Formulation And Evaluation Of Nanoemulgel Clove Leaf Oil (*Sygyzium Aromaticum*) (L.) Merr & Perry As Anti-Acne

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Abstract.
Acne also known as acne vulgaris, is an inflammatory condition of the polysebaceous layer that is brought on by the buildup of keratin. Active oil glands under the skin cause acne. Androgen hormones function as a stimulant for this activity. Cloves are herbal plants that contain essential oil chemicals. The chemical composition of clove leaf oil is mainly composed of eugenol. Eugenol has pharmacological effects as antibacterial, analgesic and anti-inflammatory. Nanoemulsion is a nanocarrier that gets a lot of attention, because of its small particle size so that it can be thermodynamically stable and can increase drug penetration. Recent advances in nanoemulsion research have created new prospects in a number of industries, including pharmaceuticals, biotechnology, food, and cosmetics. This research was conducted by formulating clove leaf oil into nanoemulgel preparations, followed by evaluation of the preparations and testing of antibacterial activity against acne-causing bacteria. The result of the nanoemulgel formulation is yellow, smells of clove leaf oil, is transparent and homogeneous. Nanoemulgel viscosity 3434-3109 M.Pas, pH 6.15-6.41 and particle size 12.3-13.3 nm. Physical studies of nanoemulgel been carried out for three cycles and there is no change in color, change in odor and phase separation. The results of the antibacterial activity of Staphylococcus epidermidis at NEG1 12.03 mm, NEG2 16.93 mm, NEG3 18.96 mm and EG 18.03 mm. The results of the antibacterial activity of Propionibacterium acnes at NEG1 11.50 mm, NEG2 16.16 mm, NEG3 18.03 mm and EG 17.33 mm. The results of the study concluded that clove leaf oil nanoemulgel with a concentration of 7% was the best formula, because it is stable during storage and has the greatest antibacterial activity compared to emulgel.

Keywords: *Clove leaf oil, nanoemulgel, antibacterial and anti-acne.*

I. INTRODUCTION

The skin is the first organ to be affected by external stimuli such as pain, touch, and other unpleasant sensations. As a result, the skin is more susceptible to disease. Acne is the most common skin disease worldwide [1]. Acne also known as acne vulgaris, is an inflammatory condition of the polysebaceous layer that is brought on by the buildup of keratin. The etiology of this condition is influenced by the metabolites that bacteria like *Propionibacterium acnes* and *Staphylococcus epidermis* produce, which can react with sebum to exacerbate the inflammatory response. Active oil glands under the skin cause acne. Androgen hormones function as a stimulant for this activity [2]. Acne has long been controlled with topical and systemic medications, including antibiotics and retinoids. In dermatology, antibiotic resistance, retinoid side effects, and drug allergies are on the rise. Alternative medicine based on medicinal plants has been investigated as a solution to this problem [3].Clove leaf is one of many herbal plants that are intended to treat acne. Clove plants contain essential oils in large enough quantities, both in flowers, bud and leaves. The main content of clove leaves is phenolic compounds, tannins, saponins, flavonoids, triterpenoids and alkaloids [4]. The chemical composition of clove leaf oil is mainly composed of eugenol, which is 70 until 98% [5].

Eugenol compounds have pharmacological activities as analgesic, anti-inflammatory, antimicrobial, antifungal, antiseptic, and local anesthetics so that these compounds are widely used in the pharmaceutical

industry. [6]. The results of a previous study stated that clove leaf oil at a concentration of 20% had antibacterial activity against *Propionibacterium acnes* bacteria with an inhibition zone diameter of 18.58 mm [7]. Nanoemulsion is a nanocarrier developed recently which has received a lot of attention due to its various benefits due to its small particle size that provides better absorption and increase drug bioavailability [8]. Nanoemulsion is a thermodynamically stable system formed from aqueous phase, organic phase, surfactant and co-surfactant. It showed better nanoemulsion stability than conventional emulsions in flocculation, sedimentation, phase separation and creaming [9]. Nanoemulsion drug delivery systems can be administered via different routes of administration, including oral [10], intranasal [11] and topical [9]. Emulsions and nanoemulsions differ mainly in particle size. The particle size in nanoemulsions is 5-200 nm and in conventional emulsions 1-20 nm [12]-[13]. Nanoemulgel has a lower viscosity than hydrogel preparations, so it is able to produce proper dispersion and good dispersion. Advantages of nanoemulsion preparations when added to a gel base are that they are easy to apply and convenient to use so they are preferred by patients [14].

