Available online on 15.08.2020 at http://ajprd.com

Asian Journal of Pharmaceutical Research and Development

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Open Access Research Article

Formulation and Antibacterial Activity Tests of Nanoemulsion Gel Black Cumin (*Nigella Sativa* L.) Ethanol Extract

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A B S T R A C T

Objective: Black cumin (*Nigella sativa* L.) is an herbal plant that has the chemical content of essential oil and pharmacological effect as a broad-spectrum antibacterial namely *thymoquinone* (TQ). This research was carried out by making ethanol extract of black cumin, formulating it into nanoemulsion gel and testing the antibacterial activity against pathogenic bacteria in the skin.

Methods: Black cumin seeds macerated with 80% alcohol and concentrated with a rotary evaporator. Black cumin ethanol extract is formulated into nanoemulsion gel using spontaneous emulsification with concentrations of 3%, 5% and 7%. The preparation is stored and evaluated for 12 weeks at room temperature. Evaluation of preparation quality includes organoleptic, viscosity, particle size, centrifugation and cycling test. Antibacterial activity test of nanoemulsion gel of black cumin ethanol extract was carried out by cup-plate technique using *Staphylococcus aureus*, *Staphylococcus epidermidis* dan *Propionibacterium acne*.

Results: the extract yield was 18,37% w/w. All formulas were stable during 12 weeks storage, viscosity 2850-3750 mPa.s, particle size around 45,85-419,78 nm, centrifugation results and cycling tests showed no sedimentation and phase separations, so it were said to be physically stable. The results of the antibacterial activity test showed the value of minimum inhibitory concentration (MIC) of *Staphylococcus aureus* bacteria in F1, F2 and F3 respectively 7,83; 8,93 and 10,00 mm, *Staphylococcus epidermidis* bacteria 6,63; 7,67 and 8,43 mm, *Propionibacterium acne* bacteria 6,43; 7,47 and 7,83 mm.

Conclusion: The results of the study concluded that nanoemulsion gel of black cumin ethanol extract with a concentration of 7% is the best formula because it is stable during storage, has the greatest antibacterial activity against pathogenic bacteria in the skin.

Keywords: Black cumin, nanoemulsion gel, antibacterial, *Staphylococcus aureus*, *Staphylococcus epidermidis* dan *Propionibacterium acne*.

A R T I C L E I N F O: Received -07 May 2020; Review Completed 11 July 2020; Accepted 18 July 2020; Available online 15 August. 2020

Cite this article as:

Syahfitri E L*, Reveny J, Nainggolan M, Formulation and Antibacterial Activity Tests of Nanoemulsion Gel Black Cumin (*Nigella Sativa* L.) Ethanol Extract, Asian Journal of Pharmaceutical Research and Development. 2020; 8(4):07-11. DOI: http://dx.doi.org/10.22270/ajprd.v8i4.777

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INTRODUCTION

The skin is the largest organ that is on the outside of the human body which is supple and soft [1]. Skin health issues are important, especially those related to bacterial activity in the body [2]. Acne is a skin health problem that attacks adolescents and adult with a prevalence of 80% [3] a condition where the pores of the skin become blocked causing inflammation of the pus sac [1]. Inflammation that occurs can be triggered by pathogenic bacterias [4] such as *Propionibacterium acne*, *Staphylococcus epidermidis*, *Staphylococcus aureus* dan *Pityrosporum ovale* [5]. *Staphylococcus aureus* bacteria including gram-positive [6] have a diameter of ±0,5-1µm, are immobile and non-spores, can grow at pH 4,0-9,8 and temperatures 35-37^{0}C [7]. *Staphylococcus epidermidis* is a gram-positive, white or yellow colony and an-aerobic [8] spherical or coccus in irregular groups, 0,8-10 µm in diameter, immovable, non-spores, growing at 37^{0}C [9]. *Propionibacterium acne* bacteria are an-aerobic, gram-positive [8] rod-shaped, non-spores, plays a role in the pathogenesis of acne vulgaris [10].

