International Pharmacy Acta. 2023; 6(1): e7
Original Research Article
OPEN ACCESS
ISSN: 2645-3266

Stability Study of Nanoparticle, Microemulsion, Multiple Emulsion and Niosome Cream from Ethyl Acetat Fraction of Green Tea (*Camelia sinensis* L.) Leaf

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Article Info:
Received: July 2024
Accepted: December 2024
Published online: December 2024

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ABSTRACT:

The stability of EGCG in green tea leaves is significantly impacted by temperature and storage duration. The formulation of w/o cream was not the best for preserving the stability of EGCG, thus it was required to change the way cosmetic preparations were delivered in a number of dosage forms. Physical and chemical tests were conducted on the ethyl acetate fraction of green tea leaves (*Camellia sinensis* L.) in the dosage forms of nanoparticles, micro particles, multiple emulsions, and *niosomes* cream. Test of accelerated stability (temperature 50, 60 and 700C). HPLC was used to evaluate EGCG. The results of the physical test preparations for nanoparticles, micro emulsions, multiple emulsions and *niosomes* met the positive control parameters, namely the dispersion power of 6.34 ± 0.76 cm; 5.26 ± 0.45 cm; 6.07 ± 0.76 cm; 6.16 ± 0.19 cm. pH of each preparation 4.73 ± 0.10; 5.30 ± 0.2; 5.46 ± 0.19; 5.05 ± 0 and viscosity 2834 ± 844.6744; 3390 ± 1.57; 2582 ± 360; 3300 ± 173.20. EGCG levels in cream preparations of nanoparticles, micro particles, multiple emulsions and *niosomes* at a temperature of 250C and 100C respectively 476.0236 ± 332.6097; 32.6651 ± 3.5385; 0.2221 ± 0.0078; 3.4320 ± 1.9533. In contrast to W/O creams, preparations including nanoparticles, micro emulsions, multiple emulsions, and *niosomes* can preserve their physical and chemical stability. Nanoparticle cream, micro emulsion, multiple emulsion type W/O/W and niosomes were proven to maintain physical stability compared to cream type O/W. Nanoparticle cream, micro emulsion, multiple emulsion type W/O/W and niosomes were proven to maintain chemical stability compared to cream type O/W.

Keywords: Stability; EGCG; Nanoparticles; Microparticles; Multiple emulsions; Niosome.

Please Cite this article as: Widyaningrum N, Taufiq H, Lestari S, Devitasari ANB, Farahiya B, Fairuzia I, et al. Stability Study of Nanoparticle, Microemulsion, Multiple Emulsion and Niosome Cream from Ethyl Acetat Fraction of Green Tea (*Camelia sinensis* L.) Leaf. Int. Pharm. Acta. 2023; 6(1):e7
DOI: https://doi.org/10.22037/ipa.v7i1.44820

1. Introduction

Current research introducing many phytochemicals to support health or fight disease is based on centuries-old traditions. Traditional ingredients are the latest advancement in the therapeutic field, green tea and its constituents are one of the important components of this strategy to promote health or cure a disease [1]. Chemical compounds called catechins, a class of polyphenols, are particularly abundant in green tea. Green tea is the least oxidized and therefore has the highest concentration of catechins. Epigallocatechin-3-gallate (EGCG) is the most abundant catechin found in green tea [2].

EGCG has several potentials in health such as anti-oxidant, anti-carcinogenic, anti-inflammatory, and antimicrobial [3]. EGCG was unstable and degrades faster in solution with an increase in pH and temperature [4]. The finding of research [5] revealed that EGCG stability would rise at a temperature of 2oC following the application of a pH 4 buffer solution. If EGCG was not kept under control at an acidic pH, phenolic chemicals would polymerize and oxidative stress would result.

Stability of EGCG in HEPES (N-2-hydroxyethylpiperazine-N-2-ethanesulfonate) in HEPES medium depends on pH, storage temperature and ascorbic acid as a reducing agent. Evaluation of EGCG in HEPES buffer has shown that this molecule cannot maintain its physicochemical properties and potential beneficial effects, as it is partially or completely degraded, depending on the concentration of EGCG. The most suitable storage temperature for EGCG to retain its its structure proved to have lower values (4 or 200C). pH 3.5 was able to provide greater stability than pH 7.4. However, the presence of a reducing agent (ascorbic acid) was shown to provide greater protection against EGCG degradation [6].