II. METHODS

2.1 Ingredients

The ingredients used in this research are incubator (Memmert), laminar air flow cabinet (Astec HLF 1200 L), magnetic stirrer (Thermo), particle size analyzer (Horiba Scientific, viscometer (NDJ-8S), micropipet (Eppendorf), pH meter (Hanna), sonicator (Elma), clove leaf oil, trietanolamin (PT. Nitra Kimia), PEG 400 (PT.Nitra Kimia), carbopol 940 (PT.Nitra Kimia) and tween 80 (PT.Nitra Kimia).

2.2 Formulation Nanoemulsion Gel and Emulsion Gel

The process of making nanoemulgel using spontaneous emulsification method consisting of an aqueous phase and organic phase. The aqueous phase consists of tween 80 (surfactant) and distilled water stirred constantly at a speed of 5000 rpm. The organic phase consisted of clove leaf oil and PEG 400 (cosurfactant) was stirred at a constant speed of 5000 rpm. The organic phase was injected into the aqueous phase with constant stirring at 5000 rpm and sonicated for 30 minutes until a clear preparation was formed. In a separate container, carbopol 940 was dispersed in distilled water, then triethanolamine was added and homogenized at 500 rpm to form a transparent gel. The final step, nanoemulsion was added slowly into the gel base and homogenized at 2000 rpm for 10 hours [15]-[16]. The nanoemulgel formula is shown in Table 1.

Emulgel formulations were made to compare stability, appearance and antibacterial inhibition with nanoemulgel. The emulsion manufacturing process consists of a water phase and an oil phase. Methyl paraben and propyl paraben were dissolved with hot distilled water, added tween 80 and then homogenized at 70^0C (Oil Phase). Furthermore, clove leaf oil was added with PEG 400 and homogenized at 70^0C (water phase). Then the oil phase was added to the aqueous phase and homogenized until the emulsion was at room temperature. The gel was prepared by dissolving carbopol in distilled water and adding triethanolamine to form a basic gel. The final step is to gently add the emulsion to the gel so that it forms a white emulgel [17]. The emulgel formula is shown in Table 2.

Table 1.Clove leaf oil nanoemulsigel formulation

Ingredients	**NEG1**	**NEG2**	**N2G3**
Clove leaf oil	2	4	6
Tween 80	30	30	30
PEG 400	15	15	15
Methyl Paraben	0,1	0,1	0,1
Propyl Paraben	0,02	0,02	0,02
Carbopol 940	1	1	1
Triethanolamin	0,1	0,1	0,1
Aquadest ad	100	100	100

Table 2. Clove leaf oil emulsigel formulation

Ingredients	**EG**
Clove leaf oil	6
Tween 80	0,5

PEG 400	2,5
Methyl Paraben	0,1
Propyl Paraben	0,02
Carbopol 940	2
Triethanolamin	0,1
Aquadest ad	100

2.3 Evaluation of Nanoemulgel and Emulgel

Organoleptic test

Organoleptic tests were carried out on nanoemulgel and emulgel which were visually observed for changes in color, smell and shape [18].

Homogeneity Test

The homogeneity test was carried out on nanoemulsigel and emulsion by applying a certain number of samples to a glass object. The sample must show a homogeneous arrangement and there are no visible coarse grains [15].

Viscosity measurement

Viscosity measurement aims to determine the viscosity of nanoemulgel and emulgel preparations. Viscosity measurement using NDJ-8S viscometer. Viscosity measurement was carried out by placing the preparation in a 50 ml beaker and using an appropriate spindle [15].

pH Measurement

Determination of the pH of nanoemulgel and emulgel was carried out using a pH meter by calibrating it first with a buffer solution (pH 7.01) and (pH 4.01) until the instrument showed the pH value. Then the electrodes were washed with distilled water and dried with a tissue. Then the electrode is dipped into the sample, until the instrument shows a constant pH value. According to skin pH, the required pH range is 4.5–6.5 [19].

