Comparison of the Antioxidant and Transmembrane Permeative Activities of the Different *Polygonum cuspidatum* Extracts in Phospholipid-Based Microemulsions

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ABSTRACT: The main purpose of this study was to investigate the transmembrane permeability of polyphenol-containing *Polygonum cuspidatum* extracts (PCE) encapsulated in phospholipid-based o/w microemulsion system. First, preparations of several PCEs using solid- or liquid-phase extraction or a combination of both, as well as evaluation of their antioxidant activities, were conducted and compared. In the antioxidant study, results indicated that PC-1 with the least extraction process exhibited the best antioxidant activity. By comparing the permeability coefficient (K_p) among all tested PCEs in microemulsions (ME-PCs), ME-PC1 also possessed the largest permeability coefficients of both resveratrol and emodin. In addition, comparison of the transmembrane permeability of several polyphenol-encapsulated microemulsions showed that resveratrol had the most competitive advantage in the microemulsion formula for the control-release process. Taken together, it can be concluded that the matrix removed from the solid-phase extraction in PC-1 not only possesses antioxidant activity but also acts as an enhancer in transmembrane permeation. The structure specificity of the polyphenol plays important roles in the mechanism of the transmembrane permeation process. These findings might provide scientific evidence for the value of developing polyphenol-containing PCEs as nutraceuticals and cosmoceutical products.

KEYWORDS: *Polygonum cuspidatum* extract, microemulsion, polyphenols, transmembrane permeation, encapsulation, extraction

■ INTRODUCTION

Polygonum cuspidatum Sieb et Zucc. (Hu Zhang) (PC) has been used as an analgesic, antipyretic, diuretic, and an expectorant in traditional Chinese medicine.[1] The major components in PC, including several stilbenes and anthraquinones as well as their glycosylated derivatives, contribute to the pharmacological basis for the treatment of various inflammation-related diseases.[2] The PC extract has received interest as an ingredient in nutraceutical formulations due to the high content of 3,4′,5-trihydroxystilbene.[3] Several in vitro and in vivo studies have indicated the multiple health beneficial effects of resveratrol, including antioxidant, anti-inflammatory, antiaging, antidiabetic, cardioprotective, and neuroprotective activities.[4] The mechanisms of action of its chemopreventive and life-extending activities have been extensively studied and reviewed.[4,5] Emodin, a major anthraquinone in PC, is a potent tyrosinase inhibitor for the application of PC as a skin-whitening cosmoceutical product.[6] It has been reported that emodin possesses chemopreventive, antitumor, and estrogenic activities.[7,8] In spite of the pleiotropic pharmacological activities of resveratrol and emodin in PC, like other polyphenolic compounds, the bioavailability is low, as indicated by pharmacokinetic studies.[9,10]

Resveratrol and emodin, which possess a polyphenolic structure similar to curcumin, are water insoluble that might impede their application in the field of nutraceuticals and functional foods. There has been growing interest in the advantages of microemulsions for solving the problems of solubility as well as stability of nutraceuticals and food additives in aqueous solutions.

Flavonoids, including quercetin and hesperidin, have been reported to be encapsulated in a microemulsion system for cosmoceutical application.[11,12] In addition, several other strategies have been developed to increase the bioavailability of polyphenolic phytochemicals, including curcumin by using nanoparticles, liposomes, and micelles[13] and isoflavones by using β-cyclodextrin and a self-emulsifying system.[14,15] Microemulsions are self-assembled mixtures of water, oil, and surfactants and have the advantages of being optically isotropic and thermodynamically stable. Microemulsions have attracted much interest in recent years as potential drug delivery systems because of their transparency, ease of preparation, and long-term stability.[16] Much attention has been given to the utilization of phospholipids in formulating pharmaceutically acceptable microemulsions.[17,18] The techniques and applications of nanoparticles in nutraceutical delivery systems have been discussed and reviewed intensely over the past few years.[19,20] Studies of food microemulsions have focused on using food-grade, nonionic surfactants derived from natural products.[21] The solubilization of lycopene and lutein derivatives in aqueous media was successfully improved by Amar et al. using microemulsion technology.[22] Another study investigated the construction of lecithin-based microemulsions with a wide range of food-acceptable surfactants in food application.[23]

Table 1. Extraction Procedures and Yields for the Preparation of Different PC Extracts

PC extract	extraction procedure	PC extraction yield[d] (%)
PC-1	raw material → MeOH extraction	17.5
PC-2	raw material → reflux ext. with MeOH	19.2
PC-3	PC-1 → SPE[a] (silica)[b]	17.9
PC-4	PC-1 → SPE (XAD-4)[c]	13.1
PC-5	PC-1 → SPE (XAD-4)[c] → SPE[a] (silica)[b]	2.6

[a] SPE, solid-phase extraction. [b] Column was washed with *n*-hexane, and ethyl acetate was used as an eluent for collection. [c] Column was washed with H_2O and then 1:1 of H_2O/MeOH; MeOH was used as an eluent for collection. [d] The PC extraction yield was calculated from each defined extraction procedure.

