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Research Article

FORMULATION OF A CREAM CONTAINING ETHOSOMAL GREEN TEA (*CAMELLIA SINENSIS* L. KUNTZE) LEAF EXTRACTS FOR IMPROVED DERMAL PENETRATION

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ABSTRACT

Objective: This study aimed to formulate the epigallocatechin gallate (EGCG) from green tea into ethosomes and measure the resulting increases in skin penetration using a rat model.

Methods: Ethosomes were formulated using ethanol concentrations of 25% (F1), 30% (F2), and 35% (F3), and those with favorable characteristics were then incorporated into a cream for the determination of penetration into Franz diffusion cells.

Results: We showed that the formulation F3 had the best spherical morphology, a Z-average value of 73.01 nm, a polydispersity index of 0.26, a zeta potential of −47.77±3.93 mV, and the highest percentage of drug entrapment (49.46%±0.62%) compared with the other formulas. The total cumulative EGCG penetration from the resulting ethosomal cream was 905.75±49.47 μg/cm^2, whereas that from the cream containing green tea leaf extract was only 413.92±52.83 μg/cm^2.

Conclusion: These data indicate a higher penetration of EGCG from ethosomal green tea cream than from cream containing non-ethosomal green tea extract.

Keywords: Epigallocatechin gallate, Ethosomal green tea leaf extract, Topical cream, Dermal penetration.

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INTRODUCTION

Since natural materials are considered safer and have fewer side effects than synthetic materials, pharmaceutical preparations containing natural active substances are increasingly desired [1]. Green tea leaves (*Camellia sinensis* L. Kuntze) are a common source of natural ingredients with health benefits for general and oral health [2,3]. Green tea leaves are credited to have antiproliferative activity and antibacterial activity [4]. Green tea leaves contain multiple polyphenols and derivative flavonoids known as catechins. Among these, epigallocatechin gallate (EGCG) is the most prevalent in green tea leaves [5] and has antioxidant activity that prevents cell damage from oxidative stress, particularly in the skin [6]. Accordingly, antioxidants from green tea may inhibit the development of cancer, heart disease, and immune suppression and could serve as anti-inflammatory, antiaging, and anti-wrinkle agents [2,5,6].

Several studies have shown that EGCG has poor bioavailability and is subject to the first-pass metabolism in the liver and very low absorption through the small intestine [7]. Most digested EGCG does not enter the blood and remains in the colon, where it is degraded by microbiota [8]. Therefore, transdermal drug delivery of EGCG may offer an alternative that overcomes the problems associated with oral administration of EGCG.

Transdermal drug delivery systems have been designed to facilitate the passage of drug compounds through the skin and into the circulation [9]. Transdermal drug delivery avoids gastrointestinal disturbances, first-line metabolism, and fluctuations in circulating drug levels that are associated with oral routes of delivery [10,11]. In addition, transdermal administration is non-invasive and convenient and leads to improved patient compliance [12,13]. However, effective delivery requires penetration of the drug through the skin and into the circulatory system. Since EGCG is hydrophilic (Log p=0.48) and has a large molecular mass (458.37 Da), its skin penetration is highly limited [14]. To address this, drug carrier systems such as lipid nanovesicles are widely considered.

Various lipid nanovesicles can be used as drug carrier systems to increase penetration of active ingredients through the skin [15]. For example, ethosomes can be used as phospholipid-based carrier systems and have high ethanol contents (20%–45%). Ethanol potently enhances skin penetration due to the interdigitation effects of ethanol on the lipid bilayers of skin tissues. Ethosomes were previously designed as modified lipid nanovesicles that contain phospholipids, ethanol, and water depending on the stability of the active ingredient. The particle sizes of ethosomes vary between 10 and 1000 nm [16], but ethosomes have higher entrapment capacities [10,13] and lead to higher transdermal flux than conventional liposomes [15,16]. In this study, we formulated EGCG from green tea into ethosomes and measured the resulting increases in skin penetration using a rat model.

In general, ethosomes are used as drug carriers in semisolid formulations such as gels and creams [17]. Creams are made as oil-in-water (o/w type) emulsions and are commonly used as preferred preparations. Since these o/w-type creams are slightly oily and can spread well, they have the advantages of convenience and ease of application to the skin [18].

In this study, ethosomes of green tea leaf extracts (*C. sinensis* L. Kuntze) were formulated into cream preparations and were then compared with cream containing green tea leaf extracts regarding *in vitro* penetration tests in Franz diffusion cells from the skin of female Sprague Dawley rats.

