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Original Article

NANOEMULSION FORMULATION OF CARDAMOM ESSENTIAL OIL (*AMOMUM COMPACTUM S.*) AND IT'S *IN VITRO* ANTI-AGING ACTIVITY

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ABSTRACT

Objective: Cardamom fruit (*Amomum compactum S.*) is one of the most famous spices in the world. Its essential oil has anti-aging activity. This study aims to formulate a stable anti-aging nanoemulsion preparation of Cardamom Essential Oil (CEO) using Tween 80 and Pluronic F127 as surfactants and co-surfactants.

Methods: Optimization began by analyzing the transmittance and pH results as affected by Tween 80 and Pluronic F127. The optimum surfactant/cosurfactant composition was used to make cardamom oil nanoemulsion serum by adding grape seed oil as the oil phase. Evaluation of nanoemulsion preparation included organoleptic, particle size, polydispersity index (PDI), zeta potential, pH, viscosity, emulsion type, and stability test by freeze and thaw cycle test and centrifugation. The most optimal and stable nanoemulsion formula was tested for its activity in inhibiting anti-aging enzymes (tyrosinase, elastase, and hyaluronidase).

Results: According to the analysis, the optimal ratio of Tween 80 and Pluronic F127 for nanoemulsion formation was 8.5 ml: 0.15 g. The nanoemulsion was pale yellow with a distinctive cardamom flavor, had a globule size of 10.8±0.5 nm with a polydispersity index (PDI) of 0.207±0.1, zeta potential of-19.33±0.5 mV, pH of 7.44±0.29, viscosity of 394 cPs, with type M/A emulsion. The nanoemulsion preparation actively inhibited tyrosinase, elastase, and hyaluronidase enzymes with IC_{50} 2.45 µl/ml, 20 µl/ml, and 4.80 µl/ml, respectively. The preparation with the strongest anti-aging effect on tyrosinase and hyaluronidase enzymes was a mixture of amomum essential oil and grape seed oil, which remained stable after the stability test.

Conclusion: It can be concluded that the nanoemulsion formula meets the characterization of nanoemulsion preparations and is active as an anti-aging.

Keywords: Cardamom essential oil, Anti-aging, Nanoemulsion. Tyrosinase, Elastase, Hyaluronidase

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INTRODUCTION

Cardamom (*Amomum compactum* Soland.) is a plant of the genus Amomum, which belongs to the ginger family (Zingiberaceae). Cardamom essential oil *(A. compactum S.)* is obtained by distillation of cardamom fruits. Based on the GC-MS chromatography profile, cardamom essential oil contains the main components eucalyptol (1,8 cineole) and 1-α-terpineol, both of which have anti-aging activity [1, 2]. Eucalyptol and 1-α-terpineol inhibited 62.5% of tyrosinase at a 0.05% concentration, supporting cardamom essential oil as a potential anti-aging candidate [1]. Cardamom also contains phenolic compounds that may inhibit skin hyperpigmentation, reduce wrinkles, and prevent skin dryness [3].

Aging is an accumulation of complex biological processes that occur and are influenced by intrinsic and extrinsic factors. Together, these two factors cause changes in each layer of the skin, both structurally and physiologically. Therefore, to protect the skin from many of these factors, many people use cosmeceuticals, especially anti-aging to restore skin rejuvenation [3].

Many anti-aging formulations, such as creams and gels, have been developed. However, the development of cream and gel formulations has limitations in terms of stability, so other dosage forms have been developed. Anti-aging preparations can be made using emulsion technology. The emulsion technology that has been developed recently is SNEDDS. Nanoemulsion is an emulsion delivery system with a globule size of 10-200 nm. Emulsions consist of 2 liquid phases in a phase dispersion system, where one liquid is dispersed very finely and evenly in the other liquid phase [4]. Tween 80 has a role in reducing interfacial tension in the emulsion. The use of Tween 80 alone is not enough to reduce surface tension, but this can be overcome by adding cosurfactant [5]. A surfactant is a substance that can reduce the surface tension of a medium and weaken the interfacial tension between two phases with different degrees of polarity. Cosurfactants can bind with surfactant molecules, which causes the formation of increasingly compressible interfacial tension. One of the cosurfactants that can be used is Pluronic F127. When added, Pluronic F127 can be associated with surfactant molecules and reduce the droplet size. In his research, Adeel reported that Pluronic F127, as a cosurfactant, could form droplet sizes of 29±7 nm. The combination of Tween 80 and Pluronic F127 is expected to form a nanoemulsion formulation (10-200 nm) [6].

