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Original Article

FORMULATION AND STABILITY STUDY OF BLACK CUMIN (*NIGELLA SATIVA* L.) SEED OIL EMULSION USING SUCROSE PALMITATE AS EMULSIFIER

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ABSTRACT

Objective: An emulsion of black cumin seed oil was developed using an orally safe surfactant, sucrose palmitate, to make it more comfortable to consume.

Methods: The emulsion was made using a 3% concentration of sucrose palmitate to emulsify 5% (F1) and 7.5% (F2) black cumin seed oil to the developed stable emulsion. The hedonic test was applied to 30 panelists, showing the accepted formulation.

Results: The pH value of each formulation degraded during 12 w of storage. The formula of 5% oil (F1) has better physical stability, and its bioactive component, Thymoquinone, showed a slight degradation on the first day. But it showed a rapid degradation after 60 d of storage due to its instability in a solution. The F1 formula (mean = 3.1667) is more preferred than the F2 formula (mean = 3) of the 1-5 hedonic scale, with the significance score (p) valued less than 0.05 and considered to be significantly different from its original form.

Conclusion: The emulsion of black cumin oil can be developed and more comfortable to consume.

Keywords: Emulsion, Thymoquinone, Sucrose palmitate, Hedonic test, Stability

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INTRODUCTION

Medicines derived from plants have amplified their use for various diseases [1]. Apart from their easy accessibility and low cost, it is believed that natural medicines have fewer side effects than synthetic drugs or modern allopathic drugs [2, 3]. Indonesia is one of the countries that apply treatment derived from medicinal plants. One of them is the utilization of the efficacy of seed oil derived from the seeds of the black cumin plant.

Black cumin (*Nigella sativa* L.) from the Ranunculaceae family is herbal. Many pharmacological activities include anti-inflammatory, antioxidant, anticancer, and immunomodulatory. It has been used for more than 2000 y [4, 5]. Among the parts of the black cumin plant, the seeds are the part that is often used and has beneficial properties. Thymoquinone from black cumin seed oil is a wide-range therapeutic bioactive component. Antioxidants and immuno-modulators are two of the famous therapeutic effect of black cumin seed oil [6]. Due to the antioxidant activity of Thymoquinone, it is currently believed to consume black cumin seed oil to increase the body's immunity against the ongoing pandemic [7].

Cumin seed oil is produced from the cold-pressed process of black cumin seeds and then filtered. The result is fixed oil and essential oil [8]. The content of Thymoquinone in black cumin seed oil is the most important ingredient that provides a therapeutic effect [9]. The highest number of bioactive constituents was present in essential oils isolated by different extraction methods from black cumin seeds. Black cumin seed oil, which is commonly consumed, namely fixed oil, also contains Thymoquinone but in a lower concentration than the concentration in an essential oil [6].

Table 1: Formulation of black cumin seed oil emulsion

No.	Composition	F1 (%)	F2 (%)
1	Black Cumin Seed Oil	5	7,5
2	Sucrose Palmitate	3	3
3	Honey	15	15
4	Propylene glycol	5	5
5	Methylparaben	0,1	0,1
6	Alpha Tocopherol	0,05	0,05
7	Vanilla Essence	2 drops	2 drops
8	Demineralized water	Ad 100 gram	

Cumin seed oil has pharmacological potentials such as antioxidant, anticancer, and antimicrobial. It also has analgesic, antipyretic, contraceptive, anti-oxytocic, antitussive, and anti-inflammatory. In traditional use, black cumin seeds are used for various diseases such as chronic headaches, respiratory diseases, diabetes, hypertension, male infertility, paralysis, infections, diseases of the digestive system, immunity boosters, and cough medicine. In current conditions, the black cumin seed oil is used as an immunity enhancer and for its antiviral effect. The cumin seed oil has been found to suppress viral load in preclinical trials of cytomegalovirus infection because it is associated with an increase in CD4+cells and interferon-gamma and suppressing viral load in HIV/AIDS patients [10].

An emulsion is a thermodynamically unstable system. It consists of at least two immiscible liquid phases stabilized by an emulsifier. It consists of a dispersed phase and a continuous phase. The dispersed phase is manifested as globules, with the size of the globule diameter of the dispersed phase generally being in the range of 0.1-10 m, with a consistency ranging from high-viscosity to low-viscosity liquids—nonpolar, such as water and oil [11].

