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ORIGINAL ARTICLE

Peel-off emulgel mask of *Cocos nucifera* L. Extract using gelling agent carbomer 940 as antiacne against *Propionibacterium acnes* ATCC 11827

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ABSTRACT

Cocos nucifera Linn., which contain lauric acid has been known had antibacterial activity against *Propionibacterium acnes* that usually improve severe of pimple. Current study investigated optimum formula of emulgel mask based on the *C. nucifera* L. Extract from Kopyor coconut. Extract were tested for antibacterial against *P. acnes* ATCC 11827. In this research, *C. nucifera* L. extract of 1 and 5% were formulated as an active agent of peel off antiacne emulgel mask-containing carbomer 940 in various concentration (1% and 1.5%). The peel-off emulgel mask of *C. nucifera* L extract was then evaluated in terms of viscosity, pH, drying time, spreadability, and antibacterial activity. The selected formula was formula containing 5% of extract and 1% of carbomer 940. This formula had pH that suitable with skin pH 4.5–6.5, had good spreadability, and also produced highest antibacterial activity against *P. acnes*.

Key words: Antibacterial activity, carbomer 940, *Cocos nucifera* L. extract, *Propionibacterium acnes*

INTRODUCTION

Cocos nucifera L is a multipurpose plant that has main component is water and soft flesh which has not been much researched as drug or cosmetic. Kopyor coconut is one type of it. To provide great benefits in developing potential as drug and cosmetic, kopyor need to be extracted and characterized. Muhammadiyah University Purwokerto Coconut Research Center Indonesia collaborated with the Faculty of Pharmacy Airlangga University to undergo research using kopyor extract as active agent for cosmetic. This study investigated the potential of kopyor extract (*C. nucifera* L.) and tested its potential and activity as an antimicrobial and cosmetic.

MATERIALS AND METHODS

Materials

Materials used in the study had a degree of pharmaceutical-grade purity including, polyvinyl alcohol, Carbomer 940, Na-ethylenediaminetetraacetic acid, phenoxyethanol, propylene glycol, Tween 80, Span 80, TEA, BHT, NaCl. Kopyor coconut was obtained from Purwokerto Research Centre, and ATCC 11827 *Propionibacterium acnes* was obtained from the Surabaya Health Laboratory Center. Nutrient agar media was obtained from the Testing Service Unit (ULP) Faculty of Pharmacy Universitas Airlangga.

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Methods

Kopyor coconut extraction with maceration method

A sample of 150 g was immersed in 96% ethanol with a ratio of 1: For 1 day and was repeated three times. Maserate was allowed to settle for sedimentation purposes. The oil sample was separated from the ethanol layer and the residue. Then, it was shaken out with a rotary evaporator to remove the remaining solvent. The yield was calculated, and phytochemical screening was analyzed.[1]

Saponification test

Volumetric method

A total of 2.5 g of sample was weighed in a 250 mL erlenmyer. 25.0 mL KOH was added to ethanol 0.5 N then continued to reflux for 30 min while turning occasionally. Then, 1 mL PP indicator was added. The excess of KOH was titrated with 0.5 N HCl. The saponification value was calculated.

N HCl = (1000 × m KHP × %KHP x Vt_2)/(Vt_1 × Mr KHP × V_HCl)

Saponification value = ([v blank titration v-sample titration] ×N HCl xMr KOH)/(m sample)

Formulation of Peel-off Mask Emulgel

Polyvinyl alcohol and Carbomer 940 solution were prepared. Into Carbomer 940, TEA was added dropwise while stirring. Tween 80 was dissolved in water and then propylene glycol was added followed by addition of phenoxyethanol and Na-EDTA. Then the water phase was put into a gel base which has expanded and was stirred homogeneously. Kopyor (*Cocos nucifera* L.) extract was mixed with Span 80 and BHT, then PVA was added and the gel mixture was stirred until homogeneous. Formula can be seen in Table 1.

