ORIGINAL ARTICLE

Preparation of Black Cumin Extract Nanoemulsion Using the Oil Phase of Virgin Coconut Oil (VCO) Tween 80 and PEG 400 Surfactants

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ABSTRACT

Introduction: Black Cumin Seed (*Nigella sativa*) is known to have anti-inflammatory activity because it contains thymoquinone. Manufacturing in the form of nanoemulsion extracts can accelerate the absorption process in the body. A Nanoemulsion is a transparent or translucent system with a uniform and very small globule size (usually in the 2-500 nm range). Nanoemulsions consist of surfactants, co surfactants, oil and water. Tween 80 is used as a surfactant, which when combined with VCO, will form a small nanoemulsion globule size (2-500 nm). PEG 400 act as a co surfactant to help lower interfacial tension. This study aims to determine the concentration of VCO, tween 80, and PEG 400, which can be formulated as nanoemulsion preparations. **Methods:** The preparation of black cumin extract nanoemulsion was carried out by a combination of the low energy emulsification method with a magnetic stirrer and the high energy emulsification method with a sonicator. **Results:** The results showed the test results droplet size nanoemulsion extract cumin, a black in colour with a concentration of 1 % produced a grain size of 139.3 nm, at a concentration of 2% obtained the particle size of 122.7 nm and at a concentration of 3% obtained the particle size of 154.8 nm. **Conclusion:** The polydisperse index is in the range of 0.57-0.59, which shows particle size uniformity nanoemulsi, because all three formulations have a similar polydisperse index, which means that the distribution of particles with particle size uniformity levels were very good.

Keywords: Black cumin, extract, nanoemulsion, tween 80, PEG 400

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INTRODUCTION

Black cumin seed (*Nigella sativa*), known as black cumin or Habbatusauda, is native to Southern Europe and is found in India. This plant has been used as an herbal remedy for more than 2000 years. The seeds of the black cumin plant are used for medical purposes and have been added to the traditional herbal medicine in Arabia and Greece. Black cumin seeds (*Nigella sativa* L.) are reported to have shown pharmacological effects which include antibacterial, anthelmintic, anticestodic, antifungal, antiviral and can increase the immune response (1).

One of the studies that carried out the extraction of the active compound from black cumin seeds stated that thymoquinone was the main active compound in black cumin essential oil (2). Black cumin extraction with the reflux method for 3 hours using ethanol solvent hasthe ability to inhibit microbial growth (3). *In vivo*, *Nigella sativa* is effective in healing burns in rats (4).

Currently, the technology of nano development improves the quality of food function. The use of nanotechnology in the food and pharmaceutical fields has a tendency to continue to increase. Nanotechnology can prepare active drug particles on a nanoscale. The shape and size of the particles is one of the factors that affects the effectiveness of the drug, because the particle size is very influential in the process of solubility, absorption and distribution of drugs (5). Increasing the amount of drug in the blood during systemic delivery will also increase the risk of side effects and reverse effects, up to the risk of reaching toxic levels. In many cases, an increase in levels of the drug in the blood is necessary for the drug to produce a pharmacological effect. Therefore, nano particles provide a good solution because they can provide pharmacological effects at smaller (efficient) doses (6).

Research using nano technology with black cumin raw materials is still limited. Ravindran et al. (7) carried out the manufacture of black cumin nanoemulsion using low energy with the composition of pure thymoquinone, acetonitrile as a solvent, and pluronate F-68 as a surfactant. Bamusa and Hujaj, did the manufacture of nanoparticles using high energy with black cumin composition, bysupercritical fluid extraction (SFE) method with palm oil, 1% Tween 80, and sorbitol (1). In another study by Tubesha et al., manufacture of black cumin nano emulsions was done using high energy with the composition of pure thymoquinone, glycerol trioleate, 2% Tween 80, and demineralized water (8).

Nanoparticles have many advantages. Nanoparticle manufacturing technology is needed for the development stage in the world of medicine. This research studies the development of black cumin extract in the form of a nanoemulsion which is expected to have better solubility and absorption properties (100%). Therefore, the purpose of this study is to make an estab-made nano emulsion from extracts of black cumin oil phase of virgin coconut oil (VCO) with a combination of surfactants with tween 80 and peg 400.