This research aims to formulate a stable anti-aging nanoemulsion preparation of Cardamom Essential Oil (CEO) using Tween 80 and Pluronic F127 as surfactants and co-surfactants. This study seeks to design and prepare nanoemulsions incorporating CEO, characterize them physicochemically, assess their stability, and evaluate their antiaging activity. To achieve these objectives, we prepared both nanoemulsion of CEO and nanoemulsion without CEO incorporation. We measured the size distribution of dispersed-phase droplets. An *in vitro* study of the anti-aging activity of nanoemulsion will also be carried out to get a candidate for cosmeceuticals, especially natural aging products.

MATERIALS AND METHODS

CEO isolated from (*A. compactum* Soland.) (Laboratorium Biota Sumatera), *grapeseed oil* (Pietro Coricelli Spa, Italy), Tween 80 (Sigma Aldrich, Merck, Germany), Pluronic F127 (Sigma Aldrich, United States), dimetilsulfoksida (DMSO) (Merck, Germany), 3,4-dihidroksifenilalanin (L-DOPA) (Sigma Aldrich, China), phosphate buffer (Merck, Germany), kojic acid (Sigma Aldrich, United Kingdom), N-Succinyl-Ala-Ala-Ala-p-nitroanilide (SANA) (Sigma Aldrich, Germany), tyrosinase enzyme (Sigma Aldrich, Germany), elastase enzyme (Sigma Aldrich, Germany), hyaluronidase (Sigma

Aldrich, Germany), hyaluronic acid (Sigma Aldrich, Germany).

Optimization of cardamom essential oil nanoemulsion formula base

The nanoemulsion base consists of grapeseed oil as the oil phase (0,4 ml), Tween 80 and Pluronic F127 as surfactants and cosurfactants, and distilled water as the solvent, with the composition shown in table 1. Pluronic F127 was dissolved in distilled water using a magnetic stirrer at a speed of 200 rpm for 25 min. Tween 80 is mixed into Pluronic F127, which is dissolved using a magnetic stirrer at a speed of 150 rpm for ten minutes. The grapeseed oil phase was added slowly and stirred at 150 rpm for ten minutes using a magnetic stirrer to form a nanoemulsion base.

Table 1: Ratio of surfactant/co-surfactant and water in nanoemulsion formula

Formula	Tween 80 (ml)	Pluronic F127 (g)	Water (ml)
F1	9	0.1	1
F2	9	0.1	1
F3	7	0.3	3
F4	5	0.5	5
F5	5	0.5	5
F6	5	0.5	5
F7	3	0.7	7
F8	1	0.9	9
F9	1	0.9	9

The percentage of transmittance analyzed with the criteria was shown in table 2, and then the best result was used to prepare the CEO nanoemulsion.

Table 2: Response criteria

	Criteria	Lower limit	Upper limit
Transmittance	Maksimum	90	99.999
pH	In range	4.5	8

Preparation of nanoemulsion of cardamom essential oil

Nanoemulsion preparations were made with three types of oil phases: an oil phase containing essential oil (0.4 ml), an oil phase containing a mixture of grapeseed oil and essential oil (0.2:0.2 ml), and a grapeseed oil phase. Pluronic F127 was dissolved with distilled water solvent using a magnetic stirrer at 200 rpm for 25 min. Tween 80 was mixed into the dissolved Pluronic F127 with the predetermined ratio (table 1), using a magnetic stirrer at 150 rpm for ten minutes. The oil phase was added slowly and stirred at 150 rpm using a magnetic stirrer for ten minutes. The method used was the titration method. This method can produce nanoemulsions spontaneously with transparent droplet dispersion characteristics.

Evaluation of nanoemulsion preparation of cardamom essential oil

Appearance of preparation

The evaluation was carried out by visually observing the color, odor, consistency, and homogeneity of the nanoemulsion [7].

