacteria to develop well because of the supportive environment.¹ To overcome the problem of acne caused by the presence of *Propionibacterium acnes* bacteria which cause many problems including youth's performances and infections,² treatment with antibacterial drugs such as benzoyl peroxide or a combination of antibiotics is required according to the severity of acne.³ However, the use of antibiotic drugs can cause resistance if used incorrectly. Rahmi *et al.*, 2015 reviewed that as many as 50% of the *Propionibacterium acnes* isolates with various strains studied from acne patients experienced resistance to the antibiotics clindamycin and erythromycin, while 20% of these isolates were resistant to tetracycline antibiotics.⁴ Therefore, the development of a new alternative medicine derived from natural resources is desirable. One of the most commonly found tropical plants in Indonesia is coconut (*Cocos nucifera* L.) (Arecaceae family) and one of them is the variety of kopyor, which is coconut that has genetic deviation. The main fatty acid contained in kopyor *endocarp* of coconut is lauric acid. It is known that lauric acid has a strong antibacterial and anti-inflammatory

effect on *Propionibacterium acnes* bacteria.⁵ *Endocarp* of coconut containing lauric acid can be extracted to obtain pure coconut oil or *Virgin Coconut Oil* (VCO). Some studies using VCO demonstrated antibacterial activity,⁵⁻⁷ however the formulation did not use a VCO peel-off method. Therefore, this research will observe the antibacterial activity of VCO in the forms of peel-off mask. According to Sia *et al.*, 2010, lauric acid is included in medium-chain saturated fatty acids which have strong antimicrobial effects.⁶ Nakatsuji *et al.*, 2009 mentioned that lauric acid has a strong antibacterial and anti-inflammatory effect against *Propionibacterium acnes* bacteria.⁵ Based on research by Santoso *et al.*, 1995, the results of the analysis of the fatty acid profile of kopyor coconut meat found lauric acid with a percentage of 46.9%.⁸ Coconut meat extracted by fermentation method, centrifugation method, or oil addition method can produce virgin coconut oil or hereinafter referred to as virgin coconut oil (VCO).⁹ According to Dumancas *et al.*, 2016, VCO contains high levels of saturated fatty acids such as lauric acid, caproic acid, and caprylic acid.¹⁰

The advantages of the peel off mask include easy to apply, does not cause greasiness and easy to clean because it will form an occlusive film that can be peeled off. Peel off masks can also increase the permeability of active agent because of their occlusivity.¹¹ One of the gelling agents commonly used for making peel off masks is polyvinyl alcohol (PVA).¹² PVA is a water-soluble synthetic polymer and has several functions, one of which is to increase the viscosity.^{13,14} The gel produced by polyvinyl alcohol dries easily and forms a strong transparent film, and adheres well to the skin.¹²

This study aims to determine the VCO concentration of kopyor plant that will be used in peel off emulgel mask to provide antibacterial activity against *Propionibacterium acnes* ATCC 11827, as well as determining the effect of concentration of *Poly Vinyl Alcohol* (PVA) on peel off mask's physical characteristics (pH, viscosity, spreadability, drying time, droplet size, size distribution and zeta potential).

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Materials and Methods

Collection of Plant Materials

VCO from Kopyor coconut were collected from Indonesian garden of kopyor coconut in 2-hectare areas of University of Muhammadiyah Purwokerto campus, Campus Dukuhwaluh, Kembaran, Purwokerto, and Central Java, Indonesia. The kopyor coconut was collected by Professor Sisunandar in 12 September 2019 (Sample voucher numbers PWTF 01-05). All pharmaceutical grades of chemicals were used for this research.