The ideal formula for green tea cream with antibacterial activity against P. acnes was at a concentration of 6 percent ethyl acetate fraction [7]. However, in this study, the cream's stability was subpar at the time of storage, necessitating the modification of the preparation. It was well established that creating niosomes, nanoparticles, microemulsions, and numerous emulsions had the benefit of improving EGCG stability [8–10]. Research on the stability test of W/O/W type multiple emulsion cream, niosomes, microemulsions, and nanoparticles of the ethyl acetate fraction of green tea leaf extract was therefore required to ascertain the stability of EGCG (Camellia sinensis L.).

2. Materials & Methods

Stainless steel pan, thermometer, pH meter Hanna HI 98121, rotary evaporator (Heildolph Germany), Thermostat water bath HH-6, Moisture balance brand Shimadzu EP-90 model Moc63u, PSA (Horiba SZ-100), Ultrasonication (Branson CPX-952 -118R), centrifugation (Hettich Zentrifuge EBA 20/20S), Homogenizer (IKA 25 T Ultra Turrax), Brookfield viscometer.

EGCG standard 93894-10MG (Sigma-Aldrich). The materials for making niosomes are technical non-ionic surfactant (Span 60®), technical 92% cholesterol (Sigma-aldrich), technical dichloromethane (Merck). The technical grade ingredients used for the manufacture of the cream are citric acid (Sigma-aldrich), triethanolamine (Sigma-aldrich), ascorbic acid (Sigma-aldrich), stearic acid (Sigma-aldrich), tween 80 (Sigma-aldrich), span 80 (Sigma-aldrich), sorbitol (Sigma-aldrich), propylene glycol (Sigma-aldrich), VCO (Sigma-aldrich: 46949), methyl paraben (Sigma-aldrich), propyl paraben (Sigma-aldrich), aqua demineralisata, aquadest (technical), and ice cubes. Multiple emulsion cream ingredients are Paraffin oil (Merck), cetyl dimethicone copolyol (Pancaran Sinar Persada Ltd.), ethyl acetate (Brataco Inc.), magnesium sulfate (Merck), aquades (technical), polyoxyethylene (20) cetyl ether (Brij ® 58 Sigma), cetomacrogol 1000 (Brij ® 58 Sigma), HPMC (Brataco Inc.).

2.1. The Process of Extracting and Fractionating Tea Leaves

Using the decoction method, 60 grams of green tea leaf were extracted with 1200 ml of distilled water at 90^0C for 30 minutes. After filtering, the extract was immediately subjected to extremely cold conditions until it reached a temperature of 5ºC. Vitamin C was then added to pH 4 and kept at 20°C [6]. The filtrate was then separated using a separating funnel and 1200 ml of ethyl acetate. To create a dry powder, the ethyl acetate fraction was subsequently thickened using a rotary evaporator. The powder underwent a moisture balance test (moisture content < 2%) [11].

2.2. The Production of Cream of Nanoparticles, Microemulsions, Multiple Emulsions and Niosomes

The fatty phases, namely stearic acid (5 g), VCO (20 g), and span 80 (2.2 g), were heated in a water bath at 70ºC until evenly mixed. Meanwhile, the liquid phases were propylene glycol (2.1 g), tween 80 (5.7 g), sorbitol (20 g), citric acid (0.7 g), triethanolamine (2.45 g), ascorbic acid (0. 06 g), methyl paraben (0.25 g), propyl paraben (0.15 g), and Aqua Demineral (ad 100 ml) were heated with a water bath at a temperature of 70ºC until thoroughly blended then added the ethyl acetate fraction of leaf extract of green tea (6 g) for 1-2 minutes. The fat phase was gradually added to the liquid phase to homogenize it. A sort of cream (O/W) was created through homogenization using a homogenizer at a speed of 1000 rpm [7].

2.2.1 Nanoparticles

The created type (O/W) cream was next homogenized for 90 minutes with an ultrasonic instrument to create nanoparticles (hereinafter referred to as nanoparticle cream). Physical testing were performed on the nanoparticle cream product, including homogeneity, dispersibility, pH, and viscosity tests, in a cleaned glass container [12].

2.2.2. Microemulsions

In order to create a microemulsion, the liquid and fatty phases were combined gradually with a magnetic stirrer at a speed of 500 rpm for an hour at 40 °C, until they were dissolved and homogeneous. The microemulsion was then homogenized at a speed of 1000 rpm using a homogenizer.