Droplet size and polydispersity index

The droplet size and polydispersity index were evaluated using a particle size analyzer (PSA) with the principle of scattering light rays by droplets. Nanoemulsion has a droplet size range of 10-200 nm. A good range for the polydispersity index value is<0.5 [11]

Transmittance

An evaluation was carried out using UV-Vis spectrophotometry. The blank used is distilled water, which is measured at a wavelength of 650 nm. The transmittance percentage that can produce a good nanoemulsion is the closest to the blank transmittance percentage or above 90% [8]

pH

The pH test is carried out using a calibrated pH meter, and then the electrode is dry and dipped into the sample for several minutes. The pH of the nanoemulsion topical preparation has a value like the skin pH range, around 4.5 – 8 [9]. When the value on the tool is stable, it means the pH of the sample has been read

Viscosity

A viscosity test was carried out using a Brookfield DV2T viscometer. The viscosity tool is turned on in the tare position, and spindle number three is installed with the rotation speed set at 100 rpm. It is ensured that the spindle is immersed in the nanoemulsion preparation up to the boundary line. Measurements were repeated three times. The range of viscosity values for nanoemulsions is 10-2000 cPs [10].

Nanoemulsion type

Evaluation of nanoemulsion type using the staining method with methylene blue and Sudan III. The characteristic of methylene blue being soluble in water determines the type of O/A, which is blue when one to two drops are added to the sample. The oil-soluble characteristics of Sudan III determine the A/M type, which is red when one to two drops of the sample are added [11].

Potential zeta

Potential zeta evaluation is carried out using a zeta sizer. This test aims to measure the long-term physical stability of the nanoemulsion preparation. The value range of+30 mV or less than-30 mV on the test can produce stable nanoemulsions [12].

Stability

Nanoemulsion stability was evaluated using freeze and thaw cycle and centrifuge. The freeze and thaw cycle method is carried out by storing the preparation at temperatures of-5 °C and 25 °C each for no less than 24 h, which is repeated for six cycles. The centrifuge method uses a centrifuge at 3500 rpm for 30 min. Then it was observed organoleptically (consistency, phase separation, and precipitation) and quantitatively (percent transmittance and pH) [13].

Anti-aging activity

Antiaging activity testing measured the IC_{50} values for anti-tyrosinase, anti-elastase, and anti-hyaluronidase from cardamom essential oil nanoemulsions. The method used to test anti-tyrosinase activity refers to the method developed by Momtaz *et al.*, (2008) with several modifications [14]. First, kojic acid was prepared as a comparison. 50 µl of each sample solution was added with 20 µl of tyrosinase enzyme (250 Units/ml in phosphate buffer pH 6.5), then made up to the volume to 100 µl by adding 30 µl of 50 mmol phosphate buffer pH 6.5. Then, incubated at room temperature for 5 min, 100 µl of 5.07 mmol L-DOPA substrate was added to each well. Microplates were incubated for 20 min at room temperature. The solution in each well was measured at a wavelength of 492 nm to determine the percent inhibition. The percentage of inhibition is calculated by:

$$\% \text{ Tyrosinase inhibition} = \frac{A-B}{A} \times 100\%$$

A = Absorbance of solution without sample

B = Absorbance of the solution with added sample

In addition, anti-elastase testing was carried out, referring to the method developed by Moon et al. (2010) with some modifications [15]. Thirty microliters of sample was put into a 96-well microplate. Then 200 mmol Tris-HCl buffer (Ph 8.0) was added, and 30 µl of elastase enzyme solution (0.038 Units/ml) was added. The plates were incubated for 20 min at room temperature. Next, 40 µl of Nsuc-(Ala)3-nitroanilide substrate (1 mmol in buffer) was added and incubated for 20 min. Absorbance was measured at a wavelength of 410 nm. The percentage of elastase inhibition is calculated by:

$$\% \text{ Elastase inhibition} = \frac{A-B}{A} \times 100\%$$

A = Absorbance of solution without sample

B = Absorbance of the solution with added sample

Then, an anti-hyaluronidase test was carried out using the method of Tu and Tawata *et al.* (2015) [16]. Five microliters of sample were loaded into a 96-well microplate. Then, 100 ml of hyaluronidase enzyme (1.5 Units/ml in 20 mmol phosphate buffer pH 7 containing 77 mmol NaCl and 0.01% bovine serum albumin (BSA) was added, and the mixture was incubated for 10 min at 37 °C. Then, 100 µl of hyaluronic acid substrate (0.03% in 300 mmol phosphate buffer pH 5.35) was added and incubated at 37 °C for 45 min. After incubation, the reaction between the substrate and the enzyme was stopped by adding 1 ml of albumin acid solution (0,1% Bovine serum albumin (BSA) in acetate buffer pH 3.75). The absorbance of the sample was measured at a wavelength of 600 nm. The percentage of enzyme inhibitory activity is measured in the following way:

$$\% \text{ Hyaluronidase inhibition} = \frac{A-B}{A} \times 100\%$$

A = Absorbance of solution without sample

B = Absorbance of the solution with added sample

RESULTS

Optimization of cardamom essential oil nanoemulsion formula base

Organoleptic evaluations were conducted on each formulation, with all tests being repeated three times to ensure the reliability and accuracy of the results with the average below (table 3).

Organoleptic results showed that the preparation was pale yellow in color with different consistencies. The more Tween 80 used, the thicker the consistency of the preparation and the more yellow the color of the preparation. This is because Tween 80 has an organoleptic yellow color with a thick consistency.

Table 3: Percentage of transmittance and pH of base nanoemulsion

No	Formula	Tween 80 (ml)	Pluronic F127 (g)	Water (ml)	Percent transmittance (%)±SD	pH±SD
1	F1	9	0.1	1	99.6±0.28	8.1±0.05
2	F2	9	0.1	1	99.5±0.15	8.1±0.01
3	F3	7	0.3	3	99.2±0.10	7.6±0.04
4	F4	5	0.5	5	98.6±0.05	7.2±0.20
5	F5	5	0.5	5	98.6±0.05	7.2±0.00
6	F6	5	0.5	5	98.5±0.05	7.2±0.04
7	F7	3	0.7	7	85.1±0.10	6.8±0.09
8	F8	1	0.9	9	1.5±0.23	6.2±0.00
9	F9	1	0.9	9	1.5±0.23	6.2±0.00

Table 4: Tukey tested the nanoemulsion criteria that influence tween 80 and pluronic F127 on percent transmittance

Percent transmittance					
	Formula	N	Subset		
			1	2	3
Tukey HSD[a,b]	Formula 6	3	98.433		
	Formula 4	3	98.567		
	Formula 5	3	98.567		
	Formula 3	3		99.100	
	Formula 1	3		99.433	
	Formula 2	3		99.467	
	Sig.		.862	.078	

Table 5: Tukey tested the nanoemulsion criteria that influence tween 80 and pluronic F127 on pH

Source	Type III sum of squares	df	Mean square	F	Sig.
Corrected Model	3.459[a]	5	.692	87.869	.000
Intercept	1018.058	1	1018.058	129322.773	.000
Formula	3.459	5	.692	87.869	.000
Error	.094	12	.008		
Total	1021.611	18			
Corrected Total	3.553	17			

Table 6: Optimal nanoemulsion formula

Formula	Tween 80 (ml)	Pluronic F127 solvent (ml)	Pluronic F127 (g)	CEO (ml)	Grapeseed oil (ml)
FA	8.5	1.5	0.4	-	0.4
FB	8.5	1.5	-	0.4	-
FC	8.5	1.5	0.2	0.2	0.2

Observing the percent transmittance shows that formulas 1, 2, and 3 have the highest percent transmittance, and based on the Tukey test ($p<0.5$), it shows that there is no difference in the average transmittance of formulas 1, 2, and 3. The Tukey test shows that there is a difference in the average pH of formulas 1, 2, and 3, which are formula 3 meets the range of pH of skin (4.5-8). Based on these tests, the ratio of Tween 80 and Pluronic F127 (8.5 ml: 0.15 g) was chosen as the optimal formula.

Preparation of nanoemulsion of cardamom essential oil

The nanoemulsion preparation was made with an optimal mixture of Tween 80 and Pluronic F127, namely Tween 80 and Pluronic F127 (0.15 g in water), with a composition of 8.5:1.5 v/v.

Evaluation of nanoemulsion preparation of cardamom essential oil

Appearance of preparation

Organoleptic results showed that the preparation was pale yellow in color with different consistencies. The more Tween 80 used, the thicker the consistency of the preparation and the more yellow the color of the preparation. This is because Tween 80 has an organoleptic yellow color with a thick consistency.