Particle size measurement

Particle size measurement was carried out by diluting 0.5 g of sample with 1 mL of distilled water. Then 1 mL was taken to test the particle size. Particle size measurements were carried out at the Institute of Technology Bandung using Horiba Scientific, Nanoparticle Analyzer SZ-100. Particle size testing was carried out to determine the size of the globules formed in nanoemulgel and emulgel [20].

Physical stability study of nanoemulgel and emulgel

The purpose of conducting a cycling test is to determine the stability. Cycling test is an accelerated test by storing the preparation at 4 ± 2^0C for 24 hours, then transferred to 40 ± 2^0C for 24 hours. This treatment is 1 cycle. The treatment was repeated for 6 cycles and the phase separation was observed. Physical conditions after the experiment were compared with before the experiment [21].

Antibacterial activity

Antibacterial activity testing was carried out on nanoemulgel and emulgel preparations. Using the agar diffusion method (Kirby-Bauer), this test was conducted. A sterile petri dish was first filled with 0.1 mL of inoculum, and then 15 mL of nutrient agar (NA) media was added while maintaining a temperature of 45–50°C and homogenized until solid. Each petri dish was put of paper disk that had the test solution drip-coated on it. After 24 hours of incubation at 36–37°C, then the diameter of the clear zone was measured using a caliper which was expressed in millimeters. Tests are carried out triple [22]-[23].

III. RESULT AND DISCUSSION

3.1 Formulation Nanoemulsion Gel and Emulsion Gel

The nanoemulgel and emulgel formulations consisted of clove leaf oil, tween 80, PEG 400, aquadest, propyl paraben, methyl paraben, carbopol 940 and triethanolamine. Clove leaf oil is used as a carrier oil and an ingredient that has antibacterial, antiseptic and anti-inflammatory activities. Tween 80 (surfactant), PEG 400 (cosurfactant), carbopol 940 (gelling agent), triethanolamine (pH balance). The results of the nanoemulgel and emulgel formulations are shown in Fig 1.

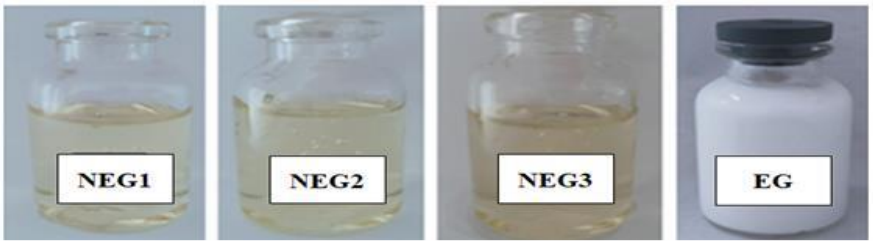

Fig 1. Formulation nanoemulgel and emulgel

3.2 Evaluation of Nanoemulgel and Emulgel

Organoleptic test

Organoleptic tests were observed visually on all formulas including color, odor and shape. The organoleptic results are in Table 1.

Table 1. Results Of Formulation Nanoemulsgel and Emulgel

Formula	Color	Shape	Odor
NEG1	Yellow	Transparant	Smell specific clove lef oil
NEG2	Yellow	Transparant	Smell specific clove lef oil
NEG3	Yellow	Transparant	Smell specific clove lef oil
EG	White	Transparant	Smell specific clove lef oil

The results of organoleptic nanoemulgel concentrations of 2%, 4% and 6% were yellow, had a distinctive aroma of clove leaf oil and were transparent. Emulgel is white in color, has a characteristic aroma of clove leaf oil and is not transparent.

Homogeneity Test

The purpose of the homogeneity test was to determine the homogeneity aspect of nanoemulgel and emulgel. The results of the homogeneity test are in Table 2.

Table 2. Results Of Organoleptic Examination Of Nanoemulgel And Emulgel

Formula	Result
NEG1	Homogeneus
NEG2	Homogeneus
NEG3	Homogeneus
EG	Homogeneus

The results of the examination of the homogeneity of nanoemulgel and emulgel are that there are no coarse grains on the object glass, which means that all preparations are homogeneous.