Production of Kopyor VCO using Centrifugation Method

Coconut meat was mixed with water in a ratio of 1:1 and was squeezed until coconut milk was obtained. Coconut milk was hushed up until two layers were formed. The two parts were then carefully separated. The part that was taken was the top coconut milk head and was centrifuged at 2500 rpm for 60 minutes. Three layers were then formed: oil in the upper part, oil pulp in the middle layer, and water in the lower layer. The oil layer was then carefully removed so that it did not mix with the oil pulp and water.¹⁵

Qualitative Examination of VCO

Qualitative VCO examination was aimed at ensuring that the VCO is of good quality. Parameter analysed includes; organoleptic properties in terms of color, odor, and taste; density; refractive index and lauric acid level, and compared to the standard reference.¹¹

Production of VCO Peel Off Emulgel Mask

Polyvinyl alcohol (PVA) and Carbomer 940 solution as formula in Table 1 were made. In Carbomer 940 solution, TEA was dripped to adjust pH and formed gel. Second, flourished gelling agent was mixed. Tween 80 was dissolved in followed by propylene glycol, phenoxyethanol, and Na EDTA as the water phase. Then, the water phase was mixed with gel base which had expanded and was stirred homogeneously. VCO was mixed with Span 80 and Butyl Hydroxyl Toluene (BHT) as the oil phase followed by addition into the base of PVA and carbomer 940 and the mixture was stirred until homogeneous.

Determination of Kopyor VCO

VCO was determined by refractive index using refractometer, density and VCO content using Gas Chromatography (GC).

Minimum Inhibitory Concentration (MIC)

Streak plates of *Propionibacterium acnes* bacteria on nutrient agar

plates were prepared. VCO concentration of 10-90% was prepared using polyethyleneglycol solvent. The positive control of 2.5% benzoyl peroxide was used. The negative control of polyethyleneglycol was prepared. The MIC was determined using disc diffusion method.^{16,17} The agar media was incubated at 35-37°C for 24 hours. The diameter of the inhibition zone was measured to determine the minimum inhibitory concentration.

Physical Characteristics Evaluation

pH and Viscosity test

The pH test was carried out using a pH meter on day 0 and 6 to check the pH stability. Measurements were replicated 3 times.¹⁸ The viscosity test on day 0 and 6 was also done using a cone and plate CP-41 spindle viscometer on the rate of shear of 1 rpm triplicates.¹⁹

Spreadability Test

The spreadability test was done using two scales of glass plates. Gel preparations were weighed ± 1 gram and was placed in the middle of a glass plate and then covered with another glass plate. At the top of the glass plate, a weight of 5 grams was given and then additional weight was given to the preparation until the preparation no longer spread. The measurement was replicated 3 times.¹⁸

Drying Time Examination

This test was done by weighing the gel of 700 mg. The gel was spread evenly on the glass plate with a size of 7.5 x 2.5 cm. The glass plate was put in an oven at 37 \pm 2.0°C to match the skin temperature. Drying time was monitored every 5 minutes until all gels had dried.^{20,21}

Particle size, polydispersity index (PDI), and Zeta potential

This test was conducted using Dynamic Light Scattering Malvern. VCO peel off mask were dissolved in a ratio of 1/100 (v/v). The particle size measurement, PDI and zeta potential (mV) was recorded.²²

Evaluation of Antibacterial Activity

Antibacterial activity test against *Propionibacterium acnes* bacteria was done in nutrient agar media disc diffusion method. The wellbore was filled with formulas of 100 mg. The agar medium was incubated at 35-37°C for 24 hours. The diameter of the inhibition zone was measured to determine the antibacterial activity.²³ Gel of 2.5% benzoyl peroxide was used as positive control.