Sucrose palmitate is a food emulsifier used in the pharmaceutical industry. Sucrose palmitic is synthesized by transesterification of sucrose and vinyl esters of fatty acids in this type of palmitic acid [12]. Sucrose Palmitate is a nonionic surfactant emulsifier in powder

form. The description is in the form of a powder with a molecular weight of 580.7 g/mol [13]. The solubility of sucrose palmitate depends on its type. Sucrose palmitate with an HLB value of 1 is soluble in oil, while sucrose palmitate with an HLB value of 15-16 is soluble in water. This type of emulsifier has good emulsification properties and is used at low concentrations [14]. Thymoquinone is a bioactive component in black cumin seed oil whose main structure is a terpenoid, and terpenoids are sensitive to heat [8].

There has been reported that the loss of thymoquinone concentration in heated black cumin seeds (with heating>150 °C) to be more than 75% [15]. Some studies reported the effect of solvent type, pH, and light on the stability of thymoquinone during storage. Thymoquinone was more stable at lower pH, but its stability decreased with increasing alkalinity [16]. Thymoquinone has a maximum wavelength of 252-257 nm, slightly soluble in water, with a solid description. The boiling and melting points of thymoquinone are at 232.0 °C and 45.5 °C [13].

MATERIALS AND METHODS

The ingredients used were pure black cumin seed oil (*Nigella Sativa* sp. Seed oil) (Rumman, Central Java, Indonesia). Al-Shifa Natural Honey (Alshifa, Sunbulah, Saudi Arabia), alpha-tocopherol (Asean Chemical, DSM Nutritional Products LLC, North America), methylparaben (Asean Chemical, Gujarat, India). Sucrose monoester sucrose palmitate Habo Monoester P-90 (Compass Foods Pte Ltd, Singapore), Propylene glycol (Brataco, Indonesia), Demineralized water (Brataco, Indonesia), Thymoquinone Standard (Sigma-Aldrich, Singapore), pro-analytical methanol (Merck, Germany), pro-HPLC methanol (Merck, Germany), and Aqua analytical grade (Onemed, Indonesia).

Characterization of black cumin seed oil

Black cumin seed oil was characterized by observing pH, specific gravity, surface tension, and interfacial tension values.

The emulsion was made by dissolving sucrose palmitate in propylene glycol, which was heated to a temperature of±40 °C and then mixed with honey. Methylparaben was added to the mixture, then homogenized at a low speed of 300 rpm for 2 min. The stirring speed was increased to 1100 rpm then black cumin seed oil, which had been dissolved in alpha-tocopherol, was poured. Warm demineralized water at the temperature of 40 °C was poured slowly and homogenized for 15 min.

Organoleptic and homogeneity test

The organoleptic test was carried out by observing the emulsion dosage form, smell, taste, and the presence of phase separation using the five senses pH test. Measurements were taken every week for 12 w, starting from week 0, 1, 2, 3, 4 to week 12, and the numbers obtained were recorded [17].

Globule size test

The Particle Size Analyzer was used to determine the globule size. One drop of the vortex preparation was dissolved in 10 ml of water, then vortexes again to obtain a homogeneous dispersion, then put into a cuvette and observed for the globule size distribution.

Viscosity and rheological test

The Cole-Parmer brand Brookfield Viscometer was used in a room at a temperature of 30±2 C with spindle number 1; the speed varied from 12,20,30,50,60,100 rpm gradually.

Storage stability test

Storage was carried out for 12 w at 4 °C±2 °C, 40 °C±2 °C, and 30 °C±2 °C. Physical appearance and phase stability were observed as periodic pH (ICH guidelines).

Cycling test

The preparation was placed at a temperature of 40±2 °C for 24 h. Then put in the refrigerator at a temperature of 4±2 °C, for 24 h, then repeat the cycle for six cycles. Physical and pH changes that occur are observed and then compared with the initial preparation.

Centrifugation test

The samples were put into a centrifuge tube, then centrifuged at a speed of 3800 rpm for 30 min, then the phase separation was observed [18].