Recent studies have indicated that phosphatidylcholine embedded microemulsion systems improve transmembrane bioavailability in both rat skins and Caco2 cells.[24,25] In our previously published studies, a polyphenols-encapsulated O/W microemulsion system using food-acceptable components, lecithin, and Tween 80 as the surfactants and ethyl oleate (EO) as the oil phase was successfully constructed for the improvement of the transmembrane bioavailability of curcumin and isoflavonoids-containing red clover.[26,27]

Most commercially available raw herbal extracts, with no further purification, have limited potential for innovation and patent protection in the development of related products. In addition, no matter what route they take, through either oral or transdermal application, the bioavailability of raw herbal extracts is poor, and unavailable bioactive ingredients are unable to reach the target tissues. The main purpose of this study was to further investigate the transmembrane permeability of polyphenols-containing PC extracts encapsulated in the previously established microemulsion system. To enhance the contents of the polyphenols in the extracts, the preparation of several PC extracts using solid- or liquid-phase extraction or a combination of both was conducted and compared. The antioxidant activities and the contents of the polyphenols of the PC extracts were also examined. Meanwhile, by utilizing biocompatible materials, including EO as the oil phase and lecithin and Tween 80 as surfactants, several polyphenols with similar structural figures were also encapsulated in a stable microemulsion system for comparison of their transmembrane efficiency with various *P. cuspidatum* extracts (PCE)-encapsulated microemulsions. These studies might provide scientific evidence for the value of developing polyphenols-containing PC extracts as nutraceuticals and cosmoceutical products.

■ MATERIALS AND METHODS

Materials and Chemicals. Lecithin (L-α-phosphatidylcholine, purity > 60%), Tween 80, EO, the reagents for antioxidant assays, including Folin–Ciocalteu reagent, 2,2-di(4-*tert*-octylphenyl)-1-picrylhydrazyl, free radical (DPPH), and horseradish peroxidase, and all of the solvents for extraction and liquid chromatography were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). Silica gel (70–230 mesh) from Silicycle and polymeric resins (Amberlite XAD) from Supelco were used for solid extractions. The purified phytochemicals in this study were obtained from Sigma-Aldrich Chemical Co. *P. cuspidatum* was obtained from a local herbal market. Deionized water (DI water) with a resistivity above 17 MΩ cm was used for preparing microemulsions.

Procedures for the Preparation of PCEs. The dried PC roots were ground into powders and strained through a 250 μm sieve (mesh no. 60 base on ASTM classifications) to afford a PC fine powder. The PC powder was further extracted using a series of extraction methods and steps, as shown in Table 1. One hundred grams of PC powder was extracted with 1 L of methanol by shaking at room temperature for 3 days. PC-1 extract was obtained after filtration and evaporation under reduced pressure. PC-2 was prepared by extraction of PC powder with a 10-fold volume of methanol under reflux for 4 h. PC-1 was further chromatographed on 20-fold of silica gel (70–230 mesh). The column was washed with hexane and then eluted with ethyl acetate. The eluent was collected until no more bright spots were detected under UV 254 nm upon thin-layer chromatography analysis. The eluent was evaporated under reduced pressure to afford PC-3. For preparing the PC-4, PC-1 was chromatographed on 20-fold of Amberlite XAD-4 polyremic resin. The column was washed with water and 50% methanol sequentially until no more color materials eluted. The polyphenols-containing fraction was then eluted with a 2–3 bed volume of methanol and then ethyl acetate to afford PC-4.

Determination of Total Polyphenols. The total polyphenols content was determined by Folin–Ciocalteu reagent. The standard curve was prepared using a series of dilutions of gallic acid in EtOH: water (10:90, v/v). The PC extracts were diluted to different concentrations and mixed with Folin–Ciocalteu reagent (1 mL:1 mL) and aqueous Na_2CO_3 (2 mL, 10%). The mixture was allowed to stand for 15 min, and the total polyphenols were determined by measurement of absorbance at 730 nm. The total polyphenols content is expressed in terms of gallic acid equivalent (GAE; mg/g of PC extract).

