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# Formulation of virgin coconut oil microemulsion with natural excipients

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### ABSTRACT

Introduction: Virgin coconut (Cocos nucifera (L.)) oil (VCO) is taking attention from researchers because of its biological activities. However, applying VCO directly on the skin often leaves an uncomfortable feeling of greasy, clogged pores and difficult to wash. Coconut oil in microemulsion is a better option since it can increase bioavailability as well as convenience for users. Furthermore, this study aims to use natural ingredients, safe for the skin and environmentally friendly.

Methods: The research started from extracting saponins from *Gleditsia australis* by using ethanol and water as extraction solvents. The extracted saponins were characterized by chemical reactions. Then we combined saponins and some other surfactants to investigate the possibility of microemulsion formation.

Results: We extracted saponins with efficiency of 7.2%. The results showed that microemulsions were formed using a mixture of surfactants including saponins and lecithin (ratio of 0.05:100 w:w) in the formula with a ratio of water phase, oil phase, surfactants respectively 3%, 48.5%, 48.5%. Microemulsion met the requirements of quality control and the product is microbiologically stable after 3 months of storage without using preservatives.

Conclusion: In this study, we have successfully prepared Virgin coconut oil microemulsion using natural excipients including Virgin coconut oil, water and a mixture of surfactants used as saponins and lecithin. The system showed stability at 30 and 40 °C upon three-month storage and the product is microbiologically stable after 3 months of storage without using preservatives.

Key words: Virgin coconut oil, Gleditsia australis, saponins, microemulsion

### **INTRODUCTION**

Skin is our first and best defense against external aggressors. It is the most visible indicator of health. However, when the skin is dry, depending on the degree of dryness these functions no longer work as well as before. Skin becomes more sensitive to the sun, more vulnerable to damage and to inflammation, and promotes cellular senescence<sup>1</sup>. Especially in cases of chronic diseases such as psoriasis and atopic dermatitis, combination of medicinal treatment and moisturizers significantly improves the therapeutic effects. Natural products containing bioactive ingredients with various benefits, such as antioxidant, antiinflammatory and antibacterial, are more and more popular<sup>2,3</sup>.

VCO has been studied for its beneficial biological effects<sup>4,5</sup>. It has long been used to moisturize and treat skin infections<sup>6</sup>. Due to its safety and efficacy, VCO is used as a therapeutic moisturizer and has been shown to be effective on mild to moderate xerosis<sup>7</sup>. There have been several studies of virgin coconut oil microemulsions (COM) using a mixture of synthetic

surfactants, the two most common were Span and Tween.

However, using a large amount of chemicals for production has a negative impact on the environment as well as human health. Therefore, the study aims to examine natural surfactants because of their high biological compatibility with the skin as well as their environmental friendliness. In which, saponins present in *Gleditsia australis* is an abundant source of raw materials in nature. Because saponins have not been commercialized, the research went from extracting saponins and then using them to study the formulation of COM.

### **MATERIALS AND METHODES**

#### Materials

VCO is a product of Luong Quoi Coconut Co., LTD. *Gleditsia australis* fruits harvested in CuChi, HoChiMinh city, Vietnam. Ethanol, acetone, *n*hexane, *n*-butanol, ethyl acetate to extract saponins were purchased from China (Xilong, research laboratory standard). Lecithin (powder, L-aphosphatidylcholine, HLB 4-7 according to supplier)

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was purchased from Lipoid (Germany). Non-ionic surfactants, polyoxyethylene sorbitan monooleate (Tween 80), sorbitan monooleate (Span 80) were purchased from China (Xilong, research laboratory standard). Distilled deionized water was used in all experiments.

### **Methods**

### Extraction of saponins from Gleditsia australis fruits

First, the medicinal herbs was broken down and the extraction was done by soaking the dried plant material in 70% ethanol with herbal:solvent ratio is 1:5 in 2 hours at room temperature and then at 80  $^{o}$ C by using the temperatured water bath. The extraction was then shaking with three time with ethyl acetate (extraction : ethyl acetate 1 : 1 v : v) to remove non-polar impurities. Discard the ethyl acetate solution and the extraction was then shaking with n-butanol. The n-butanol fraction was collected and evaporated to obtain a total saponins precipitate.

### Determination of the required HLB values for VCO and the HLB of saponins

The required HLB and HLB values were determined by monitoring the phase separation time of the emulsion using the mixture of surfactants with different HLB values. The emulsion formulation used for the tests consisted of 75% VCO, 5% water and 20% surfactants. Briefly, VCO, water and surfactant was weighted into the glass tube with the quantity of 0.75 g, 0.5 g and 2 g respectively. The tube was closed with plastic cap. This mixture were vortexed in five minutes. The most stable formultations was seledcted for rHLB and HLB calcuation. With the formulation using tween 80 as surfactant, we can calculated the rHLB of VCO. Based on this result, we calculated the HLB of saponins. The rHLB / HLB value was calulated as described in Tuba (2020)<sup>8</sup>.