Black cumin is an herbal plant that grows about 20-90 cm tall and is classified into the *Ranunculuceae* family [11]. Macroscopically including dicotyledon seeds, triangular in shape, seed size of 2-3,5 mm x 1-2 mm, black on the outside and white on the inside, slightly aromatic and bitter [12]. Black cumin has chemical properties and pharmacological effects to overcome bacterial problems [1314]. *Thymoquinone* (TQ) is a black cumin essential oil compound that has broad spectrum antibacterial activity [15].

Nanoemulsion is a heterogeneous system consisting of fluid that can not be mixed, dispersed as droplets in other liquids with droplet sizes between 20-500 nm so as to

increase stability and bioavailability [16]. Gel is a semi-solid system consisting of a dispersion composed of small inorganic particles and penetrated by a liquid [17].

MATERIALS AND METHODS

Materials

The materials used in this research are v*acuum rotary evaporator*, oven (Memmert), incubator, *laminar air flow cabinet*, *particle size analyzer* (*FRITSCH, Laser Partickle Size Analysette*), magnetic stirrer, viscometer NDJ-8S, autoklaf, micropipet (Eppendorf), pH meter (Hanna), calipers, sonicator, *Staphylococcus aureus*, *Staphylococcus epidermidis* dan *Propionibacterium acne*. Black cumin seeds, *Nutrient Agar* (Oxoid), ethanol 96% (PT. Bratacem), trietanolamin (CV. Rudang Jaya), carbopol 940, sorbitol (CV. Rudang Jaya), tween 80 (CV. Rudang Jaya), sodium carboxyl methyl cellulose (CV. Rudang Jaya), parafin liquid (CV. Rudang Jaya).

Sample preparation and extraction

As much as 1000 g of black cumin seeds were macerated with 10 liters ethanol 80% and soaked for the first 6 hours while stirring, then allowed standing for 18 hours. Maserat is stored in a dark bottle and then separated by precipitation, then filtered. The search process was carried out twice. The extract was concentrated with a rotary evaporator at a temperature of $\pm 50^0$C, to obtain a thick extract of 183,7 g (yield 18,37%) [18].

Formulation of nanoemulsion gel

The process of making nanoemulsion gel using spontaneous emulsification method by mixing the oil phase and water phase, after nanoemulsion is formed, a gel base is added. The aqueous phase consists of ethanol extract black cumin, tween 80 (surfactant) and distilled water stirred with a stirring rod to form a thick, pale yellow mass. The oil phase consists of liquid paraffin (oil) and sorbitol (cosurfactant) stirring with a stirring rod. The oil phase is added to the aqueous phase and then on the sonicator for 30 minutes until a clear preparation is formed, then homogenized with a magnetic stirrer with a speed of 2000 rpm for 1 hour (nanoemulsion). Making a gel base is done by developing carbopol 940 in aquades then adding triethanolamine then adding the CMC sodium corpus and homogenized (gel base). Nanoemulsion was added with a gel base homogenized with a magnetic stirrer at a speed of 2000 rpm for 10 hours. The formula of nanoemulsion gel ethanol extract of black cumin can be seen in table 1.

Table: 1. Nanoemulsion Gel of Black Cumin Ethanol Extract Formulas

Nanoemulsion Stuff	**F1 (g)**	**F2 (g)**	**F3 (g)**
Black cumin ethanol extract	3	5	7
Liquid paraffin	3	3	3
Tween 80	30	30	30
Sorbitol	25	25	25
Aquadest ad	75	75	75
Gel Stuff	**Formulas (g)**		
Sodium carboxyl methyl cellulosa	0,25	0,25	0,25
Carbopol 940	1	1	1
Trietanolamin	qs	qs	qs
Aquadest ad	25	25	25

Evaluation of Preparations

Organoleptic

Organoleptic tests were carried out on each formula which was stored for 12 weeks at room temperature and observations were carried out every week, the parts observed in the form of changes in color, odor, shape and phase separation.