Droplet size and polydispersity index

The droplet size test was conducted on each formulation, with all tests being repeated three times to ensure reliability and accuracy of the results. The results show that all nanoemulsion preparations have an average droplet size that falls into the range of values for nanoemulsion preparations (10-200 nm). The surfactants and cosurfactants used are Tween 80 and Pluronic F127, which are able to stabilize O/W type nanoemulsions, thereby reducing the droplet size.

Potential zeta

The average value of the formula (Grapeseed oil) and cardamom essential oil formula are into the good zeta potential value range (more than+30 mV or less than-30 mV), while the mixed formula has a zeta potential value that is slightly below the range.

Table 7: Results of droplet size and polydispersity index nanoemulsion of mixed (essential oil and grapeseed oil) formula

Formula	Average droplet size±SD (nm)	Average polydispersity index±SD
FA	10.9±0.5	0.165±0.02
FB	10.5±1.3	0.155±0.01
FC	10.8±0.5	0.207±0.08

The polydispersity index results show that all preparations fall into the nanoemulsion value range (<0.5). Meanwhile, a polydispersity index value of>0.5 indicates the presence of flocculation of droplets.

Table 8: Results of potential zeta nanoemulsion formula

Formula	Average potential zeta±SD (mV)
FA	-33.2±2.8
FB	-36.1±3.5
FC	-19.3±0.5

Viscosity

The viscosity measurement results show an average of 394.0 cPs±8.2. Each formula meets the serum viscosity range, which is 230-1150 cPs, and into the nanoemulsion viscosity range, which is 10-2000 cPs. A low viscosity value indicates that the liquid is practical to use on the skin because it is not thick.

Nanoemulsion type

All formulas dissolved and turned blue with the addition of methylene blue. The result indicated that all nanoemulsion preparations are of the O/W type.

Stability

Stability tests were carried out on the nanoemulsion preparation of a mixture of grapeseed oil and cardamom essential oil using centrifuge, freeze, and thaw cycle. Organoleptic observations were carried out on the preparations before and after testing. The observations showed that the preparation remained stable and did not become cloudy when stored at temperatures of-5 °C and 25 °C.

The optimal formula showed no instability (<90% transmittance), indicating stability during the freeze-thaw cycle (table 7)

Table 7: Freeze and thaw cycle stability percent transmittance evaluation results

Cycle	% Transmitan
0	99.43%
1	99.4%
2	99.0%
3	98.3%
4	97.43%
5	94.9%
6	94.8%

Followed by centrifuge testing to see the effect of gravity (shocks) on the nanoemulsion preparation after centrifugation. The results were obtained, which remained homogeneous, and there was no phase separation even after applying a centrifugal force to the tube wall of 1500-2000 g (speed 3500 rpm for 30 min). This indicates that the nanoemulsion preparation is stable against gravity or shock.

Anti-aging activity

The results of the anti-aging enzyme inhibitor test are shown in table 8. Kojic acid, Ascorbic acid, and linoleic acid were used to control positive enzyme activities.

Table 8: Results of enzyme inhibitor activity in the nanoemulsion formula

Sample	IC_{50} tyrosinase	IC_{50} elastase	IC_{50} hyaluronidase
Nanoemulsion of *grapeseed oil*	5.91 µl/ml	>10 µl/ml	>10 µl/ml
Nanoemulsion of CEO	5.02 µl/ml	>10 µl/ml	4.96 µl**/ml
Nanoemulsion of CEO and *grapeseed oil*	2.45 µl**/ml	>20 µl**/ml	4.80 µl**/ml
CEO	18.6 µg/ml	54.8 µg/ml	27.42 µg/ml
Grapeseed oil	4.20 µl**/ml	0.78 µl**/ml	0.26 µl**/ml
Kojic acid	4.40 µg/ml	Untested	Untested
Ascorbic acid	Untested	32.817 µg/ml	Untested
Linoleic acid	Untested	Untested	18.609 µg/ml

DISCUSSION

The present study aimed to formulate the nanoemulsion of cardamom oil and evaluate its anti-aging activity with an enzymatic test of tyrosinase, hyaluronidase, and lipase enzymes, which contribute to aging. The physicochemical characteristics (appearance, droplet size distribution, ζ-potential, polydispersion index, type of emulsion, viscosity), stability over time, and anti-aging activity were evaluated.

