INTRODUCTION

Green tea from the tea plant *Camellia sinensis* has several health benefits [1]. Bioactive compounds present in green tea leaves include alkaloids, flavonoids, and phenols [2]. The flavonoids (particularly the catechin fraction) are basic phenolic compounds responsible for antioxidant activity [3]. Epigallocatechin gallate (EGGC) is the major catechin in green tea and the primary source of its antioxidant activity [4]. EGGC is hydrophilic (log p=0.48) [5] and thus has poor skin permeability. However, skin delivery has several advantages such as the mitigation of gastrointestinal disturbances and bypass of hepatic first-pass metabolism [6]. Therefore, a delivery system that can facilitate the skin absorption of EGGC is desirable, and a formulation using transethosomes could be among the best such vehicles for EGGC-based treatment.

For the skin delivery of such compounds, several strategies have been used to bypass the stratum corneum barrier. Liposomes have been used for topical drug delivery; however, conventional liposomes do not deeply penetrate the skin [7]. To overcome this limitation, novel lipid vesicles - transethosome and transfer some - were developed. Transethosome is a modified ethosome formed into lipid vesicles that can improve penetration through the skin [8]. Transethosome contains high ethanol content together with an edge activator or permeation enhancer [7]. Transethosome can be formulated into a semi-solid form such as a cream. Creams generally have greater esthetic appeal because they are non-greasy and can be easily applied, spread, and removed [9].

In this study, transethosomes containing green tea extract were formulated into a cream (transethosome cream, TEC) and evaluated for their physicochemical characteristics and skin penetration capacity.

This formulation exhibited highly advantageous physicochemical characteristics, high entrapment efficiency (EE), and markedly enhanced skin flux than a non-TEC (NTEC).

METHODS

Materials

Green tea leaf extract (*C. sinensis* L. Kuntze) (Andy Biotech [Xi’an] Co. Ltd., China), EGGC standard (Sigma-Aldrich, Singapore), Lipoid P30 (Lipoid GmbH, Germany), solvents, and other chemical reagents were of analytical grade.

EGGC assay of green tea leaf extract

Green tea leaf extract was prepared according to the methods of Fangeiro *et al.* with some modifications [10]. Standard solutions of EGGC were prepared at 25, 30, 35, 40, 45, and 50 µg/mL for constructing a calibration curve. The solution was analyzed using reversed-phase-high-performance liquid chromatography with a UV-visible detector set at 280 nm and a flow rate of 1.0 mL/min. The mobile phase comprised 0.05% (v/v) acetic acid: acetonitrile (87:13 v/v) at a flow rate of 1.0 mL/min. The injection volume was 20 µg/mL, and retention time was approximately 16 min.

Antioxidant activity of green tea leaf extract

The antioxidant activity was measured according to the method of Mishra *et al.* [11]. Ascorbic acid standard and green tea leaf extract solutions were prepared at different concentrations. DPPH 100 µg/mL (1.0 mL) and methanol (2.0 mL) were added to 1.0 mL of each sample solution. The mixture was shaken and incubated at room temperature for 30 min. The antioxidant activity was determined using a UV-visible spectrophotometer at 514 nm.
Transehosome formulation

Transehosome was prepared in three formulations (Table 1) through the thin-layer hydration method. Lipoid P30 and Span 80 were dissolved in dichloromethane in a round-bottom flask and the dichloromethane was removed by rotary evaporation at 40°C. Nitrogen gas was applied to form a thin layer that was stored in the refrigerator for 24 h, rehydrated using a solvent containing green tea leaf extract in 95% ethanol and phosphate buffer (pH 5.5), and then shaken by rotation for 1 h at 37°C. All products were stored at 4°C in the refrigerator until testing.

Transehosome morphology was examined using transmission electron microscopy (TEM) (FEI type Tecnai G2 20 S-TWIN). All samples were analyzed with an accelerating voltage of 200 kV at 20°C and using LaB₆ filament as the electron source. Briefly, the suspension was aliquoted onto a carbon-coated copper grid and dried at room temperature for imaging at various magnifications (Fig. 1).