Hedonic test

The hedonic test of stable emulsion were carried out on 30 panelists. Panelists tried the emulsion and filled out a rating form on a scale of 1-5. The scale used is 1. Very Dislike 2. Dislike, 3. Neutral, 4. Like, 5. Very Like. The results were analyzed using the SPSS (Statistical Product and Service Solution) version 24 program.

HPLC method optimization

Optimum wavelength optimization was carried out using a UV-Vis spectrophotometer. Then the optimization of the composition of the mobile phase and the flow rate for the determination of the thymoquinone content was carried out using HPLC (High-Performance Liquid Chromatography). Using thymoquinone standards, the composition of the methanol-water mobile phase was 70:30, 75:25, 80:20, and flow rates of 0.8, 1.0, and 1.2 ml/minute [19, 20].

The analysis was carried out for five repetitions at the optimum analysis conditions. About 20 microliters of thymoquinone standard were injected into the chromatograph. Then observed for HETP, N, retention time, tailing factor, and % RSD of the retention area and time [21].

Linearity and range

For linearity, standard solutions were prepared with respective concentrations of 20, 30, 50, 100, and 200 g/ml; then, each was injected into the chromatographic system. The regression equation is made by plotting the area and concentration on the curve, then the correlation coefficient (r) is calculated. The linearity test is determined with a minimum correlation coefficient of 0.9990 [21]

Selectivity

To determine selectivity, as much as 20 l of thymoquinone standard solution was injected into the chromatography. Then injected as much as 20 µl of the black cumin seed oil emulsion sample solution.

LOD and LOQ

The LOD and LOQ values were calculated based on calibration data from the response standard deviation values.

Accuracy and precision

Thymoquinone standards with a concentration range of 80%, 100%, and 120% were added to the emulsion, then extracted using methanol and centrifuged for 15 min at 3000 pm. The supernatant was then filtered using a 0.45 µm millipore filter membrane and injected into the chromatography.

Determination of thymoquinone concentration

The emulsion was extracted using methanol and centrifuged for 15 min at 3000 pm. The supernatant was then filtered using a 0.45 µm millipore filter membrane and injected into the chromatography.

RESULTS AND DISCUSSION

Characterization of black cumin seed oil

The black cumin seed oil has a physical appearance in the form of yellow-brown oil with a characteristic oil odor, spicy taste, and low consistency. The oil has a specific gravity of 0.87724 g/ml. The degree of acidity of the oil is 5.03. The oil surface tension value is 37.082 dyne/cm, and the oil interfacial tension value is 14.5103 dyne/cm.

Black cumin seed oil emulsion formulation

The best black cumin seed oil emulsion used was the formulation that had the best stability after storage for forty-eight hours to determine whether the emulsion tended to cream. The emulsion was made using an IKA RW 20 digital homogenizer. Sucrose palmitate was dissolved using propylene glycol, which was warmed at a temperature of ±40 °C. Although it was soluble in water, the dissolving of sucrose palmitate in hot water took a long time, and

sucrose palmitate tended to form a gel. This method was applied to 5%, 7.5%, and 10% oil concentrations. Variations of honey concentrations are 15%, 20%, and 25%. However, the formulation with 10% oil underwent phase separation; at the maximum concentration of surfactant use, the oil did not bind well. In the formulation with 20% and 25% honey concentration, the emulsion experienced faster creaming. This phenomenon mostly occurs due to differences in the aqueous phase's specific gravity and the emulsion's consistency, which has a low viscosity [2]. So that in the final emulsion formulation, 3% sucrose palmitate was used, with 15% honey for each variation of 5% and 7.5% black cumin seed oil.

Organoleptic and homogeneity

Fig. 1: Organoleptic of black cumin seed oil emulsion F1 (Left) and F2 (Right)

The appearance of the emulsion is a creamy milky liquid, with a characteristic odor of oil and honey with a mixture of vanilla. The liquid is easy to flow and homogeneous, and there is no phase separation, color gradation, or creaming.