Determination of Antioxidative Activities of PCEs by the Scavenging Effect on DPPH Radicals. A 0.9 mL amount of 0.1 mM ethanolic DPPH solution was incubated with 0.1 mL of each serial diluted PCE in ethanol. The reaction was kept in the dark for 30 min. The absorption of each at 517 nm was determined by a spectrophotometric reader against the blank solution without DPPH. The percentage of inhibition = [1 − (absorption of sample + DPPH − blank)/(absorption of DPPH only − blank)] × 100. The data represent the mean ± standard error (SE) from the triplicate determinations of each sample.

Determination of Antioxidative Activities of PCEs by Hydrogen Peroxide Reduction. The assay is based on the horseradish peroxidase (HRPO)-mediated oxidation of phenol red by H_2O_2, which results in the formation of a compound showing increased absorbance at 610 nm with a pH of 12.5. A reaction mixture, containing 0.88 mL of phenol red solution (0.28 mM phenol red in PBS), 0.1 mL of serially diluted PC extract and 0.01 mL of 1 mM H_2O_2 were mixed and allowed to stand for 5 min. A 0.01 mL amount of 1 mg/mL HRPO was then added to the reaction mixture, and 0.01 mL of 1 N NaOH was added after 5 min of incubation. The absorption was measured at 610 nm to determine the reduction of hydrogen peroxide free radicals. The percentage of reduction = [1 − (absorption of reaction mixture − blank)/(absorption of reaction mixture without PC extract − blank)] × 100. The data represent the mean ± SE from the triplicate determinations of each sample.

Preparation of PCE-Encapsulated Microemulsions. On the basis of the previously published result, a ternary phase diagram of phospholipid-based microemulsion was constructed where the microemulsion region was identified by the clear and transparent appearance of the solution.[18] In our previously established system for the construction of microemulsion, it was indicated that an O/W microemulsion system using lecithin/Tween 80 (molar ratio of 0.3) as the surfactant and EO as the oil phase at a ratio of 2.5:12.5:85 (oil:surfactant:water) was the most suitable formula for the encapsulation of curcumin and isoflavonoids. The mixture was prepared by adding appropriate amounts of oil, lecithin, and Tween 80 as surfactants and PCEs in a test tube and

was kept at 50 °C and well mixed using a vortex mixer. Water was then added to the mixture in a bath sonicator at 50 °C until a transparent and isotropic microemulsion was obtained. In this study, 10 mg of PCE or 1 mg of purified polyphenol was added to a 10 g microemulsion formulated as described above. The resultant microemulsion was passed through a 0.45 μm filter to remove excess solid and then subjected to high-pressure liquid chromatography (HPLC) to analyze the loading capacity. The separation was performed on a Cosmosil 5C 18-MS column (5 μm, 25 cm × 4.6 mm i.d., Nacalai Tesque, Kyoto, Japan). The sample (20 μL) was eluted with a mobile phase composed of 0.1% H_3PO_4 (A) and acetonitrile (B) and a gradient profile as follows: 0– 5 min, from 90% A, 10% B to 70% A, 30% B; 5–20 min, from 70% A, 30% B to 60% A, 40% B; 20–40 min, from 60% A, 40% B to 10% A, 90% B; 40–45 min, from 10% A, 90% B to 5% A, 95% B. The flow rate and detection wavelength were set to be 1.0 mL/min and 280 nm, respectively. The standard curves of resveratrol and emodin in 50% ethanol ranging from 0.1 to 0.001 mg/mL were used for calculation.

Measurement of the Diameter Distributions of PCEs-Encapsulated Microemulsions. The diameter distributions of various PCE-encapsulated microemulsions in different formulations were measured by a 90Plus particle sizer (Brookhaven Instruments Co., New York), equipped with a 532 nm laser light source. The 90Plus particle sizer based on the principles of dynamic light scattering (DLS), the collection times for the autocorrelation function were 1–3 min at the 90° scattering angle. Diameter distributions were calculated using autocorrelation data analysis by using statistical analysis.

