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Research Article
Analysis of *Coffea canephora* Bean Supercritical Extract and Raffinate and Development of Active Material

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Abstract

Background and Objective: *Coffea canephora* bean contains a large amount of caffeine and chlorogenic acid, which have effects such as anti-cellulite and hair-loss improvement. This study aimed to find an optimal extraction process that maximizes active ingredients from *Coffea canephora* beans and to develop a functional material improved transdermal permeability. **Materials and Methods:** The nine extracts were quantitatively and qualitatively analyzed on caffeine and chlorogenic acid with HPLC. Polyphenol quantification assay, DPPH radical scavenging assay and ABTS+radical scavenging assay were performed. A deformable liposome encapsulating the optimum *Coffea canephora* bean extracts was prepared using propylene glycol to improve stability and transdermal permeability. The characteristics of formulations were measured and Franz diffusion cell was performed to confirm the transdermal permeability. **Results:** Based on HPLC analyses and radical scavenging assays, the supercritical extract under the condition of 150 bar and 60°C and the hydrothermal extract of the supercritical raffinate were selected as the optimum extracts. The deformable liposome system dramatically enhanced the stability and skin permeation of the formulation. The transdermal permeability was increased 15.35 fold. **Conclusion:** The analyses verified that the best extraction method for caffeine is the supercritical extraction at 150 bar and 60°C and that the best extraction method for chlorogenic acid is the hydrothermal extraction of supercritical raffinate. In addition, the results suggest that the formulation with the optimum *Coffea canephora* bean extracts could be used as an excellent functional material with abundant active ingredients, higher stability and improved transdermal permeability.

Key words: *Coffea canephora*, green coffee bean, cellulite, hair-loss, liposome, deformable liposome, supercritical extract, supercritical raffinate, transdermal permeation

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The main active ingredients of green coffee (*Coffea canephora*, *Coffea arabica*) bean are caffeine ($C_8H_{10}N_4O_2$) and chlorogenic acid ($C_{16}H_{18}O_9$). These days, caffeine has been applied as a topical agent increasingly, because it promotes blood circulation and fat decomposition by inhibiting phosphodiesterase and hair growth by inhibiting 5α-reduction activity[1-3]. Chlorogenic acid is considered a representative antioxidant indicator of coffee extracts on account of its strong antioxidant effect. A green coffee bean contains a great deal of chlorogenic acid, especially 5-Caffeoylquinic Acid (5-CQA)[4,5].

Supercritical fluid extraction is a bio-friendly extraction method that does not leave residual solvent. Carbon dioxide is the most frequently used solvent for supercritical fluid extraction. This is because carbon dioxide has a relatively low critical point (31.1°C, 73.8 bar) and there is no need to vaporize after extraction to separate extract from raffinate. In addition, carbon dioxide is an inert gas that does not adversely affect the human body even if it remains in the extract[6-8].

Recently, research on new types of liposomes has been actively conducted, such as deformable liposomes, ethosomes and noisomes[9-11]. A deformable liposome is a type of elastic liposome, whose edge activator is usually a single-chain surfactant. Edge activators function to destabilize the lipid bilayer, giving flexibility to it. It has been reported that propylene glycol belonging to the glycol group not only acts as an edge activator but also helps active ingredients to penetrate the skin[12,13].

In this study, supercritical CO_2, hydrothermal, ethanol extraction and hydrothermal extraction of supercritical raffinate, which had never been investigated before, were performed from *Coffea canephora*. Caffeine and chlorogenic acid of *Coffea canephora* extracts were analyzed by High-Performance Liquid Chromatography (HPLC). After confirming the contents of active ingredients, antioxidant capacity was directly and indirectly verified by evaluating the polyphenol quantification, DPPH radical scavenging assay and ABTS+radical scavenging assay. Furthermore, the extracts including the most active ingredients were selected and manufactured with a deformable liposome to improve stability and transdermal permeability. The characteristics of the formulation such as mean size and zeta potential were measured and the transdermal permeability was analyzed through a Franz diffusion cell. This study aim to find out the optimal extracts of *Coffea canephora* bean and to develop them into a functional material with enhanced transdermal delivery.

MATERIALS AND METHODS

Study area: The study was carried out on University of Eulji in 2023.

Materials: Dried and unroasted *Coffea canephora* bean (KH-Pharm, Korea) from Vietnam was used in this study.