2.2.3. Multiple Emulsion

Making a primary emulsion was the first step, which involved mixing paraffin oil (19.2 g) and cetyl dimethicone copolyol (3.4 g) at 75°C in a 100 ml glass

beaker. Next, add the ethyl acetate fraction of green tea leaf extract (6 g) and magnesium sulfate (0.56 g) while stirring at a speed of 2000 rpm for 15 minutes, followed by 1000 rpm for 10 minutes, proceeded by 500 rpm for 10 minutes. The second step involved combining distilled water, Polyoxyethylene (20) cetyl ether (3.75 g), Cetomacrogol 1000® (2.5 g), and Hydroxypropyl methylcellulose (HPMC) (1.25 g) at a 700 rpm stirring rate. Physical tests were conducted on various emulsion cream products using a glass container, including testing for homogeneity, dispersion, pH, viscosity, and multiple emulsion microscopically.

2.2.4. Niosome

The ethyl acetate fraction of green tea leaf extract, cholesterol, and span 60 were dissolved in 50 ml of dichloromethane, put in a round bottom flask, and evaporated using a rotary evaporator at 39°C until the dichloromethane was entirely evaporated and a dry layer was created [8]. The round bottom flask was then placed in a desiccator, vacuumed for 15 minutes, and then left at a cold temperature for 24 hours before being hydrated to make sure the organic solvent was entirely eliminated [13]. Following that, the thin layer was hydrated with a pH 5.5 phosphate buffer solution at 40°C in a rotating evaporator to create a homogeneous niosomal suspension [8].

2.3. Testing Cream Particle Size and Multiple Emulsion Globul Size and Evaluation of Niosome Vesicle Morphology

2.3.1. Testing Cream Particle Size

This test was carried out on nanoparticle cream and microemulsion then dissolved in sufficient aqua demineralisata to form a colloid then tested for particle size distribution using a PSA (Particle Size Analyzer) tool [12].

2.3.2. Testing Multiple Emulsion Globule Size and Evaluating Niosome Vesicle Morphology

SEM (Scanning Electron Microscopy) observations were carried out by diluting multiple emulsions with deionized water (1/200) of sample droplets (1 µl). Samples were stained with 2% (w/w) phosphotungstic acid which was dried at room temperature for 30 seconds. Excess liquid was filtered through Whatman filter paper. The result was positive if the multiple emulsion droplets were light in color with a dark liquid phase [14]. Good globule size ranged from more than 0.01 m or between 0.01 - 50 m [15]. However, the niosomes were closed with a slip cover on the specimen. The vesicles were coated with carbon then with gold using a vacuum evaporator. The morphology was observed with a scanning electron microscope at 15,000 kV [16].

2.4. Physical and Chemical Stability Test of Nanoparticle Cream

2.4.1. Physical Stability Test

Homogeneity test (with homogeneous parameters), dispersion (with parameters 5-7 cm), pH (with parameters 4.5-6.5) and viscosity (with parameters 2000-4000 cP) [17,18],[15].

2.4.2. Chemical Stability Test

Each cream sample was weighed 2 g in a test tube and heated at a temperature of 50°C, 60°C, 70°C using a water bath. Each cream formula from each tube was then taken at 0 hour; 0.5; 1; 2; 3; 4; 5; and 6. Then the sample was determined using HPLC.

2.5. Identification of EGCG Levels

2.5.1. HPLC Preparation of EGCG Cream Sample

Two grams of cream plus 3 ml of methanol were then centrifuged for 30 minutes at 3000 rpm. The supernatant was discarded and the aqueous phase was extracted using 3 ml of chloroform 3 times and centrifuged at 3000 rpm for 30 minutes, then the liquid phase was added to methanol until the volume was 5 ml.

2.5.2. The Condition of HPLC

The specification of the ethyl acetate fraction of green tea extract was carried out based on EGCG. The HPLC system used from the research method [20], namely the reverse phase HPLC with an isocratic elution system.

2.5.3. Production of Mobile Phase HPLC System

The mobile phase used was a mixture of 0.1% phosphoric acid: methanol: acetonitrile: distilled water with a ratio of 14:1:3:7 (v/v/v/v). The previously used distilled water was filtered with a 0.45 filter membrane after the four were mixed and then triethylamine was added until a solution pH of 4 was obtained [20].

2.5.4. Production of EGCG Mother Liquor

EGCG was weighed at 25.0 mg which was then dissolved in 25 mL mobile phase. This mother liquor was then used for linearity, repeatability, precision and system suitability tests [20].