Degree of acidity (pH)

The degree of acidity (pH) of the post-formulation F1 was 4.89, and F2 was 4.94. The pH range for preparations that are administered orally is quite large as long as the pH is still in the range of weak acid, neutral, and weak base. In this emulsion formulation, the weak acidic pH of the emulsion was caused by the heated propylene glycol component.

Globule size and zeta potential

The homogenizer's speed was influenced by particle size and stirring time of making the emulsion, where the higher the speed and the longer the stirring, the smaller the size of the globules [22]. Another factor affecting the size of the globules is the sucrose palmitate surfactant**.**

This surfactant can reduce globule size due to the sucrose palmitate hydrophilic group and will affect interfacial tension reduction. Propylene glycol acts as a cosolvent and cosurfactant [23-25]. An increase in globule size is referred to as the Ostwald ripening phenomenon [26].

Table 2: Emulsion globule size distribution and potential zeta (n=3)

	Week	PDI	Globul size Di90 (nm)*	Potential Zeta (mV)*
F1	0	0.123	291±2	-66.9±2.3
	12	0.109	317±3	-65.7±3.1
F2	0	0.119	296±2	-39.4±2.5

*Data is expressed as mean±SD, n=3

Table 3: The emulsion viscosity during storage

	Week	Viscosity (cP)		Week	Viscosity (cP)
F1	0	6.63±0.1	F2	0	7.30±0.2
	5	6.76±0.2		5	7.30±0.1
	10	6.84±0.1		10	7.40±0.2

Data is expressed as mean±SD, n=3

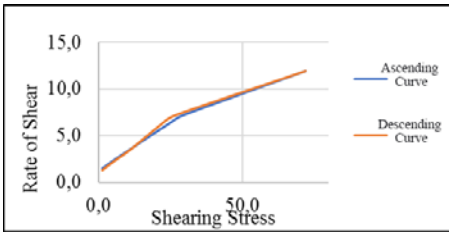

Fig. 2: Flow type curve (Rheology)

Viscosity measurements were carried out at week 0, week five, and week 10. Formulations F1 and F2 did not significantly differ in the viscosity value. Along with storage, the viscosity of the preparation increased but did not make a significant difference. The flow type or rheology of the emulsion expressed through the curve indicates that the emulsion has a Newtonian flow which shows the low viscosity of the emulsion, with most of the components that make up it being water [27].

Storage stability test

Viscosity measurements were carried out at week 0, week five, and week 10. Formulations F1 and F2 did not significantly differ in the viscosity value. Along with storage, the viscosity of the preparation increased but did not make a significant difference. The flow type or rheology of the emulsion expressed through the curve indicates that the emulsion has a Newtonian flow which shows the low viscosity of the emulsion, with most of the components that make up it being water [27].

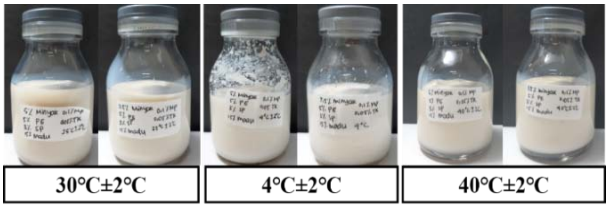

Fig. 3: Emulsion organoleptic during storage, The emulsion was stable during storage at 4 °C, 30 °C, and 40 °C, did not undergo phase separation and did not undergo creaming

The decrease in pH occurred during 12 w of storage, mainly caused by oil oxidation [30]. An increase in storage temperature increased the number of hydrogen ions [H+] [30]. The effect of degradation of propylene glycol components at high temperatures and stable pH of sucrose palmitate, which in acidic conditions tends to hydrolyze the ester group [31]. The addition of antioxidant tocopherol has not been able to reduce the oxidation reaction of the oil.

Cycling test

The cycling test results showed that both formulations were stable with no phase separation or creaming. Reversible crystallization occurs when stored at cold temperatures. The decrease in pH occurred quite drastically for six cycles.

Centrifugation test

The results showed that the preparation was relatively stable with no phase separation; there was a slight blackish-brown precipitate and the dissolved honey content of the remaining propylene glycol. Based on the centrifugation test, the formulation was considered stable.