Phytochemical screening analysis

Physical evaluation of peel-off mask

Evaluation of the peel-off mask of kopyor coconut extract was included the examination of the physical characteristics by determining pH, viscosity, dispersibility, particle size, particle size distribution, and zeta potential.[2]

pH evaluation

pH measurements used a pH meter.[3]

Viscosity evaluation

The viscosity was measured with a cone and plate DV-1 viscometer with a CP-41 type spindle. A sample of 2 g of peel-off mask was weighed. The sample must be ensured to be bubble-free and spread evenly on the surface of the cup. After that, the sample cup was reassembled on the viscometer. The viscometer was turned on at 4 rpm and then was left for a while until the reading was stable.[4]

Spreadability evaluation

One gram of peel-off mask was weighed and was placed in the middle of a glass plate and was covered with another glass plate. Additional weighting was carried out, starting from 5 g on the top glass plate. More weighting load was added out until the preparation showed no longer spreads. Deployment ability was measured by the slope of the distribution equation of the diameter of the dispersion versus weight load.[3,5]

Drying time of peel-off mask

A total of 700 mg peel-off gel mask was applied to the glass slab. The plates were left at room temperature (25°C) and at the oven (37°C). Then the mask was left to dry by checking every 5 min.[6]

Evaluation of antibacterial activity of peel-off mask

The *P. acnes* bacterial inoculum was prepared. Transmission of a bacterial inoculum was measured by a spectrophotometer at a wavelength of 580 nm until it received a 25% transmittance. The media base layer (30 mL) and seat layer (20 mL) were made with NA and then was sterilized at 121°C for 30 min. Base layer media was poured in a petri dish. It was then allowed to cool and solidify. After cooling and solidifying, the seat layer media was added to the 20 µL inoculum solution, followed by allowing cooling and solidifying. After cooling and solidifying, wells were made sterile to contain bacteria with a diameter of the center. A total well of 21 points consisted of 4 × 3 points for each sample (F1, F2, F3, and F4) including replication, 2 × 3 points for the positive control (contains 5% benzoyl peroxide concentration) and 1 × 3 points for negative control (dimethyl sulfoxide). Then, the petri dish was placed upside down and was incubated for 24 h at 37°C. After 24 h, the diameter of the zone was measured.

Statistical analysis used

Data analysis

Measurement of a significant difference between the formula with different concentrations of extract and gel base Carbopol 940 with viscosity, dispersion, and inhibitory strength of kopyor extract preparations, was performed with statistical analysis with one-way variant analysis (ANOVA). Significant differences between formulas were identified by Tukey's honestly significant difference test with a 95% confidence level ($\alpha = 0.05$). To find out the significant differences in the physical characteristics, namely viscosity before and after the thermal cycling stability test, particle size, and particle size distribution, a paired *t*-test was performed.

RESULTS

Characterization of Kopyor (*C. nucifera* L.) extract was as follows:

Table 1: Formula of peel-off mask

Components	Concentration (%) Formulas					
	1	2	3	4	5	6
Kopyor coconut extract	1	5	1	5	-	-
PVA	10	10	10	10	10	10
Carbopol 940	1	1	1.5	1.5	1	1.5
Propylenglycol	15	15	15	15	15	15
Na EDTA	0.1	0.1	0.1	0.1	0.1	0.1
BHT	0.1	0.1	0.1	0.1	0.1	0.1
Phenoxyethanol	1	1	1	1	1	1
Triethanolamine	0.5	0.5	0.5	0.5	0.5	0.5
Tween 80	4.5	4.5	4.5	4.5	4.5	4.5
Span 80	0.5	0.5	0.5	0.5	0.4	0.5
Aquadest	Ad 100	Ad 100	Ad 100	Ad 100	Ad 100	Ad 100

PVA: Polyvinyl alcohol, BHT: Butyl Hydroxy Toluene

Table 2: Standardization of 0.5 N HCl

m Na2CO3 (99,9%); Mr=106 (g)	Volume HCl (mL)	N HCl (mol/L)
264.8	6.30	0.7923
265.0	6.35	0.7866
	Average of N HCl	0.7894

Table 3: Standardization of 0.5 N KOH

Volume KOH (mL)	Volume HCl (mL)	N HCl (mol/L)
25.0	15.80	0.4989
25.0	15.85	0.5005
	Average N KOH	0.4997

$$\text{Saponification Index} = \frac{(v\ titrasi\ blanko - v\ titrasi\ sampel)\,x28.05}{m\ sampel}$$