2.5.5. HPLC Conditions for Determination of EGCG Levels

The conditions used in the determination of EGCG levels were that the sample was injected with an injection volume of 20µL, which was then eluted using a C 18 stationary phase, the mobile phase was a mixture of 0.1% phosphoric acid: acetonitrile: methanol: distilled water in a ratio of 14:3:7. :1 at a flow rate of 1.2 mL/min and detected with a UV spectrophotometer detector at (λ) 280 nm [20].

2.6. Determination of The Value of T90 with The Arrhenius Method

EGCG levels were calculated using the results of the EGCG fraction by calculating the EGCG degradation results, namely gallic acid levels. The levels that had been grouped were then averaged for each time and temperature to obtain data such as the data above and calculate 1/C (1/level) and log C (log content) at each temperature. The reaction order was determined according to the value of r which was closest to the value 1 by regressing the reaction order 0 (time vs concentration), 1 (time vs log k), 2 (time vs 1/C) by graphical method. The reaction order chosen was order 2. Because the reaction order used was order 2, the calculation of C (levels) vs. Ln C was carried out until the data was obtained like the data above to obtain the value of K at each temperature and was averaged by removing the value indicated. minus (-) because it was considered an outlier that could affect the results. The following Arrhenius equation was used.

0 : At = A_0-kt

1 : Ln At = Ln A_0-kt

2 : k = $\frac{2.303}{t\,(a-b)} Log \frac{b(a-x)}{a(b-x)}$

Temperature was converted to Kelvin, content was calculated as 1/T (1/temperature in kelvin) and log K was then searched for linear regression by finding the value of a (slope), b (intercept), and the value of r by regressing between 1/T and log k. Find the K value at 25°C (representing room temperature) and 10°C (representing the coolant temperature), 1/T, log K (with the equation y=bx+a) from the previous data and anti-log to get the K value then calculate t1/2 with the formula: 1/a.k (hours) and t90 : 0.11/a.k (hours).

3. Results & Discussion

Based on the results of extracting and fractionating green tea leaves (Camellia sinensis L.), it was obtained 9.005 grams with a fraction yield of 15.01%.

3.1. Evaluation of Morphology

The results of the SEM test proved that the multiple emulsion cream formed multiple emulsion globules. The globule results were positive if the multiple emulsion droplets were light in color with a dark liquid phase as shown in Figure 1a. The results of the niosomal morphology test showed that the vesicles were white spheres with an average of 0.95 m which were classified as multilamellar vesicles. Niosomes with diameters >0.5-10 m were classified in Multilamellar vesicles [21]. The niosomal vesicles were presented in Figure 1b.

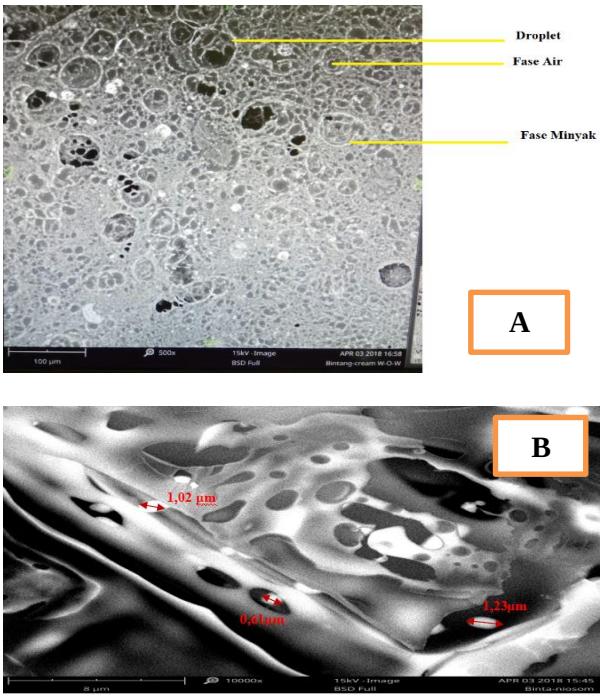

Figure 1. (A) multiple emulsion globules in cream multiple emulsion. Microscopic analysis with the SEM (FEI Quanta 400F ESEM, OR) and software image control microscopy test proved that the multiple emulsion cream formed multiple emulsion globules. **(B) vesikel niosome.** The results of the niosome morphology test using SEM showed that the vesicles were white spheres with an average of 0.95 m which were classified as multilamellar vesicles.