### Preparation of microemulsions

The ternary phase diagram that consisted of oil, water, and surfactant/co-surfactant (S/Co-S) mixtures were constructed using water titration method as described in Amol *et al.* (2021)<sup>9</sup>. The phase diagrams were prepared with fixed weight ratio of surfactants/cosurfactants (S/Co-S). This ratio was obtained based on the principle that the HLB value of the surfactant mixture was equal to the rHLB value of VCO. In cases where this ratio fails to produce microemulsion, different S/Co-S ratios are investigated. For each phase diagram, the weight of oil to surfactant or surfactant/co-surfactant (S/Co-S) ratio were varied as 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1 (w/w)<sup>10,11</sup>. Based on this principle, the study of the formula is carried out in three steps, including screening of surfactants, investigation of the ratio between oil phase and mixture of surfactants and screening of the water phase ratio in the microemulsion formula.

### Evaluate some properties of finished microemulsion

Evaluation of some properties of finished microemulsion including pH, relative density, centrifugal and thermal stability, electrical conductivity and qualitative by thin layer chromatography as described below. pH: Glass electrode pH measurements (Mettler Toledo S220K) have been carried out for our microemulsions formulation. The measurement was repeated three times.

Density of microemulsion was determined by using the pycnometer.

The thermal stability of microemulsion was characterized by thermal cycle. The microemulsion was kept at 50  $^{\circ}$ C (in water bath) for 12 hours and then at 4  $^{\circ}$ C (in refrigerator) for 12 hours and finally at room temperature for 12 hours. The cycles were repeated at least 5 times.

The conductivity of the microemulsion was measured directedly by conductivity meter (Mettler Toledo, Electrode LE438)

Thin layer chromatography was carried out on GF 254 pre-coated plate. 1 g of sample test or standard sample (coconut oil) was dissolved in 10 mL of diethyl ether and shaked well. The mobile phase was n-hexane: ethyl acetate: glacial acetic acid (8: 2: 0.5). The peaks were discovered by vanillin-sulfuric reagent.

### Preliminarey inspection of product stability

Inspection of the product stability under the influence of natural light and temperature (10, 30, 40 °C) after 3 months based on the specifications of description, pH and electrical conductivity.

### Preliminary study about skin irritancy test

Skin irritation test is conducted with three rabbits. Test substance (0.5 g solid or 0.5 mL liquid) is applied on the small area (10 cm x 15 cm), and then the treated site is covered with a patch. Patch is removed and then, signs of erythema and edema, and the responses are scored at 1, 24, 48 and 72 hours. Erythema and edema are scored with grades from 0 to 4 depending on the severity  $^{12,13}$ .

able 1. Questionnance survey	
Questionnaire (0-10 rating scale*)	
1	The fondness for the smell of the product
2	Feeling of greasy after using the product
3	Excess amount of product remains on the skin after using the product
4	Soft feeling of skin after using the product
5	Skin irritancy

\*: high score that means more possitive characteristic of product. The score was given by the sense of each volunteers.

### Inspection of product efficiency

survey is presented in Table 1.

Table 1. Questionnaire curvey

Conclusion: Choose rHLB of VCO is 8, HLB of saponins is 24.

### Assess self-preservation ability of microemulsion

Inspection of product efficiency on 25 volunteers based on questionnaire survey. The questionnaire

Assess self-preservation ability of the product after 3 months of storage based on Vietnamese specified standards in Circular No. 06/2011/TT-BYT.

### **RESULTS AND DISCUSSION**

### Extract saponins from *Gleditsia australis* fruits

Formulation of COM

### Screening of surfactants

*Mixture of surfactants including saponins and Span 80* The ability to create microemulsion with different mixture of surfactants including saponins and span 80 is shown in Table 3.

The pseu-do diagram of microemulsion with mixutre of surfactants including saponins and span 80 is shown in Figure 3.

*Mixture of surfactants including saponins, Span 80 and Tween 80 (HLB=8)* 



Figure 1: Saponins extracted from *Gleditsia australis* fruits

The extracted saponin is shown in Figure 1. Extraction efficiency: 7.2%

Description: brown powder, causing sneezing. Qualitative: The solids obtained are positive for the Liebermann - Burchard reaction (purple ring), creating stable foam in water.

## Determine the required HLB values for VCO and the HLB of the saponins

The result of experimental rHLB of VCO is shown in Table 2 and Figure 2.



Figure 4: Phase diagram using a mixture of surfactants has an HLB value of 8

The pseu-do diagram of microemulsion with mixutre of surfactants including saponins, span 80, tween 80 is shown in Figure 4.