Viscosity measurement

Viscosity measurement aims to determine the viscosity of a preparation to flow. Viscosity measurement using a Brookfield NDJ-8S. The results of the viscosity measurements are shown in Table 3.

Table 3. Results Of Viscosity Measurement Of Nanoemulgel And Emulgel

Formula	Viscosity (M.Pas)
NEG1	3434±2,30
NEG2	3375±5,19
NEG3	3109±7,54
EG	9148±2,08

Viscosity value states the amount of resistance of a liquid to flow. The higher the viscosity value, the greater the resistance to flow. Carbopol is a type of gelling agent that provides excellent stability [17].

pH Measurement

The pH test aims to determine the safety of the preparation when used so as not to irritate the skin and also to determine the stability of the preparation. The results of the pH measurements are shown in Table 4.

Table 4. Results Of pH Measurement Of Nanoemulgel And Emulgel

Formula	pH
NEG1	6,15±0,006
NEG2	6,19±0,010
NEG3	6,25±0,000
EG	6,41±0,006

The results of pH measurements showed that the higher the concentration of oil used, the pH of the preparation also increased. The pH measurement results obtained at NEG1 6.15, NEG2 6,19, NEG3 6,25, EG 6,41. The pH results obtained are in accordance with the pH of the skin, which is between 4.5 - 6.5 so it is safe to use and does not cause irritation to the skin.

Particle size measurement

Measurement of nanoemulsigel and emulsigel particles was carried out using a Particle Size Analyzer (Horiba Sz-100) at room temperature when the preparation was completed. The results of the particle size measurements are shown in Table 5.

Table 5. Results Of pH Particle Size Of Nanoemulgel And Emulgel

Formula	Particle size (nm)
NEG1	12,3
NEG2	12,9
NEG3	13,3
EG	8000

Particle size is an important parameter in nanoemulgel preparations. The smaller the particle size will increase the surface contact area, the higher the surface contact, the faster the material enters and is absorbed into the skin so that it can produce the desired effect optimally [24]. The size in the nanoscale is determined by the homogenization time, the stirring speed and the concentration of surfactants and cosurfactants used in the formulation. The particle size results of NEG1, NEG2 and NEG3 are not more than 200 nm. Small nanoemulgel particle size will be more quickly absorbed by the skin, can increase the stability of the active substance, so as to increase drug absorption. While in emulgel the particle size is 8000 nm.

Physical stability study of nanoemulgel and emulgel.

Physical observations of nanoemulgel and emulgel preparations have been carried out for three cycles and there is no change in color, change in odor and phase separation (Fig 2.). It was concluded that the nanoemulgel and emulgel preparations were stable during storage.

(a) (b)

Fig 2. Before cycling test nanoemulgel and emulgel (a) and after cycling test nanoemulgel and emulgel (b)

Antibacterial activity

Antibacterial activity testing was carried out on nanoemulgel and emulgel preparations. By using the agar diffusion method (Kirby-Bauer). The results of the diameter measurement of bacterial growth inhibition are shown in Table 6.

Table 6. The results of the diameter measurement of bacterial growth inhibition of nanoemulgel and emulgel.

Formulation	Inhibition Zone Diameter* (mm)	
	Staphylococus Epidermidis	*Propionibacterium acnes*
NEG 1	12,03±0,20	11,50±0,10
NEG 2	16,93±0,15	16,16±0,11
NEG 3	18,96±0,30	18,03±0,32
EG	18,03±0.11	17,33±0,25

Based on the table above, it can be seen that the higher the concentration of clove leaf oil used, the greater the inhibitory power produced. This proves that the eugenol contained in clove leaf oil can be used as an antibacterial, especially the bacteria that causes acne. Table 6 also shows that the 6% concentration of nanoemulgel has a greater inhibitory power than the 6% concentration of emugel.

IV. CONCLUSION

From the results of the research that has been carried out, it is concluded that clove leaf oil can be formulated into nanoemulgel preparations and is stable during the cycling test and has a particle size below 200 nm. The Nanoemulgel formulation had the greatest antibacterial activity compared to the emulgel formulation.

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