3.2. Particle Size

Droplet size testing was carried out on nanoparticle and microemulsion cream using a PSA tool which aimed to see whether the resulting preparation had a droplet size that met the microemulsion and nanoparticle droplet size criteria.

The microemulsion droplet size criteria were 10-1000 nm. Based on table 1, the particle size results in microemulsion cream with a fraction having an average size of 629.6 ± 638.7 nm were obtained. Examination of the resulting droplet size had entered the size parameter range, which was <1000 nm. Based on table 1, the particle size results in nanoparticle cream with fractions had an average size of 279.4 nm ± 260.8 nm while in cream without fractions, the average particle size was 275.7 nm ± 168.0 nm so it was included in the category SLN (Solid lipid nanoparticles) where the ideal size range was 125-500 nm [22].

Polydispersity index (PI) was used to estimate the range of particle size distribution in a sample and to find out whether there was aggregation where if the PI value was

< 0.1, the denser the distribution of the resulting particles. Based on Table 1, the PI value for cream nanoparticles obtained in cream without a fraction was 0.445 and cream with a fraction was 0.361. The PI value of microemulsion cream with a fraction of 0.852. According to research [23], the highly optimal PI value was 0.1, but some researchers stated that the PI value 0.3 or 0.5 was acceptable. Based on the results obtained, the PI of the resulting nanoparticle cream included a range of 0.5.

Table 1: Niosome modified ethyl acetate fraction formulation of green tea leaf extract. All types of cream preparation can be stored at normal temperature.

Materials	Formulation (mg)
Green Tea Leaf Extract	6,000
Cholesterol	5,070
Span 60	5,640

3.3. Physical Stability of Cream Preparation

Physical tests (on day 0) were carried out on nanoparticle cream preparations, microemulsions, multiple emulsion type W/O/W and niosomes containing ethyl acetate fraction of green tea leaf extract and cream type O/W containing ethyl acetate fraction of green tea leaf extract, while in nanoparticle cream with and without green tea fraction. Based on the homogeneity test results, it was stated that both on nanoparticle creams, microemulsions, multiple emulsions and niosomes were homogeneous. The cream was homogeneous if there was no separation between the dispersed and dispersing phases [24]. The results of the dispersion test (Table 2) on all types of cream had met the parameter requirements of 5-7 cm [25].

Table 2: Particle size test results for micro emulsion cream and nanoparticle cream. The particle size of nanoparticle and micro emulsion had entered the size parameter range (Ethyl Acetate Fraction (EAF) of Green Tea Leaf Extract).

Type of cream	Testing	The mean of particle size (nm)	PI (Polidispersi Index)
Nanoparticle	Nanoparticle Cream	279.4±260.8	0.361
	Nanoparticle Cream without EAF extract	275.7±168.0	0.445
Micro emulsion	Micro emulsion	629.6±638.7	0.852

As seen in Table 2, the results of the pH test of each cream preparation met the requirements of the pH test parameters, namely 4.5 - 6.5 according to the pH of the skin. A pH value that was too acidic would cause irritation to the skin, while a pH value that was too alkaline would cause dry skin [18]. The viscosity value of each cream preparation (Table 2.) met the viscosity test parameters of 2000-4000 cP. It was in accordance with the research [15] stating that spreadability was inversely proportional to viscosity, the lower the viscosity of a preparation, the greater the diameter of the spreadability was.

3.4. Level of EGCG

The results revealed that nanoparticle cream, microemulsion, multiple emulsion type W/O/W and niosomes were more stable than O/W cream (Figure 2, 3 and 4). The nanoparticle formulation could increase the stability of EGCG by inhibiting the decomposition of catechins [26]. Multiple emulsion cream, EGCG was protected by water phase and oil phase which was protected again by water phase so that EGCG was not easily oxidized [27]. Niosomes had a closed bilayer structure, producing vesicles with a lamellar structure that would absorb the active substance into the vesicle so as to overcome the problem of the instability of the active substance [28].