Reagents used include caffeine (DaeJung Chemicals and Metals, Korea), chlorogenic acid (5-caffeoylquinic acid, Sigma Aldrich, USA), Folin-Ciocalteu's reagent (Sigma Aldrich, USA), gallic acid (DaeJung Chemicals and Metals, Korea), (2,2-Diphenyl-1-Picrylhydrazyl) (DPPH, Sigma Aldrich, USA), L-ascorbic acid (DaeJung Chemicals and Metals, Korea), (2,2'-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid), (ABTS, Sigma Aldrich, USA), potassium persulfate (Sigma Aldrich, USA) and hydrogenated lecithin (Neuropid, Korea).

Solvents in this study are ethyl alcohol (Samchun chemicals, Korea), Dimethyl Sulfoxide (DMSO, Sigma Aldrich, USA), acetonitrile (Samchun chemicals, Korea), acetic acid (Samchun chemicals, Korea) and phosphate-buffered saline (PBS, Sigma Aldrich, USA).

All the reagents and solvents were of analytical grade.

Materials such as micropic Franz cell membrane (Apures, Korea), microplate (SPL Life Sciences, Korea) and serological pipette (SPL Life Sciences, Korea) were used, as well.

Extraction: The 500 g of D.I. water (Deionized water) was added to 50 g of *Coffea canephora* bean powder. *Coffea canephora* bean hydrothermal extract (CWE) was extracted in a thermostat at 80°C for 120 min.

The 70% ethyl alcohol 1000 g was mixed with 100 g of *Coffea canephora* bean powder. *Coffea canephora* bean ethanol extract (CEE) was extracted at room temperature for 120 min. Ethyl alcohol was evaporated for more than 3 hrs at a temperature of 50°C and a pressure of 60 mmHg with a rotary vacuum evaporator (Eyela, Japan).

Coffea canephora bean supercritical CO_2 extracts (CSEs) were extracted for 150 min under six conditions of extraction tank. The setting of the separation tank was applied equally at 40 bar and 25°C. The 99.9% ethyl alcohol was used as a co-solvent as well. The flow rate of CO_2 was 60 mL/min and the flow rate of ethyl alcohol was 5 mL/min. To completely evaporate the solvent, each of the extracts was concentrated with the rotary vacuum evaporator at 50°C and 60 mmHg for at least 3 hrs.

The raffinate remaining in the extraction tank was collected after supercritical CO_2 extraction at 150 bar and 60°C. The 500 g of DI water was added to 50 g of the raffinate. *Coffea canephora* bean supercritical raffinate hydrothermal extract (CRE) was extracted in the thermostat at 80°C for 120 min.

Table 1: Condition of supercritical CO_2 extraction

Classification	Condition		
Instrument	Agilent 1100 (Agilent, USA),		
Column	Shisheido C18 (4.6×250 mm, 5 µm, 30°C)		
Injection volume	10 µL		
Mobile phase	Time (min)	0	20
	0.2% Acetic acid in D.I. water (%) and	95	5
	acetonitrile (%)	70	30
Flow rate	1 mL/min		
Stop time/post time	20/5 min		
Detector wavelength	275, 325 nm		

Table 2: HPLC analysis condition of *Coffea canephora* bean extracts

Condition of extraction	Sample name
Hydrothermal extraction of supercritical raffinate 150 bar 60°C	*Coffea canephora* bean supercritical raffinate hydrothermal extract (CRE)
Hydrothermal extraction (80°C)	*Coffea canephora* bean hydrothermal extract (CWE)
Ethanol extraction (room temperature)	*Coffea canephora* bean ethanol extract (CEE)
Supercritical CO_2 extraction 350 bar 60°C	*Coffea canephora* bean supercritical CO_2 350 bar 60°C extract (CSE36)
Supercritical CO_2 extraction 350 bar 40°C	*Coffea canephora* bean supercritical CO_2 350 bar 40°C extract (CSE34)
Supercritical CO_2 extraction 250 bar 60°C	*Coffea canephora* bean supercritical CO_2 250 bar 60°C extract (CSE26)
Supercritical CO_2 extraction 250 bar 40°C	*Coffea canephora* bean supercritical CO_2 250 bar 40°C extract (CSE24)
Supercritical CO_2 extraction 150 bar 60°C	*Coffea canephora* bean supercritical CO_2 150 bar 60°C extract (CSE16)
Supercritical CO_2 extraction 150 bar 40°C	*Coffea canephora* bean supercritical CO_2 150 bar 40°C extract (CSE14)

All the extracts were vacuum filtered with a filter of 5 µm pore size and freeze-dried. They were stored at -20°C. The conditions of extraction and the names of the extracts were presented in Table 1.