Table 4: Saponification Index

m sample (g)	Blank titration (mL)	Sample titration (mL)	Saponification value
1.2286	15.80	13.70	47.92
1.2916	15.85	13.65	47.75
		Average	47.83
		RPD	0.30

Table 5: Minimum inhibitory concentration of extract

Concentration (%)	Inhibition zone diameter (mm)±SD
30	7.16±0.05
20	6.58±0.11
10	6.40±0.21
5	5.90±0.42
2.5	5.80±0.12
1	5.50±0.23
0.5	0.0±0.0

SD: Standard deviation

Phytochemical screening

Alkaloid, saponin, and flavonoid test are shown in Figure 1.

Saponification index

Saponification index values results are as calculation in Tables 2-4.

Saponification index = 47.83

MIC of the extract against *Propionibacterium Acnes*

MIC of the extract is shown in Table 5.

Antibacterial activity of extract

Antibacterial activity of extract against *Propionibacterium Acnes* as shown in Table 6.

pH test

pH of the formula was demonstrated in Table 7.

Viscosity test

Viscosity of the formula was demonstrated in Table 8.

Spreadability test

Spradability of the formula was demonstrated in Table 9.

Slope of Spreadability is shown in Table 10.

Drying time

The drying time of the formula is shownin Table 11.

Activity test of peel-off mask formula against propionibacterium acnes ATCC 11827

Zone of Inhibition of formula is shown in Table 12.

DISCUSSION

Kopyor coconut has been investigated worldwide using some parts of plants have been used for the treatment of various pathological conditions such as antiinflammation, antioxidant, antihelminthic, antimalarial, and antiviral activity,[7-9] thus this current research studied antiacne

Table 6: Antibacterial activity of extract against *Propionibacterium Acnes*

Sample	Concentration (%)	*Propionibacterium Acnes* inhibition zone diameter (mm)
Kopyor Extract	1	6.20
	2.5	6.50
	5	8.20
Control (+)	2.5	6.20
Control (−)		5.30
Neutrogena mask (contains benzoyl peroxide)	2.5	6.15

Table 7: pH formula

Formula	pH±SD
F1	4.89±0.02
F2	4.83±0.03
F3	5.45±0.02
F4	5.21±0.03
F5	5.79±0.07
F6	5.45±0.14

SD: Standard deviation

Table 8: Viscosity of formula

Formula	Viscosity (cps)±SD
F1	42,540±471.27
F2	46,320±559.42
F3	44,470±256.22
F4	48,789±925.84
F5	29,953±352.04
F6	33,190±115.30

formulation from the extract of *C. nucifera* L. from flesh part.

Saponification index

Saponification index values results are shown as below calculation on Tables 2-4.

The saponification index value was 47.83 and consisted of lauric acid of 29.20%.

Physical characteristics of peel-off mask formula

pH test

From these results, it showed that the pH of the peel-off mask of kopyor extract fulfilled the pH of the skin, namely pH 4.5–6.5 and was safe for use on the skin.[10] To ensure the difference in pH during the storage process, it was also advisable to look for free fatty acids that may be oxidized in the kopyor extract content that can allow an increase in free fatty acids when exposed to high temperatures.[11]

Viscosity test

The results of viscosity between formulas showed that an increase in the extract and gelling agent carbomer showed an increase in the viscosity of the preparation in line with similar studies.[12,13]

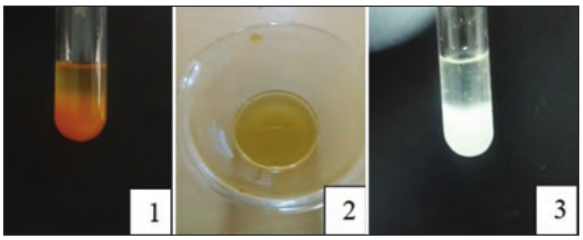

Figure 1: (1) Alkaloid test: Positive; (2) Saponin test: Negative; (3) Flavonoid test: Positive