MATERIALS AND METHODS

Materials

Green tea leaf extract (*C. sinensis* L. Kuntze; Andy biotech, China), EGCG standard (Sigma-Aldrich, Singapore), Lipoid P 30 (kindly provided by Lipoid GMBH, Germany), stearic acid, cetyl alcohol, and isopropyl myristate (pharmaceutical grade), triethanolamine (Croda, Singapore), dimethicone and propylene glycol (Dow Chemical Pacific, Singapore), sodium sulfite anhydrous and butylated hydroxytoluene (BHT; Merck,

Germany), 1,1-diphenyl-2-picrylhydrazyl (DPPH; Sigma-Aldrich), ethanol 96% and methanol (Merck), ascorbic acid (Sigma-Aldrich), potassium dihydrogen phosphate, sodium hydroxide, glacial acetic acid, and acetonitrile (Merck), and Sprague Dawley rats (Bogor Agricultural Institute, Indonesia) were purchased from their respective suppliers.

Determination of EGCG contents in extracts using high-performance liquid chromatography (HPLC)

The EGCG contents were determined using HPLC with C18 (250×4.6 mm) columns and an optimized mobile phase of acetic acid solution (0.05%, v/v) and acetonitrile (87:13, v/v). At pH 3.5–4.0, 10 mg samples were dissolved in 100 mL aliquots of mobile phase with 5 min sonication, filtered through 0.45 µm microfilters, and diluted 2.5 times with mobile phase. Subsequently, 20 µL aliquots of the resulting solutions were injected into the HPLC instrument at a flow rate of 1.0 mL/min and were then detected at a wavelength of 280 nm using an ultraviolet (UV)-visible detector. All samples were measured 3 times.

Antioxidant activity tests of green tea leaf extracts

A Vitamin C solution was generated by mixing 1.0 mL of a stock Vitamin C solution with 1.0 mL of 100.0 µg/mL DPPH and 2.0 mL of methanol. The extract test solutions were obtained by mixing 1.0 mL of green tea leaf extract solution (ES) with 1.0 mL of 100.0 µg/mL DPPH and 2.0 mL of methanol. The blank solution was produced by mixing 3.0 mL of methanol with 1.0 mL of 100.0 µg/mL DPPH. All mixtures were shaken for a few seconds and then incubated at room temperature for 30 min. The antioxidant activities were then determined by measuring absorption using a UV-visible spectrophotometry at DPPH wavelength maximum.

Preparation of ethosomes

Ethosomes were prepared using the mechanical dispersion method described by Maheshwari *et al*. [19] with slight modifications (Table 1). Briefly, lipoid P 30 was dissolved in 25 mL aliquots of dichloromethane and the resulting soluble phospholipids were then placed in dry round flasks. Dichloromethane was then evaporated at 37°C±2°C using a rotary evaporator equipped with a vacuum. On the formation of the thin layers of phospholipids on the bottoms of the flasks, a flow of N_2 gas was applied and the samples were stored in the refrigerator for up to 24 h. Thin layers of phospholipids were then hydrated in hydroethanol solutions containing phosphate buffer (pH 5.5), propylene glycol, ethanol, and green tea leaves at 37°C with rotation at 150 rpm. On the formation of the ethosomal suspensions, ultrasonication was performed for 2 min with an amplitude of 25, and the ethosomal suspensions were then cooled at room temperature and stored in a refrigerator. The characteristics of the ethosome formulations are listed in Table 1.

Characterization of ethosomes

Morphology

Ethosome morphology was examined using a transmission electron microscope after placing single drops of sample on a carbon-coated copper grid. The droplets were then dried at room temperature and stained using a phosphotungstic acid solution. The specimens were viewed under a microscope at a working voltage of 200 kV.

Determinations of particle size distributions, polydispersity indexes, and zeta potentials

Particle size distributions and zeta potentials were measured using dynamic light scattering methods with a computerized inspection system (Malvern Zetasizer). Z-average values and emerging polydispersity indexes were recorded, and zeta potentials were measured using fresh cuvettes.

Entrapment efficiency

The entrapment efficiency was determined using indirect methods in which the concentrations of EGCG were determined according to those remaining outside the vesicles. Briefly, 0.5 mL samples of ethosome suspensions (ET) were mixed with 1.0 mL aliquots of hydration solution and centrifuged at 14,000 rpm 4 times for 30 min each. The supernatants were then analyzed using HPLC with a detector wavelength of 280 nm. The entrapment efficiencies were then calculated by dividing (Qt–Qs) with Qt, then times 100%, where Qt is the total EGCG concentration in ethosome suspensions (µg/mL) and Qs is the EGCG concentration in the supernatants (µg/mL).