HPLC analysis: The 10 mg of the extract was dissolved in 1 mL of 50% DMSO. The solutions were filtered through a 0.2 µm filter into an HPLC vial and were analyzed by HPLC. Detailed analysis conditions are shown in Table 2.

Total polyphenol content quantification: The total polyphenol content was quantified by Folin-Ciocalteu assay[14]. The 10 µL of sample and 40 µL of Folin Ciocalteu's reagent were prepared and reacted for 3 min. Thereafter, 150 µL of 10% Na_2CO_3 was added. The absorbance was measured at 760 nm using a Multi-Mode Microplate Reader (BioTek, USA). The standard curve was calculated using gallic acid as a standard and the contents were converted into mg gallic acid equivalents (GAE)/g extract.

DPPH radical scavenging assay: The DPPH radical scavenging activity was evaluated through the Blois' method[15]. The 10 µL of sample and 190 µL of DPPH solution were mixed so that the final concentration of DPPH was 0.1 mM. After the reaction in a dark room at room temperature for 30 min, the absorbance of the solution was measured at 520 nm. The L-ascorbic acid, a representative antioxidant, was used as a positive control:

$$\text{DPPH radical scavenging activity (\%)} = \frac{1 - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

ABTS+radical scavenging assay: Referring to the methods of Re *et al.*[16] ABTS and potassium persulfate were dissolved in PBS and mixed so that each of their final concentrations was 7.4 and 2.6 mM[16]. This ABTS solution was reacted at 4°C for 18 hrs to obtain ABTS+radical. The 10 µL of sample and 190 µL of ABTS solution with an absorbance of 0.70±0.01 were mixed and reacted in a dark room at room temperature for 20 min. The absorbance of the solution was measured at 734 nm. The L-ascorbic acid was used as a positive control:

$$\text{ABTS+radical scavenging activity (\%)} = \frac{1 - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Preparation of the deformable liposome: Ingredients of the liposome and control solution were presented in Table 3. The deformable liposome was manufactured using propylene glycol as an edge activator. The phase A and B were, respectively heated to 80°C and completely dissolved with a homogenizing dispers (Primix, Japan) and the phase (B) was added to the phase (A) and dispersed at 3000 rpm for 30 min with a homogenizing mixer (Primix, Japan). This emulsified formulation was treated three times with a high-pressure homogenizer (Micronox, Korea) at 1300 bar to obtain the deformable liposome.

Table 3: Composition of formulations containing *Coffea canephora* bean extracts

Phase	Deformable liposome (wt%)	Control solution (wt%)
A	Glycerin (60%)	D.I. water (49%)
	D.I. water (29.7%)	Ethyl alcohol (49%)
	Hydrogenated lecithin (2%)	*Coffea canephora* bean supercritical raffinate hydrothermal extract (1%)
	1,2-Hexanediol (2%)	*Coffea canephora* supercritical $CO_2$150 bar 60°C extract (1%)
	Propylene glycol (0.3%)	
	Coffea canephora bean supercritical raffinate hydrothermal extract (1%)	
B	Medium chain triglycerides (4%)	
	Coffea canephora supercritical CO_2 150 bar 60°C extract (1%)	

Characterization of formulations: Mean size, polydispersity index (PDI), zeta potential and pH were assessed to compare the characteristics of the deformable liposome with those of the control solution. Each formulation was diluted 1/10 in D.I. water. Mean size and PDI were measured by a nano particle size analyzer (Microtrac MRB, USA). Zeta potential was examined with a particle charge analyzer (Particle Metrix, Germany) and pH was determined with a pH meters (Mettler Toledo, Switzerland).

Transdermal permeation studies: Transdermal permeation experiments were carried out through a Franz diffusion cell. The temperature of circulating water was set to 32°C to be similar to the human skin surface and PBS was put in the receptor part. After fixing the skin between the donor part and the receptor part, 0.5 mL of formulation was applied to the skin of 1.309734 cm^2 area. To evenly mix the substances permeated to the skin, the mixture was stirred at 500 rpm using a stirrer (PermeGear, USA). After 1, 2, 4, 8, 16 and 24 hrs, 0.5 mL samples were collected from the receptor part and the same volume of PBS was supplemented. To examine the transdermal permeability, one experiment was conducted with the full-thickness skin and samples were collected. To analyze the epidermal permeability, the skin was reacted at 60°C for 1 min and then only the epidermis was separated by tweezers. The other experiment was performed with it and samples were collected in the same way.

The amount of permeation was determined through caffeine presented in the sample. The collected samples were immediately filtered through a 0.2 µm filter and analyzed by HPLC. The HPLC analysis was conducted under the conditions of Table 2. Considering the solvent of the sample, caffeine was dissolved in PBS to calculate the standard curve.