The organoleptic test results show that the nanoemulsion is pale yellow due to Tween 80, transparent, with no phase separation, and remains homogeneous, stabilized by Tween 80 and Pluronic F127. The distinctive cardamom odor comes from the cardamom essential oil used in the formulation.

The droplet size results show an average range of 10-200 nm, confirming that the formulations meet the size criteria for nanoemulsions. This is critical for enhancing the stability and bioavailability of the active ingredients. Tween 80 and Pluronic F127 as surfactant and co-surfactant, respectively, play a pivotal role in achieving this droplet size. Tween 80, with its high hydrophilic-lipophilic balance (HLB), is well-suited for stabilizing oil-in-water (O/W) nanoemulsions, ensuring that the oil droplets remain dispersed in the aqueous phase. Pluronic F127, a block copolymer with amphiphilic properties, complements Tween 80 by further reducing interfacial tension and enhancing emulsion stability. Together, these surfactants minimize coalescence and maintain small droplet sizes, contributing to the overall stability and performance of the nanoemulsion, which is essential for its potential application in drug delivery, cosmetics, and other fields. The polydispersity index (PDI) results, all below 0.5, confirm the uniformity of the nanoemulsion droplets, indicating a stable formulation. A PDI above 0.5 would suggest droplet flocculation, compromising stability. Therefore, the low PDI values reflect well-dispersed and stable nanoemulsions [17]. The zeta potential for the grapeseed oil and cardamom essential oil formulas falls within the ideal range for stable nanoemulsions (>+30 mV or<-30 mV), ensuring good colloidal stability. However, the mixed formula has a slightly lower zeta potential, likely due to interactions between components, particularly the free fatty acids in grapeseed oil. While this may still provide adequate stability, further optimization could enhance stability in complex formulations. In the context of preparing nonionic surfactants, it has been established that zeta potential values exceeding±20 mV are adequate for the stabilization of colloidal nanodispersion systems. This stabilization is primarily achieved through the steric stability conferred by the presence of polar moieties within the surfactant molecules. Such molecular interactions play a crucial role in maintaining the dispersive characteristics of the nanodispersion, thereby contributing to the overall stability of the formulation [18].

The viscosity measurements averaged 394.0 cPs±8.2, falling within both the serum viscosity range (230-1150 cPs) and the nanoemulsion range (10-2000 cPs). The relatively low viscosity suggests that the formulation is easy to apply to the skin, offering a lightweight texture without being overly thick.

Nanoemulsion stability assessments by the freeze and thaw cycle method revealed that nanoemulsion CEO remained stable at temperatures of-5 °C and 25 °C each for no less than 24 h, which is repeated for six cycles with no signs of physical instability. The high surfactant concentration is required to form an optimum formula with spontaneous emulsification. Generally, a more than 30% surfactant concentration is required in the formula to stabilize the nanoemulsion [13]. In the preparation, the surfactant concentration is 85%, which can produce a stable nanoemulsion. The assistance of cosurfactants also helps reduce interfacial tension so that the nanoemulsion preparation appears to remain clear and stable after stability testing.

The anti-aging enzyme inhibition test showed that the preparation and oil phase inhibited 50% of tyrosinase activity, with the nanoemulsion of grapeseed and cardamom essential oils having the lowest IC_{50} value, indicating the strongest anti-tyrosinase potential. The results data show that the mixture of grapeseed oil and cardamom essential oil has the greatest activity in inhibiting the tyrosinase enzyme compared to the grapeseed oil preparation and the cardamom essential oil preparation. In the mixture of grapeseed oil and cardamom essential oil, there is 0.04 µl/ml of essential oil and 0.04 µl/ml of grapeseed oil in the preparation, which inhibits the tyrosinase enzyme. Compared with grapeseed oil, the tyrosinase inhibitor activity is greater in the nanoemulsion dosage form, which contains grapeseed oil, namely 0.23 µl/ml. From the results obtained, grapeseed oil was able to inhibit the tyrosinase enzyme with an inhibition percentage of 57.00% at a concentration of 0.5 µl/ml. This is the same as research by Khotapali (2023), which reported that grapeseed oil inhibited 80.10% of the tyrosinase enzyme [19]. This is thought to be due to the content of gallic acid and flavonoids, which actively play a role in inhibiting the tyrosinase enzyme [20]. The anti-hyaluronidase test showed an IC_{50} of 4.96 µl/ml for the nanoemulsion preparation of cardamom essential oil, and for the mixture of grapeseed oil and cardamom essential oil, the IC_{50} was 4.80 µl/ml. Meanwhile, the IC_{50} value for grapeseed oil preparations was not obtained. This is thought to be due to the phenolic compound content in cardamom essential oil, which plays an active role as an inhibitor of the hyaluronidase enzyme [21]. Apart from that, this is thought to be due to the content of phenolic and flavonoid compounds in grapeseed oil as inhibitors of the hyaluronidase enzyme.