Cream preparation

The cream formulations were made as ethosomal creams (CET) and creams without ethosomes (CE; Table 2). Initially, oil phase ingredients including stearic acid, cetyl alcohol, isopropyl myristate, and dimethicone were completely melted in a water bath at 70°C and then mixed with BHT and stirred until homogeneous. Aqueous phases were then prepared by adding a solution containing sodium sulfite, propylene glycol, triethanolamine, and distilled water and then heating in a water bath at 70°C. The oil and water phases were then mixed using a homogenizer with stirring at 950 rpm for 15 min. After cooling to 30°C, the green tea leaf and the ethosomal green tea extracts were added to the creams.

Evaluations of the creams

The ethosomal green tea extract and green tea extract creams were evaluated in terms of organoleptic properties, pH, homogeneity, viscosity, and rheology.

***In vitro* penetration tests**

Skin penetration tests were conducted using Franz diffusion cells with membrane areas of 2.01 cm^2 and a receptor compartment containing 15 mL of phosphate buffer at pH 5.5. The liquid compartment was maintained at 37°C±0.5°C and was stirred with a magnetic stirrer at 250 rpm. The skin specimens were placed between the donor and receptor compartments with the stratum corneum facing upward. Subsequently, 1.0 g samples of the creams were applied to the skin surfaces, and up to 2.0 mL samples were taken from the receptor compartment using a syringe after 10, 30, 60, 120, 240, 360, 480, 600, 720, 840, 960, 1080, 1200, 1320, and 1440 min. The EGCG concentrations were then determined in these samples using HPLC. *In vitro* penetration tests were performed 3 times.

Table 1: Ethosome formulations

Materials	Concentrations (%)		
	F1	F2	F3
Green tea leaf extract	Equal to EGCG 3.0	Equal to EGCG 3.0	Equal to EGCG 3.0
Lipoid P 30	6.0	6.0	6.0
Ethanol	25	30	35
Propylene glycol	10	10	10
Phosphate buffer pH 5.5	ad 100	ad 100	ad 100

EGCG: Epigallocatechin gallate

Table 2: Cream formulations

Materials	Concentrations (% w/w)	
	CET	CE
The sediment of ethosomal suspension green tea leaf extract	Equal to EGCG 1.0	-
Green tea leaf extract	-	Equal to EGCG 1.0
Stearic acid	10.0	10.0
Cetyl alcohol	2.3	2.3
Isopropyl myristate	3.05	3.05
Triethanolamine	2.0	2.0
Dimethicone	10.0	10.0
Propylene glycol	10.0	10.0
Anhydrous sodium sulfite	0.1	0.1
Butyl hydroxytoluene	0.1	0.1
Distilled water	ad 100	ad 100

EGCG: Epigallocatechin gallate

Table 3: Physical characteristics of ethosomes

Sample	Morphology	*Z*-average (nm)	Polydispersity index	Zeta potential (mV)	Entrapment efficiency (%)
F1	Spherical	220.5	0.34	−31.13±0.38	37.29±0.24
F2	Spherical	123.8	0.43	−24.33±1.12	42.75±1.00
F3	Spherical	73.01	0.26	−47.77±3.93	49.46±0.62

RESULTS AND DISCUSSION

Determinations of EGCG contents in extracts using HPLC

The EGCG contents in the green tea extracts were determined after optimizing and validating the HPLC analyses as described by Fangueiro *et al*. [20]. The analyses were performed using reverse-phase HPLC with a stationary phase C18 column and a mobile phase of 0.05% acetic acid: acetonitrile (87:13 v/v) at a flow rate of 1.0 mL/min. Since EGCG is easily degraded and unstable at high pH, the analyses were performed at pH 3.5–4. The EGCG contents in the green tea leaf extracts were 53.50%±0.23%.

Antioxidant activity tests of green tea leaf extracts

The half inhibitory concentration (IC_{50}) of the green tea leaf extract was 1.312 μg/mL and was much lower than that of the positive control ascorbic acid (IC_{50} 2.735 μg/mL), indicating greater antioxidant activity of EGCG. These antioxidant properties of the green tea leaf extract indicate potential as a source of active ingredients in creams.

Formulation and characterization of ethosomes

Ethosomes were formulated using a mechanical dispersion method, and the best ethosome products were used as active ingredients in the cream formulation. The parameters of ethosome quality were spherical shape in analyses of particle morphology (Fig. 1), small size (73.01 nm), a polydispersity index of 0.26, a zeta potential of <−30 mV or >+30 mV, and the highest entrapment efficiency (49.46%±0.62%). Based on the data in Table 3, the best ethosome formulation was F3 and this was selected as an active ingredient for cream formulations, which were evaluated as described below.