Skin Permeation of PCEs-Encapsulated Microemulsions. The permeabilities of resveratrol and emodin through the BALB/c mouse skin were investigated using Franz diffusion cells with an effective diffusional area of 0.785 cm^2. The hair of the mice was removed. The skins were excised and then clamped between the donor and the receptor chamber with 5.6 mL of cell volume. The receptor chamber was filled with 50% ethanol to ensure sink conditions. The receptor chamber was thermostatted at 37 °C, and the solution was stirred continuously at 300 rpm. One milliliter of formulation was pipetted into each donor compartment and sealed with paraffin to prevent evaporation. At time intervals of 2, 4, and 6 h, 500 μL of the receptor medium was taken to determine the permeated amount of resveratrol and emodin using HPLC analysis at 280 nm as described previously. The cumulative amount of the two major components in the PC extracts permeated through mouse skins was plotted as a function of time. The permeation rate of polyphenol (J_s) through mouse skin was calculated from the slope of the linear portion of the cumulative amount per unit area versus time plot (μg/cm^2 per h). The permeability coefficient K_p ($\times 10^{-3}$ cm/h) was calculated from the equation, $K_p = J_s/C_0$, where C_0 represents the concentration of polyphenol in the microemulsion solution. The statistical differences of K_p between the purified polyphenol and the various PCE-encapsulated microemulsions were analyzed by one-way analysis of variance (ANOVA), followed by Tukey's range test for multiple comparison.

RESULTS AND DISCUSSION

Comparison of the Contents of Major Bioactive Components, Polyphenols, and Antioxidant Activity of the Different PC Extracts. To understand the effects of different extraction techniques on the contents and bioactivity of the PC extracts, liquid extraction with methanol followed by solid-phase extraction, including using silica gel (PC-3) and polymeric resins (Amberlite XAD) (PC-4) as adsorption materials or a combination of both (PC-5), were conducted and compared. The nonionic polymeric resins XAD have been widely used for the recovery of flavonoids from plant extracts or the elimination of water-soluble contaminants in the past few years.[28] The

Table 2. Comparison of the Contents of the Two Major Components in the Different PC Extracts[a]

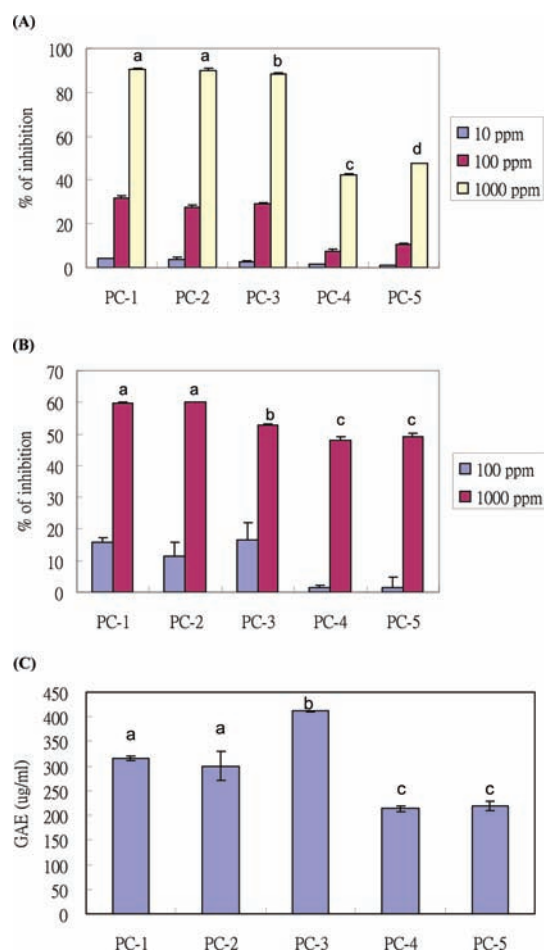|	% of major components	
PC extract	resveratrol	emodin
PC-1	6.1 ± 0.1	8.0 ± 0.7
PC-2	6.5 ± 0.1	8.6 ± 2.7
PC-3	22.2 ± 0.7	30.6 ± 6.2
PC-4	17.4 ± 1.0	21.7 ± 9.5
PC-5	33.7 ± 0.5	40.1 ± 1.1

[a] Data are represented by the mean ± SD (n = 3).

Figure 1. Comparison of the antioxidant activities and the contents of polyphenols in different PC extracts: (A) DPPH scavenging assay, (B) H_2O_2 reduction assay, and (C) gallic acid equivalence. Data are represented by the mean ± SD (n = 3). Means not sharing a common letter were significantly different ($P < 0.05$) when analyzed by ANOVA and Tukey's test.