Data analysis and statistics: The experiment was repeated three times. The results was expressed as Mean and Standard deviation (Mean±SD). For the significance test, a t-test was performed using Excel 365. It was considered to be significant that the p-value was less than 0.05.

RESULTS

Active ingredient of *Coffea canephora* bean extract

Contents of caffeine and chlorogenic acid: The HPLC analysis was performed for all nine *Coffea canephora* bean extracts and the results were given in Table 4. Caffeine showed the highest content in CSE16. The caffeine content of CSE16 was 22.00±0.26%, which was measured about 2.13~4.80-fold higher than that of the other extracts. Chlorogenic acid was found to have the highest content of 15.00±0.16% in CRE. Chlorogenic acid was not detected in CSEs. The optimal condition for caffeine extraction was the supercritical CO_2 extraction at 150 bar and 60°C and the optimal condition for chlorogenic acid was the hydrothermal extraction of the supercritical raffinate at 150 bar and 60°C. Caffeine and chlorogenic acid had very similar retention time (RT), which makes it difficult to completely separate. Therefore, the wavelength that a single peak can be detected at was found and analyzed at the same time (Table 5).

Total polyphenol contents: The total polyphenol contents of *Coffea canephora* bean extracts can be seen in Table 6. The extract which consist of the most polyphenols per unit weight was CRE. The quantified polyphenol content was 406.72±13.90 mg GAE/g, which means that 40.67±1.39% of the extract was polyphenol similar to gallic acid. The CSEs contained a comparatively small amount of polyphenol with an average of 1.51±0.49%.

Antioxidant capacity of *Coffea canephora* bean extract

DPPH radical scavenging activity: High DPPH radical scavenging activity was indicated in CRE, CWE and CEE. In particular, CRE and CWE suppressed more than 90.00% of DPPH radical at a maximum concentration of 100 µg/mL. Half Maximal Inhibitory Concentration (IC_{50}) of CRE, CWE, CEE, CSE36, CSE34, CSE26, CSE24, CSE16 and CSE14 was calculated as 19.83, 21.56, 41.86, 1316.76, 1136.15, 1370.27, 2329.23, 1119.76 and 1580.49 µg/mL. Full results were shown in Fig. 1.

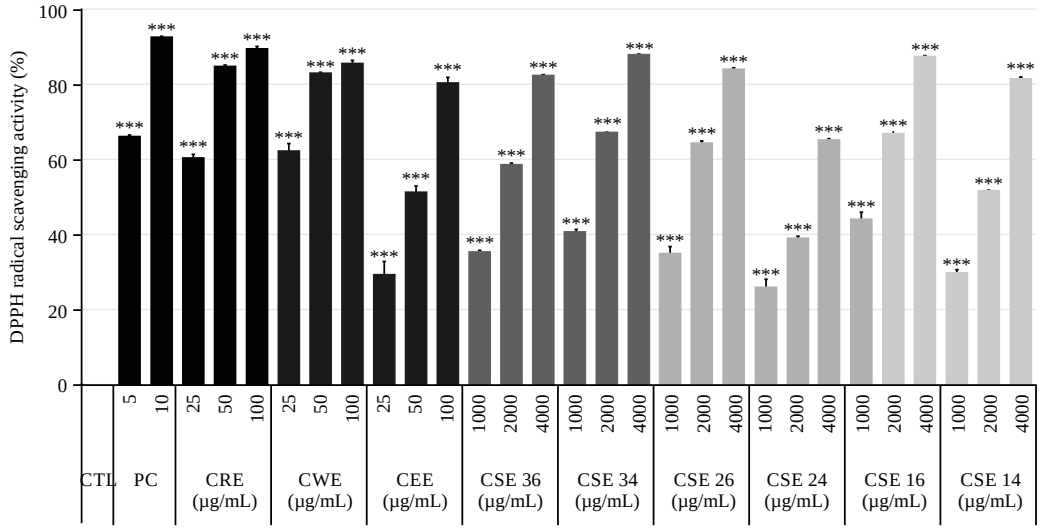

Fig. 1: DPPH radical scavenging activity of *Coffea canephora* bean extracts

Positive control: L-ascorbic acid 95.59±4.52% (radical scavenging activity) at a concentration of 10 μg/mL, ***p<0.001 vs control, CTL: Control, sample untreated group, PC: Positive control, CRE: *Coffea canephora* bean supercritical raffinate hydrothermal extract, CWE: *Coffea canephora* bean hydrothermal extract, CEE: *Coffea canephora* bean ethanol extract and CSE: *Coffea canephora* bean CO_2 supercritical extract