Table 1: Formula of Kopyor VCO Peel Off Emulgel Mask

Chemicals	Functions	Content (%) in Formula					
		1	2	3	4	5	6
VCO	Active agent	20	30	20	30	-	-
PVA	Gelling agent / Film forming	8	8	10	10	8	10
Carbomer 940	Gelling agent	18	18	18	18	18	18
Triethanolamine	Alkalizing agent	0.5	0.5	0.5	0.5	0.5	0.5
Propylenglycol	Humectants, enhancers	15	15	15	15	15	15
BHT	Antioxidant	0.1	0.1	0.1	0.1	0.1	0.1
Tween 80	Surfactants	4.5	4.5	4.5	4.5	4.5	4.5
Span 80	Surfactants	0.5	0.5	0.5	0.5	0.5	0.5
Na-EDTA	Chelating agent	0.1	0.1	0.1	0.1	0.1	0.1
Phenoxyethanol	Preservative	18	18	18	18	18	18
Water	Solvent	Ad 100	Ad 100	Ad 100	Ad 100	Ad 100	Ad 100

Notes: Formula 1: 8% PVA with VCO 20%, Formula 2: 8% PVA with 30% VCO, Formula 3: 10% PVA with 20% VCO, Formula 4: 10% PVA with 30% VCO, Formula 5: 8% PVA gel base without VCO, Formula 6: 10% PVA gel base without VCO.

Evaluation of Physical Stability

The physical stability test of peel off mask were conducted at $4^{\circ}\text{C}\pm 2^{\circ}\text{C}$ and $40^{\circ}\text{C}\pm 2^{\circ}\text{C}$ for 3 cycles, during 6 days and observation was done on organoleptic, viscosity, pH, and zeta potential.

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Statistical analysis

Statistical analysis was carried out using the one-way analysis of variance (ANOVA) method and to determine the significant difference between formulas, a confidence level of 0.95 was used ($\alpha = 0.05$).

Results and Discussion

VCO Physical Qualitative and MIC

Results of organoleptic, refractive index, and density was found as same as APCC standard (Asian and Pacific Coconut Community). Kopyor VCO had a clear yellow color, fresh coconut scented, and has no taste. The refractive index of VCO was 1.4537 (20°C) and the value was in accordance with the standard refractive index of VCO at a temperature of 20°C that was 1.4480-1.4541.

The result of VCO density was 0.921 g/cm^3 . This value approaches the value of the standard density of VCO which is $0.915\text{-}0.920\text{ g/cm}^3$. However, For VCO lauric acid content, it showed 24.96%. This result was not in accordance with APCC standards regarding lauric acid content in VCO which is 45-56%. This can be caused by differences in coconut varieties used.²⁴ Apart from differences in coconut varieties, the amount of lauric acid is also influenced by the age of the coconut. Young coconut has lower lauric acid content compared to old coconut. For antibacterial activity test, VCO showed inhibition (MIC) against *Propionibacterium acnes* ATCC 11827 bacteria at VCO concentration of 20%. This research compared effect of 20 and 30% VCO on the physical characteristics of peel-off mask.

pH and Viscosity Evaluation

Based on the results on figure 1, there was a decrease in pH in the entire formulas when compared with the pH of the peel-off mask before the stability test was done. However, the pH before and after the stability test was remained in the skin pH range of 4.5-6.5.²⁴ A decrease in pH in the stability test can be caused by an increase in free fatty acid in VCO oil during the storage process. Free fatty acid can be oxidized thereby increasing free fatty acid when exposed to high temperatures.²⁵

From figure 2 viscosity results, statistical analysis using ANOVA was conducted and it demonstrated the comparison results between formulas had significantly different values. This showed that an increase in VCO levels and an increase in the PVA base level can increase the viscosity of the preparations.^{26,27} Based on the results of statistical tests using T-Test in pairs to compare the viscosity of the

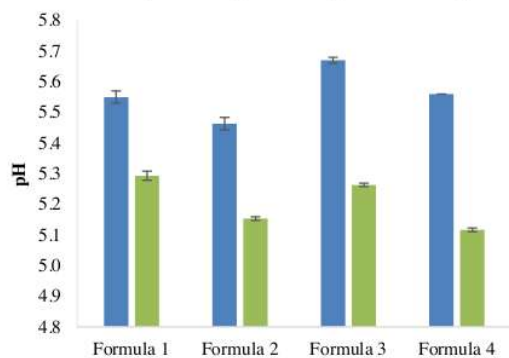


Figure 1: pH of the VCO peel off emulgel mask on day 0 (Blue) and 6 (Green).