preparation procedures vs extraction yields and the contents of the two major components of the five PC extracts are shown in Tables 1 and 2. First, the PC powders were extracted with methanol at room temperature or under reflux to afford PC-1 and PC-2, respectively. No significant differences in extraction yields and the contents of major components were observed by comparing the two processes. Further solid-phase extraction from PC-1 resulted in remarkable increases in the two major
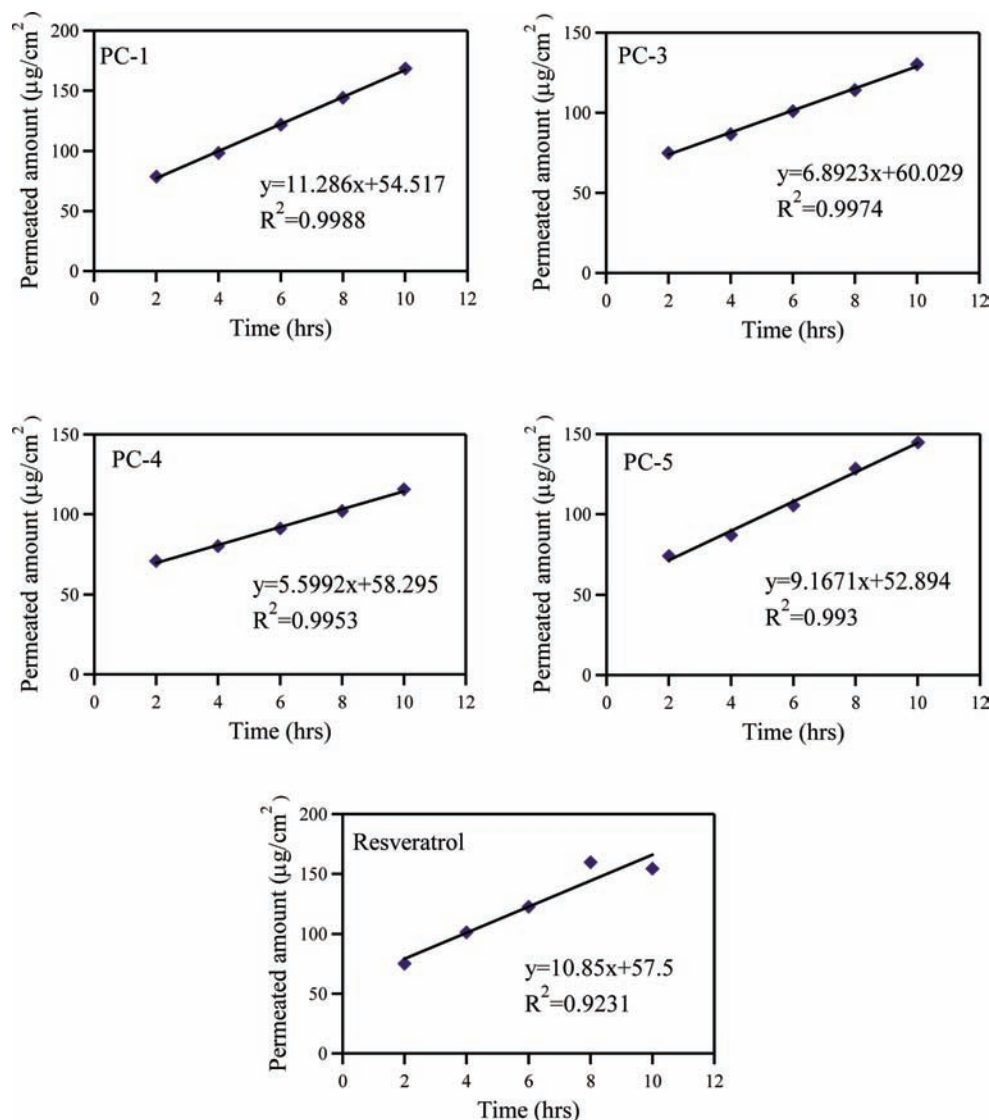
Figure 2. Linear regression of the permeated amount of resveratrol vs time in the in vitro mouse skin permeation experiment.

components. The results indicated that the contents of the bioactive components, resveratrol and emodin, in PC-3, PC-4, and PC-5 were enhanced by 3—5-fold after further treatment with column chromatography. However, in the measurement of the total polyphenols of the different PC extracts, the order of the calculated GAE among PCEs was PC-3 > PC-1, PC-2 > PC-5, PC-4 (Figure 1C). It is interesting to find that PC-1—PC-3 extracts possessed more polyphenols than PC-4 and PC-5 extracts. In particular, the content in PC-3 was the most abundant among all of the PC extracts. In the antioxidant study, both of the scavenging effect on DPPH radicals (lipophilic assay) and reduction of hydrogen peroxide free radicals (hydrophilic assay) were examined for the sake of comparison (Figure 1A,B). From the comparison of the percentage of inhibition at 1000 ppm of PCEs, the result indicated that PC-3 extract showed better antioxidant activity than PC-4 and PC-5 extracts, and PC-1 and PC-2 with less extraction process exhibited the best antioxidant effects. The order of the relative activity among PC extracts is PC-1, PC-2 > PC-3 > PC-5 > PC-4. The result also implies that the water-soluble fraction was removed by the solid-phase extraction using XAD, including those of the glycosylated

derivatives contributed partially to the antioxidant activity of the PC extracts.