MATERIALS AND METHODS

Material

The principal material that was used in the research is the seeds of black cumin obtained from Makkah, Saudi Arabia. The material emulsifier and the coating that are used are Tween 80, PEG 400 (Sigma Co., USA), and Virgin Coconut Oil (PT Citra Prima Organic).

Black Cumin Seed Extract

Extracts were made in accordance with the guidelines for implementing traditional medicine clinical trial guidelines. The ethanol extract was made using the Sokhletation method using 95% ethanol. Black cumin seeds were purchased from traditional medicine supply stores. Before extraction of black cumin seeds, identification was carried out by experts to determine the authenticity of the test material at the Nanomaterials for Renewable Energy Research Center Laboratory. Ripe black cumin seeds were selected, dried and then made into powder. Each 50 g of dry black cumin seed powder was wrapped in filter paper and then put in a soxhlet apparatus to which 300 mls of 95% ethanol was added and connected to a cooler bag. The extraction process is carried out until the black cumin seed juice runs out, which is marked by the extracted liquid, which has a clear color. The extract obtained was concentrated with an evaporator until the ethanol evaporated and, lastly, the extract was stored in a sterile bottle.

Black Cumin Extract Nanoemulsification

Manufacture of nanoemulsions was done using spontaneous emulsification technique. The emulsion system consists of an organic phase (oleoresin and 70% ethanol) and an aqueous phase (water and nonionic surfactant). The organic phase was prepared by mixing black cumin viscous extract and 70% ethanol solvent to reach a total dissolved solid of 20° brix.

Mechanical emulsification is spontaneously carried out by adding the right phase of the organic to the phase of the water through dripping (drop by drop) and this is mixed by using a magnetic stirrer. The organic phase was distributed to the phases of water, which contains a surfactant and produces nanoemulsion spontaneously by diffusion faster than organic solvents. The surfactants used are Tween 80 at a concentration of 17.3% and the cosurfactant PEG 400 at a concentration of 8.7%. The best emulsion is determined based on the size of the Particle Size Analyzer (PSA).

RESULTS

Black Cumin Extraction Analysis

Black cumin (*Nigella sativa*) from 50 grams of seeds obtained 100 ml of oil extract (essential oil). Data from the analysis of black cumin extract can be seen in table I. The yield of black cumin extract produced 18.39% of yield. This yield is much higher than the yield of research conducted by Kiswandono (9), namely 9.98% using hexane 11.86% and methanol 80% as the solvent. This difference in yield could be due to differences in the solvent used. Ethanol yielded 95% higher yield of cumin extract with hexane and methanol.

Table I: Data from the Analysis of Black Cumin Extract

Characteristics	Score
Color	Dark chocolate
Form	Fluid
Scent	Characteristic scent of cumin
Density (g/mL)	1.16
Yield (%)	18.39%

Black Cumin Extract Nanoemulsion Analysis

Nanoemulsions are prepared by spontaneous emulsification mechanism, which occurs when the organic phase and aqueous phase are mixed. The organic phase is a uniform solution of organic solvent (ethanol 70%) and black cumin eleoresin. In this study, emulsion preparation was carried out using Tween 80 and PEG 400 with a composition ratio of 0:4, 1:3, 2:2, and 3:1 using the spontaneous emulsion method (Table II).