Viscosity measurement

Measurements were made by inserting a nanoemulsion gel into a 100 mL glass beaker and selecting the appropriate spindle. This measurement uses a NDJ-8S viscometer [19]. Measurements were made every 2 weeks at room temperature.

Determination of particle size

Test carried out using a particle size analyzer (PSA) by adding 500 mg of the preparation in 1 mL aquadest, then taken 1 mL. this test is carried out at weeks 0, 6 and 12.

Centrifugation test

The nanoemulsion gel was put into a centrifugasion tube then centrifuged at 3750 rpm for 5 hours [19].

Cycling test

The preparation is stored at 4 ± 2^0C for 24 hours, then transferred to 40 ± 2^0C for 24 hours. This treatment is one cycle. The experiment was carried out as many as 6 cycles and observed separation [20].

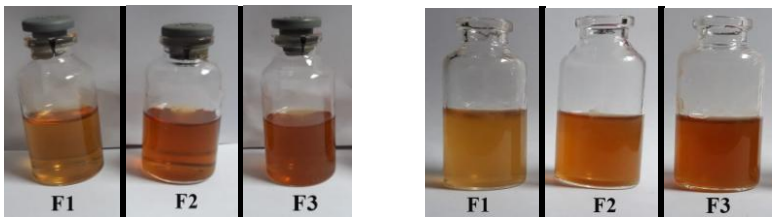
Antibacterial activity

This test is done by cup-plate technique. 0,1 mL of bacterial inoculum is inserted and 15 mL of NA has been thawed, homogenous until solidified. Every petri dish is made in the well, then put 0,1 mL of the preparation into the wellbore. Incubated at $36\text{-}37^0$C for 24 hours. Inhibitionzone diameter measurements were taken. Testing is done with three repetitions [21].

RESULTS AND DISCUSSION

Organoleptic

Evaluation of the quality of the preparation by organoleptic observation was carried out for 12 weeks of storage with observations every 1 week. Nanoemulsion gel was stored at room temperature and observed changes in color, odor, shape and phase separation by comparing the initial preparation after it was made and the preparation after 12 weeks of storage.



A B

Figure: 1. Black cumin ethanol extract nanoemulsion gel. A: week 0, B: week 12

Based on Figure 1 shows that in F1, F2 and F3 stored for 12 weeks at room temperature there is no change in odor, shape and phase separation but there is a slight turbid change in color which is due to the increasing size of the particles.

Viscosity measurement

The purpose is to measure the viscosity to determine the the thickness of a preparation. This measurement uses an NDJ-8S viscometer with rotor 2, speed 6 rpm at room temperature for 12 weeks of storage. Viscosity values obtained in F1, F2 and F3 decreased during 12 weeks of storage.

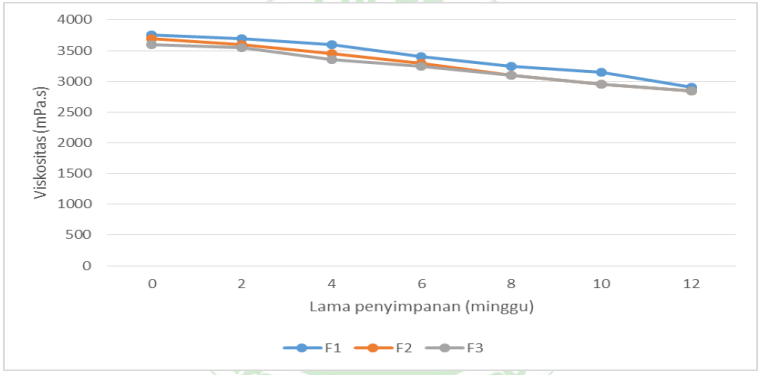


Figure 2. Effect of storage duration on the viscosity of black cumin ethanol extract nanoemulsion gel

Determination of particle size

Determination of particle size of ethanol extract nanoemulsion gel preparation was carried out using a Particle Size Analyzer (FRITSCH) at room temperature at weeks 0, 6 and 12. The result of the particle size test for nanoemulsion gel of black cumin ethanol extract in size with the length of time of storage. The particle size of F1 at weeks 0, 6 and 12 was 39,28; 110,05 and 279,32 nm. The particle size of F2 was 53,65; 129,90 and 356,01 and the particle size of F3 was 66,12; 219,31 and 389,95.