The particle size distribution, PDI, and zeta potential were determined through dynamic light scattering using a particle size analyzer (Malvern Zetazizer). The three formulations were added to 10 mL of distilled water and measured in triplicate.

EE was measured through the indirect method. Drug content was measured first in the transehosome preparation using HPLC and then in the supernatant after the separation of the transehosome component using centrifugation. Briefly, 0.5 mL of the transehosome suspension was added to 1.0 mL of phosphate buffer (pH 5.5), and the mixture centrifuged at 14,000 rpm 4 times for 30 min each. The precipitate was discarded, and the supernatant was collected and mixed with phosphate buffer (pH 5.5) and ethanol. The percentage EE of EGGC was calculated by dividing (Qt-Qs) with Qt, then times 100%, where Qt is the concentration of EGGC in transehosome suspension (µg/mL) and Qs is the concentration of EGGC in the supernatant (µg/mL). Based on predetermined criteria, the suspension with the most optimum concentration was chosen as the ingredient for the preparation of the cream. Table 2 presents the formulas for TEC and NTEC.

The cream was prepared by separately heating an oil mixture (stearic acid, cetyl alcohol, isopropyl myristate, dimethicone, and BHT) and an aqueous mixture (TEA, propylene glycol, sodium sulfite anhydrous, and distilled water) in a water bath at 70°C. The oil phase was then added to the aqueous phase and mixed using a homogenizer at 500–1000 rpm. Then, active ingredients were added to the cream base (1%, v/v) and homogenized. The cream was then evaluated for its organoleptic properties, pH, homogeneity, viscosity, rheological properties, and in vitro skin penetration capacity using a Franz diffusion cell test. This latter study was approved by the Ethics Committee of Cipto Mangunkusumo Hospital, Faculty of Medicine, Universitas Indonesia, Number. 313/UN2.F1/ETIK/2017. Abdominal skin from female Sprague-Dawley rats (2-3 months old, 200 g) was used as the test membrane. The surface area of membrane was 2.0096 cm². The receptor had a compartment volume of 15 mL filled with phosphate buffer (pH 5.5) at 37°C.

For flux analysis, 1 g of the cream was applied to the membrane and 3.0 mL aliquots were obtained from the receptor compartment after 10, 30, 60, 120, 180, 240, 360, 480, 600, 720, 840, 960, 1080, 1200, 1320, and 1440 min. The removed volume was replenished with an equal volume of the receptor media. The amount of EGGC in the receptor sample was determined using HPLC. This in vitro penetration test was conducted in triplicate for all samples.

RESULTS AND DISCUSSION

Antioxidant activities of plant extracts are mainly contributed by polyphenols [12-16]. Green tea contains phenolic compounds that act as powerful chain-breaking antioxidants, which might directly contribute to antioxidant activity. Indeed, there are strong correlations between antioxidant activity and the total phenolic and flavonoid contents

| Material | Concentration (%) |
|----------|-------------------|
| Green tea leaf extract equal to EGGC | 3.0  3.0  3.0 |
| Lipoid P30 | 4.0  4.0  4.0 |
| Span 80 | 0.5  0.75  0.5 |
| Ethanol 95% | 30  30  35 |
| Phosphate buffer pH 5.5 | ad 100  ad 100  ad 100 |

EGGC: Epigallocatechin gallate

**Table 1: Transehosome formulas**

**Table 2: Cream formulas**

| Material | Concentration (%) |
|----------|-------------------|
| Transehosome/green tea leaf extract (EGGC content) | 1.0 - |
| Green tea leaf extract (EGGC content) | - 1.0 |
| Stearic acid | 10.0  10.0 |
| Cetyl alcohol | 2.3  2.3 |
| Isopropyl myristate | 3.05  3.05 |
| TEA | 2.0  2.0 |
| Propylene glycol | 10.0  10.0 |
| Sodium sulfite anhydrous | 0.1  0.1 |
| BHT | 0.1  0.1 |
| Dimethicone | 10.0  10.0 |
| Distilled water | Ad 100  Ad 100 |