Table 4: Contents of caffeine and chlorogenic acid in *Coffea canephora* bean extract

Sample	Caffeine (%)	Chlorogenic acid (%)
CRE	5.55±0.36	15.00±0.16
CWE	7.06±0.14	12.46±0.18
CEE	6.34±0.05	3.15±0.61
CSE36	5.51±0.14	ND
CSE34	8.13±0.07	ND
CSE26	9.63±0.14	ND
CSE24	4.58±0.06	ND
CSE16	22.00±0.26	ND
CSE14	10.32±0.15	ND

Values present the Mean±Standard Deviation of three independent experiments. ND: Not detected, CRE: *Coffea canephora* bean supercritical raffinate hydrothermal extract, CWE: *Coffea canephora* bean hydrothermal extract, CEE: *Coffea canephora* bean ethanol extract and CSE: *Coffea canephora* bean CO_2 supercritical extract

Table 5: Linear range, linear regression equation, correlation coefficient, retention times of the analytical method for caffeine and chlorogenic acid

Analyte	Linear ranges (μg/mL)	Standard curve	R^2	RT (min)	λ (nm)
Caffeine	50~800	y = 0.0377x-7.0939	0.9989	11.44	275
Chlorogenic acid	50~800	y = 0.067x+3.4121	0.9998	11.47	325

RT: Retention time, R2: Correlation coefficient, y: Concentration of compound and x: Peak area

ABTS+radical scavenging activity: All the extracts showed ABTS+scavenging activity. Especially, CRE had an IC_{50} value of 5.99 μg/mL, which was equivalent to 3.97 μg/mL of positive control (PC). The IC_{50} values of CWE, CEE, CSE36, CSE34, CSE26, CSE24, CSE16 and CSE14 were calculated as 6.71, 16.37, 461.02, 543.37, 717.29, 394.50 and 614.90 μg/mL. The CSEs showed high antioxidant capacity as well. However, relatively excellent antioxidant capacity was found in hydrothermal and ethanol extracts. Detailed results were presented in Fig. 2.

Characteristics of formulations: Table 7 presented the mean size, PDI, zeta potential and pH results of the formulations. The deformable liposome was pH 6.14±0.01 and the control solution was pH 6.20±0, so none of them are expected to cause pH irritation. The absolute zeta potential value of the deformable liposome was slightly larger than that of the control solution. Nevertheless, it is more than 30 mV, which means a very stable formulation, so neither is likely to agglomerate easily[17].

The average particle size of the deformable liposome was 484.00±6.00 nm, which was 4.8-fold smaller than that of the control solution. The PDI represents the homogeneity of the particle size distribution. If it is 1, it usually means the monodisperse formulation. In liposome and nano-liposome