In Vitro Skin Permeation Studies of PCEs-Encapsulated Microemulsion. In our previously established system for the construction of microemulsion, it was indicated that an O/W microemulsion system using lecithin/Tween 80 (molar ratio of 0.3) as the surfactants and EO as the oil phase at the ratio of 2.5:12.5:85 (oil:surfactant:water) was the most suitable formula for the encapsulation of curcumin and isoflavonoids.[26] The microemulsion remained transparent with an average diameter size of 60—80 nm during 2 weeks of storage. Therefore, a further investigation for encapsulation of other polyphenols and evaluation of their transmembrane permeation was conducted.

In our study, the microemulsion formulations (ME-PCs) prepared from loading approximately 10 mg of PC extract into the previously described formula were tested for their transmembrane permeation into the mouse skin. Because of the poor solubility of polyphenols in water, 50% ethanol was used as the donor medium to provide the sink condition. The amounts of resveratrol and emodin permeated were determined at 2 h time intervals for the five formulations. Next, the transmembrane rates
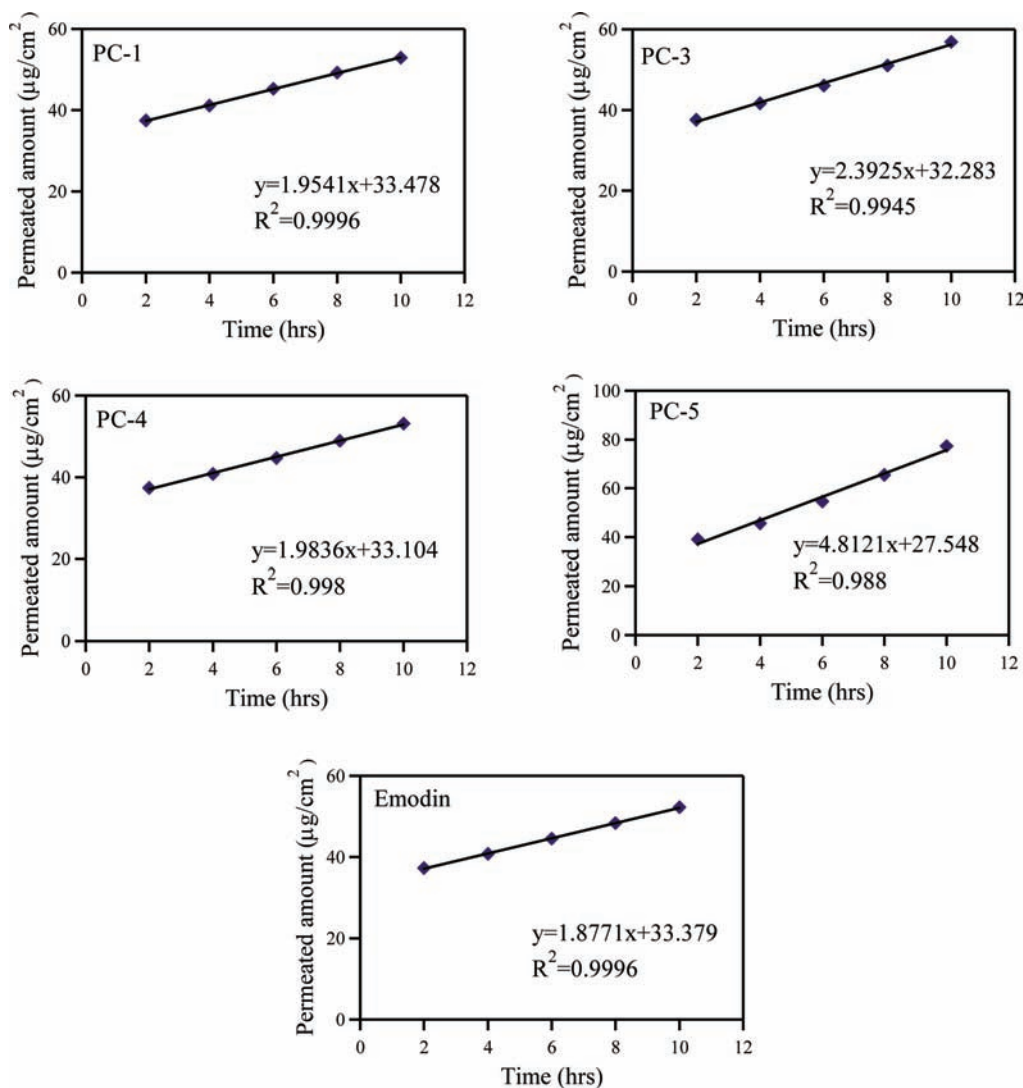
Figure 3. Linear regression of the permeated amount of emodin vs time in the in vitro mouse skin permeation experiment.