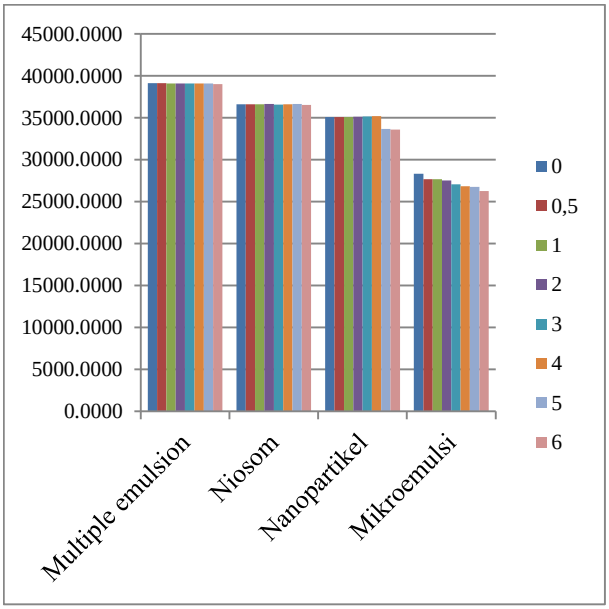

Figure 2 emulsion is more stable of EGCG concentration at 50^0C than niosome, nanoparticle and micro emulsion.

The levels analyzed from the HPLC chromatogram results in this study were EGCG which was degraded to gallic acid, so that the determination of stable levels of

EGCG (undegraded EGCG). According to the research [29], an increase in temperature would decrease the concentration of catechins while the isomers increased. EGCG could be epimerized at high temperatures to convert to their non-epicatechin epimers, namely GCG and gallic acid. The results of the analysis of stable EGCG levels (Figure 2, 3 and 4) when compared between temperatures, the best stable profile of stable EGCG levels was at 500C, while at 600C and 700C, irregular EGCG profiles were obtained. It was required to examine the physical stability of the cream under various temperature variations with real-time stability tests due to the limitations of this study, which included the less than ideal findings of time retention and chromatogram on the EGCG test.

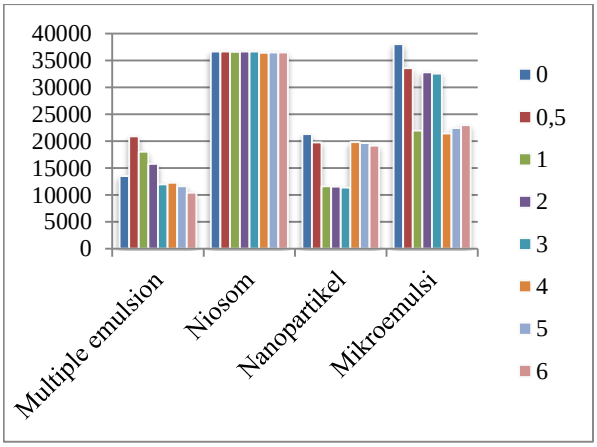

Figure 3. Profile of EGCG Level at 60^0C. The stability of micro emulsion is more stable of EGCG concentration at 60^0C than niosome, nanoparticle and multiple emulsion.

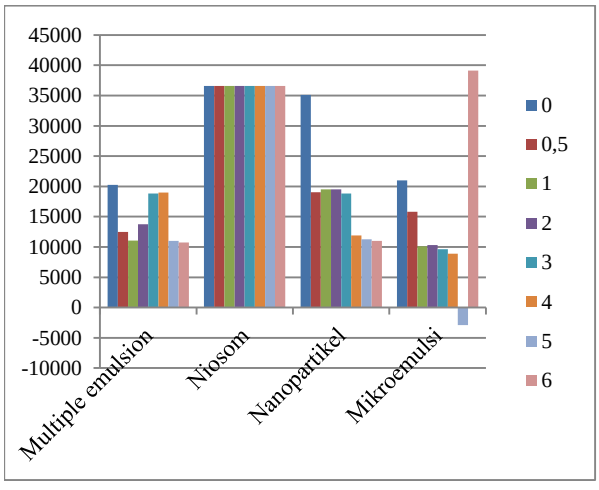

Figure 4. Profile of EGCG Level at 70^0C. The stability of micro emulsion is more stable of EGCG concentration at 70^0C than niosome, nanoparticle and multiple emulsion.

4. Conclusion

In conclucion, nanoparticle cream, micro emulsion, multiple emulsion type W/O/W and niosomes were proven to maintain physical stability compared to cream type O/W. Nanoparticle cream, micro emulsion, multiple emulsion type W/O/W and niosomes were proven to maintain chemical stability compared to cream type O/W.

Acknowledgements

The author is highly appreciated to the Pharmacy Study Program at the Medical Faculty of Universitas Islam Sultan Agung (UNISSULA) for their assistance with this study. The author is appreciative extended to the Medical Faculty for providing the pharmaceutical, chemical, and biological laboratory support.

Conflict of interest

There is no conflict of interest.

Ethics

None.

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