*Mixture of surfactants including saponins and lecithin* The ability to create microemulsion with different mixture of surfactants including saponins and lecithin is shown in Table 4.

The pseu-do diagram of microemulsion with mixutre of surfactants including saponins, lecithin is shown in Figure 5.

The comparison between formulations using mixtures of different surfactants is shown in Table 5.

Table 2: Survey results rHLB value of VCO												
HLB	4.3	5	6	7	8	9	10	11	12	13	14	15
Phase sepa- ration time (minutes)	6	8	9	9	11	10	9	9	7	6	6	3



Figure 2: The chart shows the phase separation time of the coconut oil emulsion with hypothetical HLB values of saponins

### Table 3: Investigation of the formation of COM with a mixture of surfactants including saponins and Span 80

Saponins : Span 80	$\geq$ 3:100	2:100	1:100
Ability to create microemulsions	-	+	+

(-): Cannot create microemulsions (+): Can create microemulsions





#### Table 4: Investigation of the formation of COM with the mixture of surfactants including saponins and lecithin

Saponins : Lecithin	> 0.05:100	0.05:100
Ability to create microemulsion	-	+

(-): Cannot create microemulsion (+): create microemulsion

#### Table 5: Comparison between formulations using mixtures of different surfactants

Mixture of surfactants	Physical appearance	Microemulsion forming zones	Natural excipients
Saponins, Span 80	Translucent	+	-
Saponins, Span 80, Tween 80	Transparent	+++	-
Saponins, Lecithin	Transparent	++	+



**Figure 5:** Phase diagram using the mixture of surfactants including saponins and lecithin

Select the mixture of surfactants including saponins and lecithin for further investigation as it meets the requirements of all-natural ingredients and a relatively wide microemulsion forming zones.

### Investigation of the ratio between oil phase and mixture of surfactants

The investigation of the ratio between oil phase and mixture of surfactants is shown in Table 6. Selected the ratio of VCO and active substance mixture is 5:5 for further investigation because it contains the highest percentage of VCO and still meets the requirements of stability after the thermal cycle test.

### Investigation of the water phase ratio in the microemulsion formula

The investigation of the water phase ratio in thermal stability is shown in Table 7.

The selected ratio of water in the formula is 3% because it contains the highest percentage of water and still meets the requirements of stability after the thermal cycle test. This formulation is shown in Figure 6.



Figure 6: Virgin coconut oil microemulsion

### Evaluation of some properties of finished microemulsion

The evaluation of some properties of finished microemulsion is presented in Table 8.

### Inspection of product stability

The result of product stability is shown in Table 9.

### **Skin irritancy test**

The result of skin irritancy test is shown in Table 10.

### **Examination of product efficiency**

The result of the survey is shown in Figure 7. The microemulsion is far more acceptable than the VCO.

VCO : S-CoS	9:1	8:2	7:3	6:4	5:5	4:6	3:7	2:8	1:9
Stability after thermal cycles	-	-	-	-	+	+	+	+	+

### Table 6: Investigation of the ratio between oil phase and mixture of surfactants

### Table 7: Investigation of the water phase ratio

Ingredients (%)	Formula 1	Formula 2	Formula 3
VCO (%)	49	48.5	48
Water (%)	2	3	4
Saponins (%)	0.0233	0.0231	0.0229
Lecithin (%)	48.98	48.48	47.98
Stability after thermal cycles	+	+	-

### Table 8: Evaluation of some properties of finished microemulsion

Properties		Results		
Description		Microemulsion is transparent, yellow-brown color, ther- modynamically stable, isotropic liquid		
рН		6.047		
Relative density		0.9733 g/ml		
Stability	Thermal cycles	Microemulsion remains stable after 3 thermal cycles		
	Centrifugation	Microemulsion remains stable after centrifuging at 3500 rpm		
Electrical conductivity		0.6 µS/cm		
Qualitative by thin layer chroi	natography	The principal spot in the chromatogram obtained with the test solution corresponds to the spot in the chro- matogram obtained with the reference solution in the colour, the size and the retardation factor ( $Rf = 0.37$ )		

### Table 9: Inspection of the influence of natural light and temperature

Properties	Results		
	Description	рН	Electrical conductivity
Influence of natural light	Transparent, homoge- neous, yellow-brown color	$6.09\pm0.07$	0.6 (µS/cm)
Influence of temperature (10, 30, 40 °C)	Transparent, homoge- neous, yellow-brown color	$6.13\pm0.11$	0.6 (µS/cm)

Table 10:	able 10: Results of skin irritancy test															
Rabbit No.	Time															
	1 hour			24 hours			48 ho	48 hours			72 h	72 hours				
	Т		R		Т		R		Т		R		Т		R	
	ER	Е	ER	Е	ER	Е	ER	Е	ER	Е	ER	Е	ER	Е	ER	Е
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Average	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.33	0

T: Test solution (COM)

R: Reference solution (VCO)

ER: Skin erythema

E: Skin edema

Conclusion: COM is non-irritating



#### **Preliminary** assessment of selfpreservation ability of microemulsion

The preliminary assessment of self-microbiological preservation ability of microemulsion is shown in Table 11.