Table 3. Diameter Sizes and in Vitro Skin Permeation Parameters of the Different PCE-Encapsulated Microemulsions[a]

vehicles	diameter (nm)	K_p resveratrol ($\times 10^{-3}$ cm/h)	K_p emodin ($\times 10^{-3}$ cm/h)
ME-PC1	81.9 ± 1.5	62.1 ± 2.6 a	12.9 ± 0.2 a
ME-PC3	154.1 ± 0.4	17.7 ± 0.4 b	6.5 ± 0.1 b
ME-PC4	117.0 ± 1.4	19.1 ± 0.4 c	5.7 ± 0.1 c
ME-PC5	68.9 ± 1.1	10.7 ± 0.3 d	5.9 ± 0.2 c
ME-Res	67.6 ± 1.3	34.6 ± 1.0 e	
ME-Emo	60.9 ± 1.1		7.3 ± 0.1 d

[a] Data are represented by the mean $\pm$ SD ($n = 3 - 6$). The statistical differences of K_p between the purified polyphenol and the various PCE-encapsulated microemulsions were analyzed by ANOVA and Tukey's test. Means not sharing a common letter were significantly different ($P < 0.05$). Res, resveratrol; Emo, emodin.

of the two components in each ME-PC were examined by calculating the slope of the linear portion of the cumulative amount versus time (Figures 2 and 3). To examine the effect of matrix materials on the release of resveratrol and emodin from the microemulsion system among the ME-PCs, the permeability coefficient K_p was further calculated, and the result is shown in Table 3. The averaged diameters for ME-PCs were also measured and compared. The results indicated that the particle sizes of ME-PCs were distributed within the range of 50—150 nm. Among these, ME-PC5, the extract that was obtained from two-step extraction process, possesses the smallest averaged diameter (approximately 70 nm). In the case of comparing the transmembrane permeability, it is surprising to find that ME-PC1 possessed the largest permeability coefficients for both resveratrol and emodin ($K_{p\ resveratrol} = 62.1 \pm 2.6$; $K_{p\ emodin} = 12.9 \pm 0.2$), even better than the pure components ME-Res (34.6 ± 1.0), ME-Emo (7.3 ± 0.1), or further purified extract ME-PC5 ($K_{p\ resveratrol} = 10.7 \pm 0.3$; $K_{p\ emodin} = 5.9 \pm 0.2$). However, ME-PC3, PC4, and PC5 exhibited poor transmembrane permeabilities, as compared to those of the purified components. In comparison with the results from the transdermal permeation study, it is concluded that the particle sizes of ME-PCs are not the factor that contributes to transmembrane permeability through mouse skin, as indicated by the result showing that ME-PCs
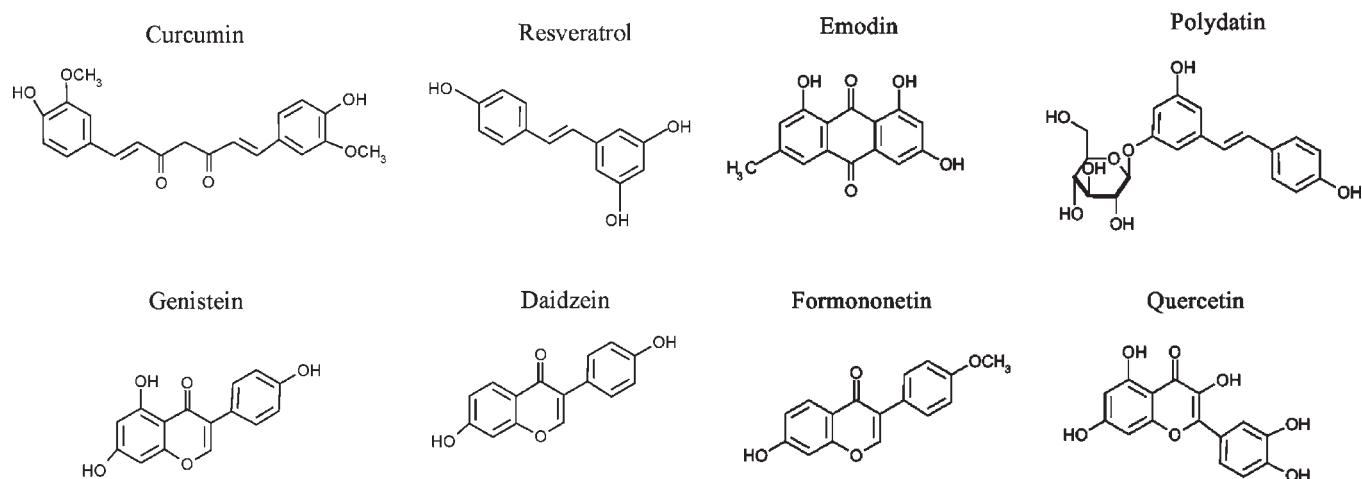
Figure 4. Structures of several polyphenols used in this study.