Spreadability test

The spread diameter was shown between 4.37 and 7.70 for antiacne formulas contain extract. This was confirmed with other researchers for mask capacity of 5–7 cm.[14] Based on the value of the slope and the load to achieve maximum dispersal capacity, it can be concluded that the base formula without an active agent was more easily spread than the preparation formula. It was appropriate that an increase in the viscosity of the preparations will reduce the spreadability of the preparations.[13] Another explanation may be due to the effect of formula, which still contains a large amount of water content in each formula that can slow the evaporation and the formation of a film layer in a peel-off mask as similar to a study conducted by Phindo.[15]

Drying time

Based on the results of the drying time, the preparation of kopyor extract mask formulas 1–4 had a drying time of 40–50 min. The formula containing kopyor extract had a longer drying time compared to the two mask base formulas without active agent. This showed the drying time of the four formulas was longer than the peel-off mask drying time in general, which was <30 min.[16] This result was maybe due to kopyor extract in which there was oil content that can still resist the evaporation of water in the preparation therefore it took longer to dry.

Activity test of peel-off mask formula against *Propionibacterium acnes* ATCC 11827

Based on the results of the peel-off mask activity test, all formulations showed inhibition against *P. acnes* ATCC 11827. However, the comparison of inhibition diameter between formulas that had low *C. nucifera* L. extract concentrations,

Table 9: Spreadability of formula

Formula	Weight (g)	Spreadability diameter (cm)±SD
F1	-	4.37±0.15
	5	4.60±0.17
	10	4.83±0.15
	15	5.13±0.23
	20	5.23±0.23
F2	-	6.03±0.06
	5	6.27±0.21
	10	6.33±0.23
	15	6.47±0.21
	20	6.53±0.25
F3	-	4.63±0.15
	5	4.87±0.11
	10	5.07±0.11
	15	5.13±0.06
	20	5.20±0.10
F4	-	6.80±0.26
	5	7.23±0.29
	10	7.43±0.38
	15	7.67±0.58
	20	7.70±0.61
F5	-	3.83±0.05
	5	4.00±0.11
	10	4.10±0.11
	15	4.17±0.12
	20	4.20±0.10
F6	-	3.97±0.06
	5	4.20±0.17
	10	4.43±0.11
	15	4.57±0.15
	20	4.57±0.15

SD: Standard deviation

Table 10: Spreadability slope of Formula

Formula	F1	F2	F3	F4	F5	F6
Slope (cm^2/g)	0.3406	0.2372	0.2168	0.5130	0.2102	0.2208

inhibitory activity was as large or not significantly different from positive control of benzoyl peroxide. The inhibitory activity against acne bacteria increased with increasing levels of kopyor extract. This can be influenced by the content of lauric acid, which was not maximum in the content of kopyor extract, and hence, it was necessary to optimize the extraction and selection of different types of kopyor coconut.[17]

CONCLUSIONS

Kopyor (*C. nucifera* L.) extract had antibacterial activity against *P. acnes* ATCC 11827 at concentration of 1 and 5% and has been successfully formulated into peel-off mask formula using gelling agent carbomer 940 at a concentration of 1 and 1.5%. The variation of kopyor extract and gelling agent concentration affected the viscosity, pH, and spreadability.

Table 11: Drying time of formula

Formula	Drying time (min) Temperature (37°C)
F1	40:00
F2	40:05
F3	45:07
F4	50:03
F5	40:15
F6	50:00

Table 12: Zone of inhibition of formula

Sample	Inhibition zone diameter (mm)±SD
F1	10.05±0.13
F2	11.68±0.30
F3	9.90±0.05
F4	11.43±0.50
Control +	9.55±0.25
Control−	6.50±0.00

SD: Standard deviation

However, the antibacterial activity between formulas in masks with a low extract concentration of 1% did not show different results of its activity, therefore it was suggested to increase concentration of extract of higher than 5% or carbomer of more than 1.5%, it was predicted that there would be a significant increase in antibacterial activity.

Based on the physical evaluation and antibacterial activity of the mask preparations, a formula which has good characteristics was selected. The chosen formula was formula, which was a formula containing 5% extract and 1% carbomer produced formula with suitable skin pH, good dispersion, and sufficient viscosity. Moreover, it had a high antibacterial activity. Further recommendation of the peel-off mask formula included study the stability of peel-off mask for a longer period.

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Conflicts of interest
There are no conflicts of interest.

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