Hedonic test

In Scent and Flavor parameters, F1 emulsion was preferred with mean values of 3.73 and 3.13 over F2 emulsion, although the two were not significantly different. In appearance parameters, F2 emulsion was preferred over F1 with a mean value of 4.06, although it was not significantly different. Both emulsions were considered capable of masking the Scent and Flavor of black cumin seed oil, with a significant difference between the black cumin seed oil blank and the black cumin seed oil emulsion.

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Method optimization

The optimum analytical wavelength of 253 nm was obtained for the detection of Thymoquinone. It preferred the composition of the methanol-water mobile phase at a ratio of 80:20 by considering the faster retention time and lower column pressure. The flow rate was chosen as 0.8 ml/min based on the lower Tf (tailing factor) value.

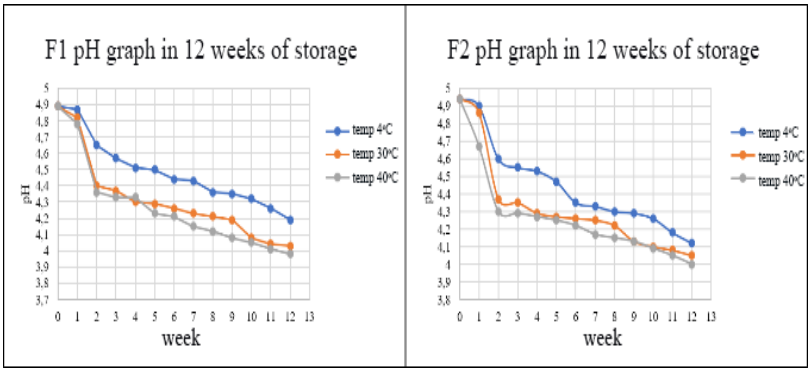

Fig. 4: Graph of decrease in emulsion pH during 12 w of storage

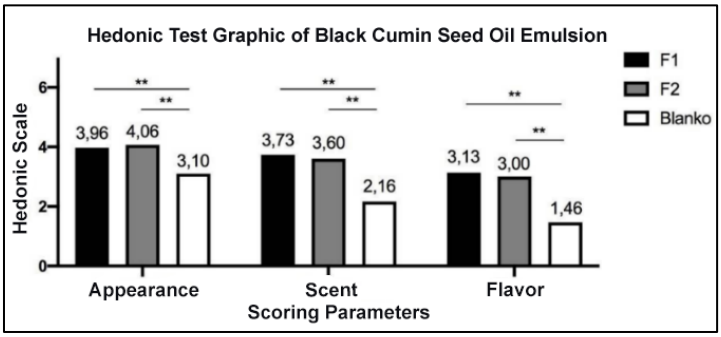

Fig. 5: Graph of hedonic test of black cumin seed oil emulsion

Table 4: Optimization of the comparison of the mobile phase composition

Mobile phase (Methanol: Water)	70:30	75:25	80:20
Area (mV)	482695	254877	323701
tR	6.146	4.694	3.797
N	4370	3610	3463
HETP	0.034329	0.0556	0.043424
Tf	1.147	1.248	1.222

Table 5: Flow rate optimization

Flow rate (ml/min)	0.8	1.0	1.2
Area (µV/s)	384547	323701	239715
tR	4.703	3.797	3.175
N	3911	3463	3132
HETP	0.038351	0.043424	0.04788
Tf	1.183	1.222	1.263

System compliance test

The analysis showed that the system is appropriate, with each value meeting the acceptability. The N value less than 2500, a minimum HETP value, and has an % RSD value of less than 2% on each area, retention time, and tailing factor.

Calibration curve and linearity test

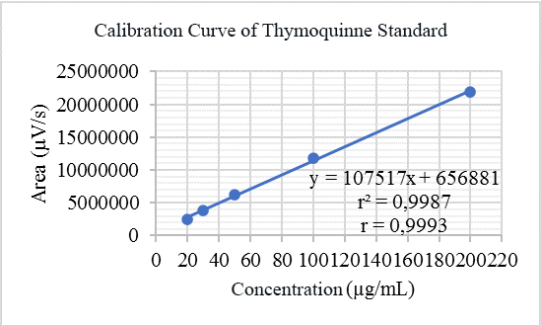

Fig. 6: Thymoquinone standard calibration curve

LOD and LOQ

Based on the 5 concentrations injected on 20, 30, 50, 100, and 200 g/ml, the calibration curve equation *y* = 107517x+656881 was obtained. With linearity or correlation coefficient r = 0.9993.