Centrifugation test

The centrifugation test is carried out at the beginning after the preparation is made with a measurement of the 1 time and visually observed phase separation occurs. This is doneto test the mechanical stability of the preparation. In this test the speed used is 3750 rpm for 5 hours which is analogous to the gravity that will be experienced by the preparation for one year [22]. The results of the tests carried out, it was found that F1, F2 and F3 did not experience preparation phase separation, his shows that the nanoemulsion gel preparation of black cumin ethanol extract is mechanically stable.

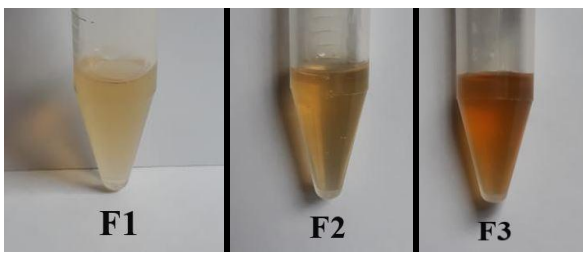


Figure: 3. Centrifugation test results of black cumin ethanol extract nanoemulsion gel

Cycling test

Cycling test was observed on nanoemulsion gel of black cumin ethanol extract which was stored at 4±2^{0}C in the

refrigerator for 24 hours and then transferred and stored at 40±2^{0}C in the climatic chamber for 24 hours, this treatment was calculated 1 cycle. The cycling test method was repeated for 6 cycles, then observed the physical conditions in the form of changes color, odor, shape and phase separation of the preparation visually by comparing the preparation before treatment and after treatment. From the test results obtained f1 and F2 did not experience.

Antibacterial activity

Testing the antibacterial activity of nanoemulsion gel ethanol extract of black cumin by cup-plate technique, this method is used because it allows the nanoemulsion gel test material to contact directly with the agar wall, so measuring the diameter of the inhibition zone will be easier and can be seen visually [23]. The test bacteria used were *Staphylococcus aureus*, *Staphylococcus epidermidis* dan *Propionibacterium acne*.

Table: 2. Results of measurements of diameter of bacterial growth inhibition against nanoemulsion gel preparation of black cumin ethanol extract

No	Formulas	Inhibition Area Diameter (mm) (n=3)		
		Staphylococcus aureus	***Staphylococcus epidermidis***	***Propionibacterium acne***
1	F1	7,83±0,058	6,63±0,153	6,43±0,208
2	F2	8,93±0,058	7,67±0,116	7,47±0,208
3	F3	10,00±0,100	8,43±0,153	7,83±0,208

Information:

F1: Naoemulsion gel of black cumin ethanol extract 3%, F2: Naoemulsion gel of black cumin ethanol extract 5%

F3: Naoemulsion gel of black cumin ethanol extract 7%

Based on these results, concentration 7% or F3 is more effective against *Staphylococcus aureus* bacteria by showing a zone of bacterial growth inhibition that is greater than the zone of bacterial growth inhibition of *Staphylococcus epidermidis* dan *Propionibacterium acne*.

CONCLUSION

The results of the study concluded that nanoemulsion gel of black cumin ethanol extract with a concentration 7% was the best formula because it was stable during storage, particle size below 500 nm, had the greatest antibacterial activity against pathogenic bacteria in the skin.

ACKNOWLEDMENT

Researchers appreciate thanks to the pharmaceutical department, faculty of pharmacy, University of North Sumatera.

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