TEC: Transehosome cream, NTEC: Non-transehosome cream, EGGC: Epigallocatechin gallate

Fig. 1: Morphology of transehosome green tea extract
Table 3: Physicochemical characteristics of the three transethosome green tea extract formulations

| Formulation code | Morphology         | Particle size (nm) | PDI   | Zeta potential (mV) | EE (%)  |
|------------------|--------------------|--------------------|-------|---------------------|---------|
| F1               | Irregular spherical| 46.24              | 0.165 | −32.73±4.25         | 34.73±14.62 |
| F2               | Irregular spherical| 35.35              | 0.319 | −29.97±3.05         | 45.26±8.15  |
| F3               | Irregular spherical| 632.0              | 0.449 | −47.77±4.93         | 38.75±4.05  |

PDI: Polydispersity index, EE: Entrapment efficiency

Fig. 2: Cumulative amount of epigallocatechin gallate penetrated of C. sinensis. In this study, the EGCG content of green tea leaf extract was 53.66%±0.35%, and the antioxidant IC₅₀ value was 1.38 µg/mL, which was substantially lower than that of ascorbic acid (2.71 µg/mL), indicating a stronger antioxidant activity.

Transethosome containing green tea leaf extract was prepared in three formulations using Lipoid P30, Span 80, ethanol 95%, and phosphate buffer (pH 5.5). Transethosome contained ethanol together with an edge activator or permeation enhancer, which was expected to improve permeation [7]. Morphological analysis through TEM revealed that transethosome particles were irregular spheres (Fig. 1). This conformation may be due to rearrangement in the lipid bilayer of vesicles due to the combination of ethanol and edge activator [8].

Results of physicochemical characterization are summarized in Table 3. The rank order Z-average values of the transethosome formulas were F2>F1>F3. Formula F2 with the highest surfactant concentration (lower surface tension) had the smallest mean particle size, in accordance with the findings of previous studies [13]. A PDI of <0.5 indicates that transethosome vesicles are homogenous; therefore, based on the criteria of particle size and homogeneity using Malvern particle size analyzer, all three formulations satisfy public standards. Zeta potential is an important physical parameter for the prediction of vesicle stability. All transethosome formulations exhibited a negative zeta potential due to the charge transition of vesicles induced by ethanol [17]. All three formulations also exhibited reasonable entrapment efficiencies, with F2 exhibiting the highest efficiency (45.26%±8.15%). Thus, higher surfactant (Span 80) concentration was associated with greater EE, in accordance with Srivastava et al. [6]. Based on physicochemical characterization, transethosome formula F2 with the smallest particle size, PDI <0.5, good zeta potential, and the highest EE was chosen as the active ingredient for the cream formulation.

Both TEC and NTEC were pale yellow (Pantone 9120 U), had the aroma of tea, and exhibited similar pH values (5.63±0.02 and 5.34±0.02, respectively). TEC had a higher viscosity than NTEC as measured at 20 rpm (12400 vs. 12000 cps), which was likely to the presence of phospholipid, surfactant, and ethanol. Both creams had similar rheology properties (thixotropic plastic). However, TEC exhibited a substantially greater delivery capacity for EGCG into rat skin (cumulative amount: 1133.90±48.15 vs. 436.26±28.52 µg/cm²; first phase flux: 60.56±4.52 vs. 25.69±0.83 µg·cm⁻²·h⁻¹ and second phase: 23.13±1.38 vs. 7.36±1.59 µg·cm⁻²·h⁻¹) (Fig. 2).

Based on Fig. 2, TEC promotes greater EGCG penetration into rat skin than NTEC. The superior delivery capacity of transethosome appears to reflect a synergism among ethanol, surfactant, vesicle, and skin lipids [6].

CONCLUSION

TEC facilitated a superior skin penetration of EGCG than NTEC. Thus, this formulation may prove effective for topical antioxidant treatment or an efficient systemic delivery.

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CONFLICTS OF INTEREST

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

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