Table 6: LOD and LOQ calculation results

Description	Value (µg/ml)
$S_{y/x}$	3.1143
LOD (Limit of Detection)*	10.2773±0.02
LOQ (Limit of Quantification)*	31.1436±0.03

*Data is expressed as mean±SD, n=3

Selectivity

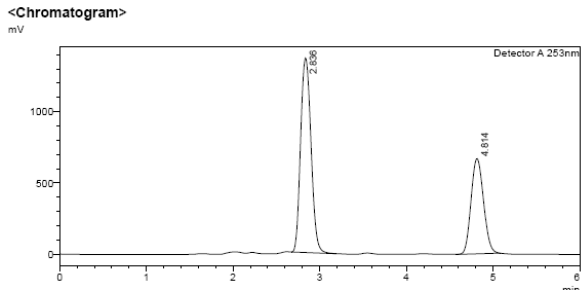

Fig. 7: Analyte separation chromatogram (re: Methylparaben tR: 2.836; Thymoquinone tR: 4.814)

The absence of a chromatogram will interfere with the thymoquinone chromatogram on the retention time, which indicates a selective method.

Accuracy

Table 7: Calculation results of accuracy test and recovery value of emulsion samples

Accuracy	Average measured concentration (µg/ml)	Average theoretical concentration (µg/ml)	Recovery (%)
80%	70.08	70.11	99.96
100%	80.91	80.11	101.00
120%	91.09	90.11	101.09

Thymoquinone is wholly separated, with good separation resolution; chromatograms appear at the same wavelength with different retention times.

Precision

Table 8: Calculation results of emulsion samples containing thymoquinone

Precision	Average measured concentration (µg/ml)	Average theoretical concentration (µg/ml)	%RSD
80%	71.14	71.78	0.02
100%	81.83	81.78	0.39
120%	91.83	91.78	0.13

Determination of thymoquinone concentration

Table 9: Calculation results of determination of timoquinone levels

Sample	Conc. (µg/ml)	Conc % (in 100 g Emulsion)	Conc % (in 1 g oil)
Pre treatment control	149.04	-	1.491
F1 d+0	72.71	0.073	1.454
F1 d+60	42.77	0.043	0.855

The results showed that the % RSD value of each injected sample is less than 2%. In the emulsification process, warm conditions with temperatures<40 °C did not drastically reduce the thymoquinone levels. After 60 d of storage, measurement of Thymoquinone levels showed a decrease in F1 content from 1.45% in 1 g oil (in the emulsion) to 0.85% in 1 g oil (in the emulsion). A decrease in thymoquinone concentration occurs in the presence of degradation. Thymoquinone is an unstable compound against exposure to light and high temperatures. Thymoquinone tends to be more stable at acidic pH than in neutral or alkaline pH conditions. The decrease in pH (oxidation) in the prepared preparations only contributed to the reduction of thymoquinone levels in the sample. Exposure to other rays that occurred during the analysis, such as light and sunlight, contributed to the degradation of Thymoquinone. Thymoquinone tends to be degraded and form another structure with a different wavelength when exposed to light and high temperatures, namely dithymoquinone [16]. Previous studies have shown that Thymoquinone is degraded by almost 70% to dithymoquinone based on the evidence through the LC-MS spectrum.

CONCLUSION

The black cumin seed oil emulsion was prepared using emulsifier sucrose palmitate. This formulation contains 5% black cumin seed oil with 3% sucrose palmitate. Observed through stability test during storage at various temperatures, cycling test, centrifugation, and globule size and obtained F1 as the formulation with the best

physical stability. The obtained formulation could mask the unpleasant odor and taste of black cumin seed oil through the hedonic tests conducted on 30 panelists. The stability of the Thymoquinone concentration using high-performance liquid chromatography showed that Thymoquinone levels decreased slightly on day 0 after formulation if compared to the pre-treatment control of thymoquinone levels in oil drastically reduced after 60 d of a storage factor value.

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AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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