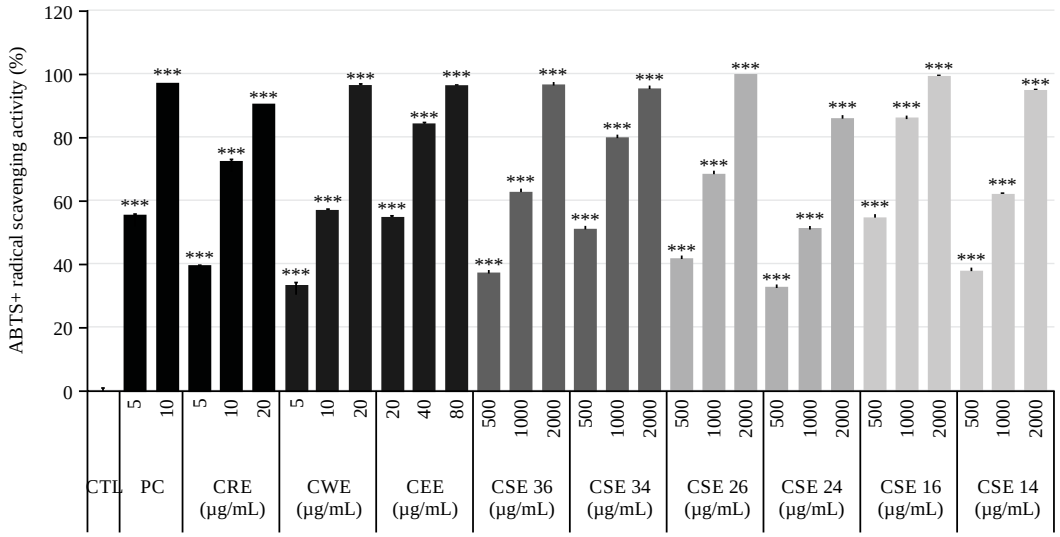

Fig. 2: ABTS+ radical scavenging activity of *Coffea canephora* bean extracts

Positive control: L-ascorbic acid 99.84±0.07% (radical scavenging activity) at a concentration of 10 μg/mL, ***p<0.001 vs control, CTL: Control, sample untreated group, PC: Positive control, CRE: *Coffea canephora* bean supercritical raffinate hydrothermal extract, CWE: *Coffea canephora* bean hydrothermal extract, CEE: *Coffea canephora* bean ethanol extract and CSE: *Coffea canephora* bean CO_2 supercritical extract

Table 6: Total polyphenol contents of nine extracts from *Coffea canephora* bean

Sample	Total polyphenols (mg GAE/g) (%)
CRE	406.72±13.90 (40.67±1.39)
CWE	224.18±6.24 (22.42±0.62)
CEE	93.08±26.05 (9.31±2.61)
CSE36	2.51±0.01 (0.25±0.00)
CSE34	1.31±0.22 (0.13±0.02)
CSE26	1.30±0.52 (0.13±0.05)
CSE24	1.34±0.97 (0.13±0.10)
CSE16	1.29±0.44 (0.13±0.04)
CSE14	1.28±0.37 (0.13±0.04)

Values present the Mean±Standard Deviation (mg GAE/g) of three independent experiments, CRE: *Coffea canephora* bean supercritical raffinate hydrothermal extract, CWE: *Coffea canephora* bean hydrothermal extract, CEE: *Coffea canephora* bean ethanol extract and CSE: *Coffea canephora* bean CO_2 supercritical extract

Table 7: Characteristics of formulation with *Coffea canephora* bean extracts

Formulation	Mean size (nm)	Polydispersity index	Zeta potential (mV)	pH
Formulation 0	2327.67±296.31	1.74±0.60	-35.05±0.06	6.20±0
Formulation 1	484.00±6.00	0.22±0.05	-40.63±0.15	6.14±0.01

Values present the Mean±Standard Deviation of three independent experiments, Formulation 0: CSE16 1% and CRE 1 in 50% ethanol and formulation 1: CSE16 1 and CRE 1% in the deformable liposome

formulations, however, PDI lower than 0.3 means the monodisperse systems[18]. Thus, the control solution was multi-disperse and the deformable liposome was monodisperse.

Permeation of formulations

Transdermal permeability: The transdermal permeation amount per unit area of each formulation was depicted in Fig. 3. The control solution did not penetrate enough to be detected until the collection after 24 hrs and the deformable liposome was also detected after 8 hrs. On the sample after 24 hrs, the accumulated permeation rate of the deformable liposome was 3.53±0.11%, about 15.35-fold higher than 0.23±0.06% of the control solution.

Epidermal permeability: The epidermal permeation amount per unit area of each formulation was described in Fig. 4. The deformable liposome permeated about 90% of the total permeation amount in the first 8 hrs. On samples after 24 hrs, the accumulated permeation rate of the deformable liposome was calculated to be 5.43±0.11%, about 2.18-fold higher than 2.49±0.26% of the control solution.

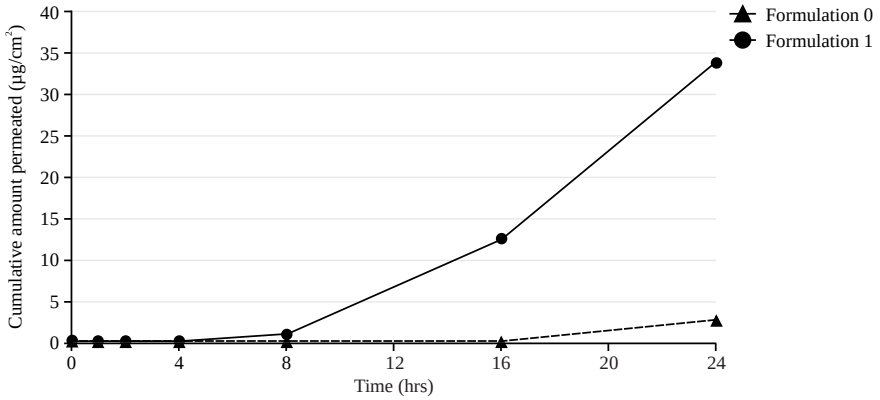

Fig. 3: Cumulative amount of full thickness skin permeated caffeine
Formulation 0: CSE16 1% and CRE 1% in 50% ethanol and formulation 1: CSE16 1 and CRE 1% in the deformable liposome