Conclusion: COM meets the requirements of the product for children under 3 years old.

### **CONCLUSION**

In this study, we have successfully prepared Virgin coconut oil microemulsion using natural excipients including virgin coconut oil, water and a mixture of surfactants used as saponins and lecithin. The system showed stability at 30 and 40°C upon three-month storage and the product is microbiologically stable after 3 months of storage without using preservatives.

The microemulsion with coconut oil has been published elsewhere, however, this is the first time the coconut microemulsion was made with natural excipients. Although there is still many questions such as microemulsion type, the scale up steps... this result is the first step to reach to the national market and to make value for our country's product.

### CONFLICTS OF INTEREST

The authors declare no conflict of interest.

### AUTHORS' CONTRIBUTION

Conceptualization, Van-Thanh Tran; Exepriments: Thi-Tuyet-Nhi Pham Methodology: Ngoc-Quynh Le, Ngoc-Son Nguyen, Xuan-Truong Le, Van-Thanh Tran

#### Table 11: Results of the assessment of self-preservation ability of microemulsion

Specified standards in Circular No. 06/2011/TT-BYT								
Types of microorganisms	Products for children under 3 years old	Other products						
Total Aerobic Mesophilic Microorgan- isms (Bacteria, Yeast and Molds)	$\leq$ 500 CFU/g or CFU/ml	$\leq$ 1000 CFU/g or CFU/ml	< 10 CFU/g					
P. aeruginosa	Absent in 0.1 g or 0.1 ml test sample	Absent in 0.1 g or 0.1 ml test sample	Not found					
S. aureus	Absent in 0.1 g or 0.1 ml test sample	Absent in 0.1 g or 0.1 ml test sample	Not found					
C. albicans	Absent in per 0.1 g or 0.1 ml test sample	Absent in 0.1 g or 0.1 ml test sample	Not found					

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### Nghiên cứu điều chế vi nhũ tương chứa dầu dừa với thành phần hoàn toàn tự nhiên

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### ABSTRACT

Tổng quan: Dầu dừa nguyên chất (*Cocos nucifera* (L.)) (VCO) đang được các nhà nghiên cứu quan tâm bởi các hoạt tính sinh học. Tuy nhiên, thoa VCO trực tiếp lên da thường để lại cảm giác nhờn rít khó chịu, bít lỗ chân lông và khó rửa sạch. Dầu dừa dạng vi nhũ tương là một lựa chọn tốt hơn vì nó có thể làm tăng sinh khả dụng cũng như tiện lợi cho người sử dụng. Hơn nữa, nghiên cứu này hướng đến việc sử dụng các thành phần tự nhiên, an toàn cho da và thân thiện với môi trường. **Phương pháp:** Nghiên cứu bắt đầu từ việc chiết xuất saponin từ Gleditsia australis bằng cách sử

dụng ethanol và nước làm dung môi chiết xuất. Các saponin chiết xuất được đặc trưng bởi các phản ứng hóa học. Sau đó, chúng tôi kết hợp saponin và một số chất hoạt động bề mặt khác để khảo sát khả năng hình thành vi nhũ tương.

**Kết quả:** Chúng tôi đã chiết được saponin với hiệu suất 7,2%. Kết quả cho thấy vi nhũ tương được hình thành khi sử dụng hỗn hợp chất hoạt động bề mặt gồm saponin và lecithin (tỷ lệ 0,05:100 w:w) trong công thức với tỷ lệ pha nước, pha dầu, chất hoạt động bề mặt lần lượt là 3%, 48,5%, 48,5 %. Vi nhũ tương đạt yêu cầu về kiểm soát chất lượng và sản phẩm ổn định vi sinh sau 3 tháng bảo quản mà không sử dụng chất bảo quản.

**Kết luận:** Trong nghiên cứu này, chúng tôi đã bào chế thành công vi nhũ tương dầu dừa nguyên chất sử dụng tá dược thiên nhiên là dầu dừa nguyên chất, nước và hỗn hợp các chất hoạt động bề mặt sử dụng là saponin và lecithin. Hệ thống cho thấy sự ổn định ở 30 và 40°C sau 3 tháng bảo quản và sản phẩm ổn định về mặt vi sinh sau 3 tháng bảo quản mà không sử dụng chất bảo quản. **Key words:** Dầu dừa nguyên chất, Gleditsia australis, saponin, vi nhũ tương

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