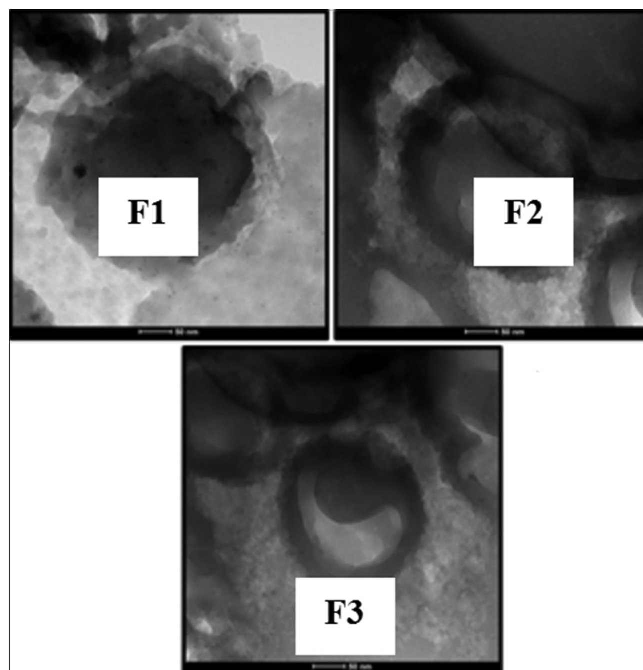

Fig. 1: Morphology of ethosomal vesicles

Evaluation of cream dosage forms

Organoleptic tests showed that CET had a pale yellow color (PANTONE 607 CP) and CE had a brownish-yellow color (PANTONE 461 CP) based on Pantone I Color Book [21]. The CET and CE creams were homogeneous when applied and had pH values of 5.40 and 5.45, respectively, indicating compatibility with the physiological pH of skin and the stability of EGCG. The viscosity values of the CET and CE creams were 11,800 and 11,200 cps, respectively, and both creams had thixotropic plastic rheology properties.

In vitro penetration tests

After applying the topical preparations, the cumulative amounts of EGCG that penetrated the Franz diffusion cells were 2442.57±93.47 μg/cm^2 for ES, 3897.67±1380.29 μg/cm^2 for ethosome suspensions (ET), 413.92±52.83 μg/cm^2 for CE, and 905.75±49.47 μg/cm^2 for CET (Fig. 2). We also calculated the flux of the active substance (EGCG) through rat skin and expressed these data as circulating EGCG per unit area per unit time. The flux values for ES, ETS, CE, and CET were 109.032±21.969, 131.65±66.03, 12.66±1.45, and 40.96±5.56 μg.cm^{-2}/h, respectively (Figs. 2-3). Based on these results, we suggest that CET achieves better penetration than CE. In addition, the ethosomal suspension had better penetration than the ES, indicating that ethosomes in topical creams can improve EGCG penetration through rat skin. The present ethosomal suspensions have a high ethanol content, which enhances penetration through hydrogen bond interactions with the phospholipid layers of the stratum corneum. Ethanol also facilitates the passage of the active ingredient into deeper skin layers by enhancing flexibility of following fusion of the ethosome vesicles with lipids in skin membranes [13,15,16].

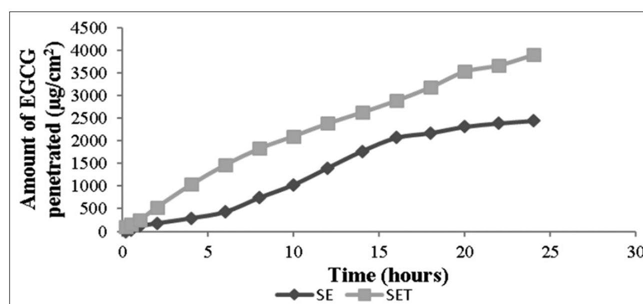

Fig. 2: Cumulative epigallocatechin gallate penetration from SE and ethosomal suspensions

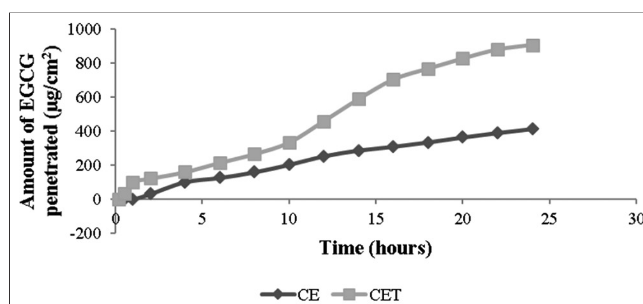

Fig. 3: Cumulative epigallocatechin gallate penetration from CE and CET

CONCLUSION

Based on the data presented herein, we conclude that the F3 ethosome formula has the best characteristics of all formulas. The ethosomal cream with the F3 formula resulted in greater EGCG penetration through rat skin than the non-ethosomal cream.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest in this study.

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