Skin aging occurs through biochemical processes involving the degradation of collagen, elastin, and skin pigmentation. Anti-aging, or slowing down the aging process, can be achieved by inhibiting several key enzymes involved in aging, such as tyrosinase, hyaluronidase, and elastase [22, 23]. Tyrosinase is an enzyme involved in the synthesis of melanin, giving skin color. Excessive activity of this enzyme can lead to hyperpigmentation such as age spots or melasma [24]. The main component of cardamom essential oil, such as eucalyptol, is known to work by inhibiting the conversion of tyrosine into melanin by reducing tyrosinase activity. Additionally, this compound reduces oxidative stress, which is one of the factors that can increase tyrosinase activity [25]. A molecular docking study reported that 1-α-terpineol interacts with several amino acids at the active site of tyrosinase, such as ASP-344 and THR-345, which are important for enzyme activation. 1-α-terpineol forms hydrogen bonds with these residues, blocking the enzyme activity [26]. Regarding elastase, which is responsible for breaking down elastin, the protein that provides elasticity to the skin, excessive degradation of elastin contributes to the loss of skin elasticity and wrinkle formation. Therefore, elastase inhibition is important in slowing down the skin's aging process related to the loss of firmness and elasticity [27]. The eucalyptol component in cardamom essential oil has been shown to inhibit elastase activity actively. This is explained by molecular docking, where hydrogen bonding occurs between eucalyptol and the amino acid group ThR A: 41 found at the active site of elastase. Additionally, other residues interact with HIS A: 57 and CYS A: 42. Van der Waals interactions were also detected with amino acids CYS A: 58, SER A: 195, GLY A: 193, and GLN A: 192 [28].

The development of anti-aging formulations, such as nanoemulsions made from cardamom essential oil, which work by inhibiting key enzymes such as tyrosinase, elastase, and hyaluronidase, is an innovative approach to slowing down the signs of aging on the skin. Several compounds are commonly used as anti-aging agents, such as arbutin, kojic acid, and ascorbic acid [29]. The development of this nanoemulsion has the potential to offer a more effective alternative compared to conventional products, as it can provide benefits in terms of faster absorption and higher stability due to smaller particle sizes. Further research and comparisons in clinical trials are needed to strengthen the claims of the effectiveness of this nanoemulsion formulation.

CONCLUSION

The optimal ratio of Tween 80 and Pluronic F127 solvent from the Tukey test is 8.5: 1.5 with the nanoemulsion characteristics having a transmittance percentage of 99.2±0.3%, pH 7.44±0.3, particle size 10.8 nm±0.5%, particle size distribution 0.207±0.1 nm, zeta potential-19.33±1.5 mV, viscosity 394 cP, o/w nanoemulsion type with a characteristic cardamom aroma. A mixture of grapeseed oil and essential oils that actively inhibits anti-tyrosinase has an IC_{50} value of 2.45 µl**/ml, inhibits 47.6% of the action of the elastase enzyme at a concentration of 20 µl**/ml, and actively inhibits the hyaluronidase enzyme with an IC_{50} of 4.80 µl**/ml and Nanoemulsions are thermodynamically and kinetically stable.

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AUTHORS CONTRIBUTIONS

All authors give a contribution to this research. Conceptualization, FI., and RA; methodology, R. A. and S. A.; formal analysis, R. A, S. A, and F. I; investigation, R. A.; writing-original and draft preparation, R. A.; writing—review and editing, F. I.; supervision, F. I. All authors have read and agreed to the published version of the manuscript

CONFLICT OF INTERESTS

The authors declare there is no conflict of interest.

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