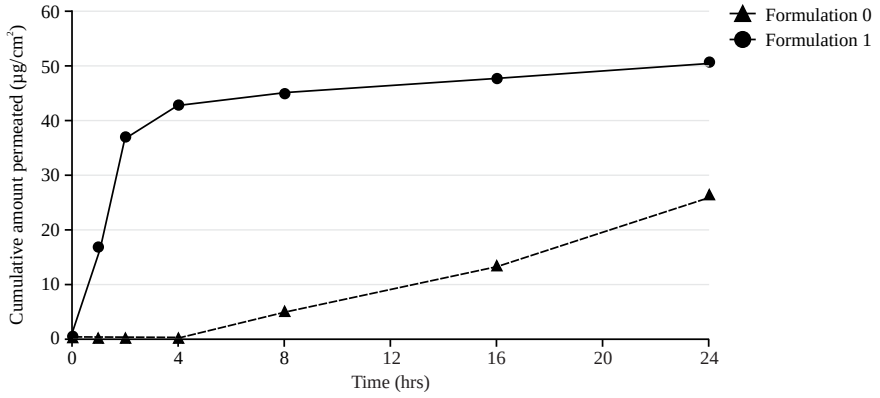

Fig. 4: Cumulative amount of epidermis permeated caffeine
Formulation 0: CSE16 1% and CRE 1% in 50% ethanol and formulation 1: CSE16 1 and CRE 1% in deformable liposome

Table 8: Permeability parameters of applying formulation with *Coffea canephora* bean extracts for 24 hrs

Formulation		Formulation 0	Formulation 1
Amount caffeine applied (µg/cm²)		543.65	480.06
Caffeine retention (µg/cm²) [%]	Dermis	24.54±2.24	18.26±1.43
		[4.51±0.41]	[3.80±0.30]
	Receptor liquid	2.55±0.63	33.91±1.03
		[0.47±0.12]	[7.06±0.22]
Total permeated (%)		27.09±2.87	52.17±2.46
		[4.98±0.53]	[10.87±0.51]
Flu×(µg/cm²/h)		0.11	1.41
[µg/cm²/min]		[1.83×10^{-3}]	[2.35×10^{-2}]
Kp (cm/h)		0.97×10^{-4}	1.47×10^{-3}
[cm/min]		[1.68×10^{-6}]	[2.45×10^{-5}]

Values present the Mean±Standard Deviation of three independent experiments, Total permeated: Retention of caffeine permeated through the epidermis, Flux: (Retention of caffeine permeated through full thickness skin)/24 hrs, Kp: Permeability coefficient, Formulation 0: CSE16 1% and CRE 1% in 50% ethanol and formulation 1: CSE16 1% and CRE 1% in the deformable liposome

Permeability parameters: The total permeated caffeine of the control solution was 4.98±0.53% and there was 0.47±0.12% in the receptor liquid. When applying the control solution, most of the caffeine permeated did not penetrate the dermis. However, applying the deformable liposome, about 65% of the whole amount permeated and penetrated the dermis. The deformable liposome was 13-fold more than the control solution for flux. The Kp values represent that the deformable liposome penetrated more than 15-fold faster than the control solution (Table 8).

DISCUSSION

The HPLC analysis confirmed that the optimal extraction method of caffeine is the supercritical CO_2 extraction at 150 bar and 60 and the optimal extraction method of chlorogenic acid is the hydrothermal extraction of supercritical raffinate. It is explained by the correlation between a solvent and an active ingredient. In the molecular structure of caffeine, three Methyl groups (-CH_3) are attached to xanthine. As can be predicted from the molecular structure, caffeine tends to dissolve easily in non-polar solvents[19,20]. In supercritical CO_2 extraction, active ingredients tend to be selectively extracted depending on temperature and pressure conditions. It explains a large amount of caffeine was extracted especially at 150 bar and 60°C[20]. On the other hand, the molecular structure of chlorogenic acid has many hydrophilic groups. Hence, chlorogenic acid is more soluble in polar solvents so a great quantity of chlorogenic acid can be extracted from hydrothermal extraction[21-23].

The extraction of *Coffea canephora* supercritical raffinate is a method that has never been investigated before. This is similar to the de-caffeination process. After going through this process several times, caffeine can be almost completely removed[24,25]. Since supercritical CO_2 extraction is performed at a low temperature, it is expected that chlorogenic acid would remain in the raffinate without being destroyed. Since it is easy to separate raffinate in supercritical CO_2 extraction, chlorogenic acid high-content extracts could be obtained through hydrothermal extraction from the raffinate from which caffeine has been completely removed.