preparations before and after the stability test treatment, the result showed that all formulas had significantly different values, and it can be concluded that formulas 1 to 4 produced an increased viscosity after the stability test. This can be caused by the lack of adding emulsifier to the formula with higher VCO levels, causing a difference in viscosity between before and after the stability test treatment. An increase in emulgel consistency can also be caused by the evaporation of the largest solvent component in the emulgel, which is water.¹¹

Spreadability and Drying Evaluation

Based on the slope value and the load to achieve maximum dispersion capacity from figure 3, it can be concluded that the base mask without active agent was easier to spread easier compared to the formula. It is appropriate that an increase in the viscosity of the preparations will reduce the spreadability of the preparations.²⁷

It can be seen from figure 4, there was an increase in the drying time for formula 1 to 4 from 30 to 35 minutes. VCO-containing peel-off mask had a longer drying time compared to the two gel base formulas without active agent. This showed the drying time of the four formulas were longer than the gel mask drying time in general, which was less than 30 minutes.¹⁹ This result was because VCO can withstand the evaporation of water in the preparation therefore it takes longer time to dry.

Antibacterial Activity of Peel-off Mask

All formulas showed inhibition against *Propionibacterium acnes* ATCC 11827 bacteria. The results of the antibacterial activity evaluation were shown in Table 2.

Based on one-way ANOVA statistical analysis test results, the ratio of inhibition between formulas did not significantly differ. This can be influenced by the low content of lauric acid in kopyor VCO, so it is necessary to optimize the selection of coconut based on its variety and the preparation technique of processing VCO.^{22,28}

Stability Evaluation

Observation of the stability was carried out about 3 cycles by storing in a temperature of $4^{\circ}\pm 2^{\circ}\text{C}$ and $40^{\circ}\pm 2^{\circ}\text{C}$ for 6 hours, this method was modified from Iradhati.²⁹ Furthermore, organoleptic, pH, viscosity, particle size, PDI, and zeta potential of the formulas were re-observed.³⁰

Table 3 showed the particle size of all formulas. Based on the statistical analysis, the average droplet size value of the formula increased after days 6 of stability test compared to day 0. During the storage, the droplets from emulsions in the gel have a large surface area so that the surface energy becomes large and reduce the energy therefore droplets tend to reduce their surface area by combining between droplets.

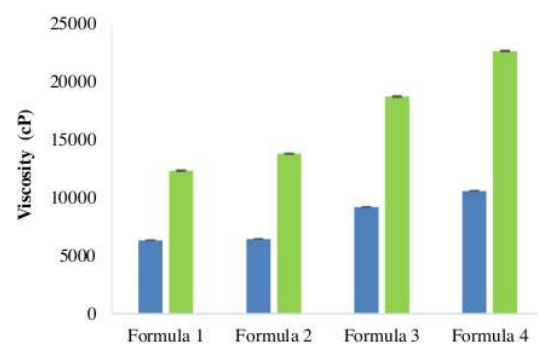


Figure 2: Viscosity of VCO peel off emulgel mask on day 0 (blue) and 6 (green)