The aqueous phase is prepared by dissolving water and a nonionic surfactant. Non-ionic surfactants are most commonly used to stabilize emulsions (10).Tween 80 as a non-ionic surfactant has a high hydrophilic/lipophilic (HLB) balance value (15.0±1.0) so that it is stable in oil-in-water emulsion systems (11). The mixing of water and Tween 80 as the aqueous phase was carried out with a magnetic stirrer for 30 minutes at room temperature. Meanwhile, the organic phase was prepared as much as 10% of the total emulsion. Emulsification occurs when the organic phase is injected into the aqueous phase

Table II: Preparation of Black Cumin Extract Nanoemulsion

Sample	EO : VCO (4%)	Tween 80 (17.3%)	PEG 400 (8.7%)	Water (70%)	Sonification
T1	1:3 (0.2 mL: 0.6 mL)	3.46 mL	1.74 mL	14 mL	
T2	2: 2 (0.4 mL : 0.4 mL)	3.46 mL	1.74 mL	14 mL	30 minutes
T3	3 : 1 (0.6 mL: 0.2 mL)	3.46 mL	1.74 mL	14 mL	

EO: Essential Oil (Black cumin extract)
VCO: Virgin coconut oil

(12).

Mixing the oil phase and water phase was done using a homogenizer (Wiggens D-50 0) at 30,000 rpm for 30 minutes. The nanoemulsion at the end of this phase was analyzed by checking droplet size with a Particle Size Analyzer (PSA).

The results of the characterization of nanoemulsions produced through the spontaneous emulsion method in this study can be seen in table III. All three formulations have a similar polydisperse index ranging from 0.57-0.59, which shows that all three formulations have a good particle size uniformity level. This indicates that the T3 formulation has much higher stability than the other two formulations.

Table III: Black cumin emulsion nano particle size based on surfactant concentration

Sample	Particle Size (nm)	Polydispersity Index	Zeta Value Potential (mV)
T1	139.3 nm	0.57	62.5
T2	122.7 nm	0.59	66.9
T3	154.8 nm	0.57	313.2

DISCUSSION

Oil-in-water nanoemulsions are oil droplets with a diameter of 1-100 nm, dispersed in the aqueous phase continuously and the droplets are surrounded by emulsifying molecules (13).The resulting droplet size depends on several factors, including the type of homogenizer used, manufacturing temperature, energy intensity, and time, as well as the condition of the sample in the form of oil, oil concentration, type of emulsifier/surfactant used, and the physicochemical properties of the sample (interface tension and viscosity) (14).

The best emulsion formulation is indicated by the smallest droplet size based on the results of particle size analysis (Particle Size Analyzer- HORIBA SZ-100). Table 3 shows the results of the droplet size test of black cumin extract nanoemulsion with a concentration of 1% producing a grain size of 139.3 nm. At a concentration of 2%, a particle size of 122.7 nm was obtained and at a concentration of 3%, a particle size of 154.8 nm was obtained. The Polydisperse index shows the particle size uniformity of nanoemulsion. From table 3, it can be seen that all three formulations have a similar polydisperse index at 0.57-0.59, which means that all three formulations have a good particle size uniformity level.

It is different from the value of zeta potential (mV), which is a parameter of electric charge between colloidal particles. The higher the value, the more stable the zeta potential of a colloid solution due to the higher value of zeta potential, which will further prevent the occurrence of flocculation or the incorporation of colloidal particles. In this case, the nanoemulsion with formulation 1 (T1) has the lowest zeta potential value, while T3 has a much higher zeta potential value. This indicates that the T3 formulation has much higher stability than the other two formulations. The swivel used in this study can produce nanoemulsion the size of an average particle at less than 200 nm. The collision between molecules during mixing causes differences in the size of the resulting nanoemulsion. The higher the speed of the homogenizer and the longer the rotation, will increase the intensity of intermolecular touch, so that the resulting nanoemulsion grain size is smaller (15).

CONCLUSION

Black cumin extract nanoemulsion was made using the spontaneous method with a concentration of 2% produced a better nanoemulsion than the 1% concentration and the 3% concentration. The polydisperse index in the range of 0.57 -0.59 showed particle size uniformity nanoemulsion, because all three formulations had a similar polydisperse index, meaning that the distribution of particles with particle size uniformity level was very good.

As the study compiles all the knowledge needed to develop nanoemulsions which are ecologically friendly extract, the translation of nanoemulsion formulations, on the other hand, is more reliant on the results of clinical investigations that can scientifically demonstrate their therapeutic potential. More studies are needed over time that will incorporate need for the inclusion of best formulations and in vitro evaluation.

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