According to the HPLC analysis, 15.00±0.16% of CRE was chlorogenic acid. In the total polyphenol content quantification, 40.67±1.39% of CRE was composed of polyphenols. It indicates that 25% of CRE was polyphenol, apart from chlorogenic acid. In the DPPH radical scavenging assay and the ABTS+ radical scavenging assay, it was proved that the more the extracts contained chlorogenic acid and polyphenol the more oxidation was suppressed. However, the tendency among supercritical extracts was not completely consistent, which resulted from the small polyphenol contents and differences within the error range.

The deformable liposome manufactured in this study is fully expected to be a functional material for cellulite treatment or hair-loss relief, containing high amounts of caffeine and chlorogenic acid by combining CRE and CSE16. Cellulite treatment mechanisms include improving blood flow, inhibiting body fat synthesis, promoting fat decomposition, reconstructing the structure of the dermis and subcutaneous fat layer and preventing the generation of free radicals[2,26]. The deformable liposome is supposed to be an excellent solution to cellulite as it can enhance blood flow, inhibit body fat synthesis with caffeine and prevent the generation of free radicals with chlorogenic acid. Concurrently, caffeine is known to improve hair-loss by inhibiting 5α-reductase activation, enhancing blood circulation and increasing nutrient delivery[27,28]. On the other hand, it has been reported that oxidative stress causes aging in the scalp and hair cells resulting in hair-loss[29-31]. Although, there are no direct studies on chlorogenic acid, its antioxidant effect may prevent aging and create synergy in hair growth.

Given the mechanisms, it is considered ideal that the skin delivery of *Coffea canephora* extracts proceeds more than the dermis. The formulation was prepared as the deformable liposome so that they sufficiently penetrated the transdermal. This improved flux for the transdermal permeation by 13-fold. In addition, the Kp increased from 1.68×10^{6} to 2.45×10^{5} cm/min. Under Marzulli's definition, the permeation speed of the control solution was moderate, ranging from 10^{6}~10^{5} cm/min section[32]. In contrast, the deformable liposome was fast speed, belonging to 10^{5}~10^{4} cm/min section. It is of great significance that the deformable liposome not only has a transdermal permeation speed 15-fold higher than that of the control solution but also has a fast speed objectively. Besides, the result of the epidermal permeation experiment showed a tendency for the deformable liposome to penetrate the epidermis rapidly within the first 8 hrs. Therefore, it could permeate capably if it is applied to the human skin.

It is presumed that the decrease in particle size and the use of propylene glycol as an edge activator influenced the increase in transdermal permeation. The appropriate particle size for transdermal drug delivery is known to be 10~600 nm[18]. The average particle size of the deformable liposome was 484.00±6.00 nm, a 5-fold decrease in size could affect the transdermal permeation. Additionally, propylene glycol's ability to give flexibility to liposomes as edge activators and its properties to promote transdermal penetration of active ingredients might have affected the transdermal permeation improvement[12,13,33]. Further research was needed to determine the direct relationship between propylene glycol and permeability by comparing the permeability of conventional liposomes and deformable liposomes using different edge activators.

CONCLUSION

The HPLC analysis revealed that CSE 16 contained abundant caffeine (22.00±0.26%), while CRE had plentiful chlorogenic acid (15.00±0.16%) and polyphenol (406.72±13.90 mg GAE/g). Antioxidant capacity tests ranked

CRE>CWE>CEE>CSEs. Consequently, CRE was chosen for its antioxidant capacity, while CSE 16 was selected for its caffeine content. Deformable liposomes incorporating these extracts demonstrated enhanced stability, reduced particle and increased transdermal permeability. The liposomes, rich in caffeine and chlorogenic acid, show potential for alleviating cellulite and hair loss, with propylene glycol as an effective edge activator. This study suggests the utility of *Coffea canephora* bean extracts as transdermal permeable functional materials.

SIGNIFICANCE STATEMENT

Green coffee bean has a lot of caffeine and antioxidants. It is known to help fat loss and hair growth. In this study, the maximum amount of caffeine was extracted using supercritical CO_2 extraction. The best extraction process was of antioxidants by using remainders. Extracts usually do not dissolve easily and neither do these extracts. So, we solved this problem with a flexible drug carrier. It helped the formulation to be better absorbed into the skin. The data can help develop a new extraction process for green coffee beans and make effective functional cosmetics.

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