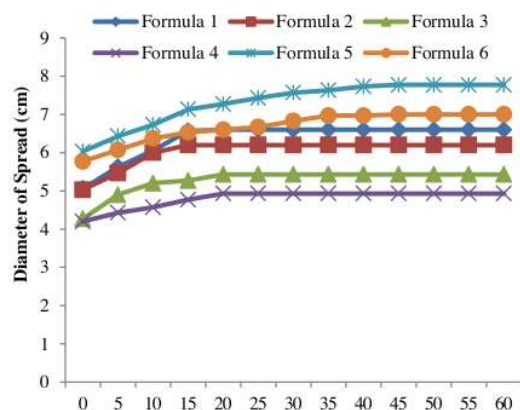


Figure 3: Spreadability test of VCO peel off emulgel mask

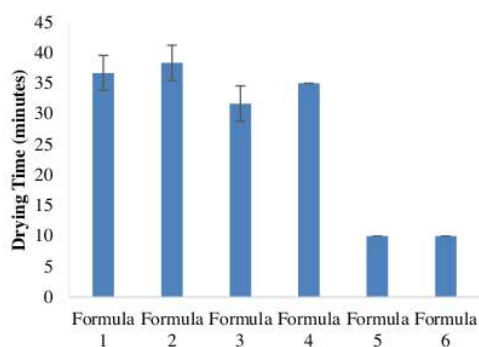


Figure 4: Drying time of VCO peel off emulgel mask

Table 2: Antibacterial Activity Test of VCO peel off Emulgel Mask

Formula	Inhibitory Zone Diameter (mm)
1	10.77 ± 0.03
2	11.0 ± 0.11
3	11.8 ± 0.10
4	12.0 ± 0.16

Table 3: Particle Size and Polydispersity Index on Day 0 and 6

Day	Particle Size (nm)				Polydispersity Index			
	F1	F2	F3	F4	F1	F2	F3	F4
0	1126 ± 7.12	986.13 ± 22.81	723.47 ± 3.51	674.03 ± 13.58	0.450 ± 0.03	0.498 ± 0.01	0.412 ± 0.02	0.414 ± 0.02
6	1308.33 ± 26.41	1285.33 ± 26.13	1147 ± 1.63	732.77 ± 7.53	0.497 ± 0.03	0.518 ± 0.04	0.414 ± 0.02	0.384 ± 0.01

Table 4: Comparison of Zeta Potential on Day 0 and 6

Day	Potential Zeta (mV)			
	F1	F2	F3	F4
0	-23.40	-22.70	-27.70	-22.70
6	-23.20	-18.70	-21.20	-18.30

As for the results of the PDI on table 3, all four formulas have poly dispersity index of 0.3-0.5 which falls within the range of the monodispersity category of 0.01-0.7, thus indicating that the prepared droplet has a good level of uniformity and tend to be more stable.³¹

Zeta potential is one of the partial indicators to show the stability of a system containing dispersed droplets through the existence of repulsive forces between droplets with the same charge when close together. According to Honary and Zahir, 2013, if there is steric or electrostatic stabilization, the potential zeta value of at least ± 20 mV is considered stable. In the formulas, there was polyvinyl alcohol that has properties as a steric stabilizer that can adsorb the surface and act as a barrier on the surface of the droplet therefore it will prevent the droplet from reuniting.³²

Based on zeta potential results, four formulas were categorized having sufficient stability (Table 4). However, after 6 days on the stability test, the Zeta potential decreased with the values of formulas 1 and 3 of above of above ± 20 mV and formulas 2 and 4 approaching ± 20 mV after day 6 storage. From the results of this study, it was suggested to choose the right kopyor varieties and optimize the VCO processing method in order to achieve the high levels of lauric acid in accordance with the standard. Furthermore, it is suggested to take longer activity test at room temperature.

Conclusion

Kopyor Virgin Coconut Oil (VCO) was successfully formulated into a peel off gel mask with the concentration of 20% - 30% VCO and PVA at concentration of 8% to 10% and have shown antibacterial activity against *Propionibacterium acnes* ATCC 11827. Increased kopyor VCO levels from 20% to 30% and polyvinyl alcohol levels from 8% to 10% had no significant effect on antibacterial activity. Formula 1 which contained 8% PVA and 20% VCO was the optimum formula considering its pH, spreadability, zeta potential and antibacterial activity. For the development of formulas, it is recommended to produce peel off gel mask consisting of a minimum of 30% VCO and 10% PVA. Longer stability test is highly recommended to optimized the VCO peel-off mask.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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