Table 4. In Vitro Skin Permeation Parameters (K_p) of Various Polyphenol-Encapsulated Microemulsions[a]

vehicles	K_p ($\times 10^{-3}$ cm/h)	vehicles	K_p ($\times 10^{-3}$ cm/h)
ME-Cur	2.5 ± 0.3	ME-Res	34.6 ± 1.0
ME-Gen	1.9 ± 0.4	ME-Emo	7.3 ± 0.1
ME-Dai	13.1 ± 5.2	ME-Pol	ND
ME-For	5.1 ± 1.1	ME-Que	14.0 ± 2.6

[a] Data are represented by the mean ± SD ($n = 3 - 6$). Cur, curcumin; Gen, genistein; Dai, daidzein; For, formononetin; Res, resveratrol; Emo, emodin; Pol, polydatin; and Que, quercetin.

possess mean diameters within the range of 50−150 nm. Several studies on the transdermal mechanism have concluded that there are three key factors that may contribute to the enhancement of skin permeation, that is, the mobility of the bioactive ingredient in the designed formulation, the concentration gradient, and the particle diameter.[29−32] The choice of oil components, surfactant/cosurfactant in the formulation should influence the ease of releasing the ingredient across the barrier. The matrix resulted from different solid-phase extractions performed differentially, indicating the potential role of the matrix as a barrier or enhancer in transmembrane studies. In addition, another relevant study indicated that reducing agents, that is, ascorbate, were found to enhance the penetration of diclofenac in rat skin.[33] Thus, it might explain that the high antioxidant activity of PC-1 might lead to a greater permeability in ME-PC1.

Comparison of the Transmembrane Permeability of Several Polyphenol-Encapsulated Microemulsions. We further prepared the other poorly dissolved polyphenols encapsulated in the established microemulsion system. The structures of these polyphenols are shown in Figure 4. By comparing the permeability coefficient (K_p) of these polyphenols-encapsulated microemulsions, the order of the K_p is ME-Res (34.6) > ME-Que (14.0) > ME-Dai (13.1) > ME-Emo (7.3) > ME-For (5.1) > ME-Cur (2.53) > ME-Gen (1.9) > ME-Pol (ND) (Table 4). It can be concluded that (1) the structure of resveratrol exhibited the most competitive advantage in the microemulsion formula for the control-release process. On the other hand, the polydatin, the glucoside of resveratrol, is not capable of forming a transpermeable microemulsion in our system, as indicated by the nondetected

permeation. (2) It is surprising to find that there is a great discrepancy among the flavonoids, especially for the results from isoflavonoids, daidzein, formononetin, and genistein. It is speculated that the polyphenols, that is, curcumin, genistein, emodin, and formononetin, bearing methoxyl or possessing intramolecular hydrogen-bonding and not exposing more hydroxyl functional groups impede their transmembrane permeation. A recent study by Hathout et al. used laser confocal scanning microscopy and the spectroscopic method to examine the penetration mechanism by measuring dermatopharmacokinetic parameters of a microemulsion system consisting of oleic acid, Tween 20, and Transcutol.[34] Their study proved the breakage of the microemulsion during the penetration process and further proposed a molecular mechanism by the preferential hydrogen bonding of oxygen-containing enhancers with ceramide head groups to break the hydrogen-bonding network of lipid bilayers. Our results also showed that the available polyphenolic hydroxyl groups are relevant to the transmembrane permeation, and the structure specificity of the polyphenols plays an important role in the mechanism of the transmembrane permeation process. This is the first report on the effect of the structural specificity of polyphenols on the control of release in a microemulsion system.

In summary, the investigation of the antioxidant and transmembrane permeative activities of the different PCEs in the previously established phospholipid-based microemulsions was conducted and compared. In the antioxidant study, the result indicated that PC-1 with the least extraction process exhibited the best antioxidant activity. By comparing the permeability coefficient (K_p) among all of the tested PC extracts, ME-PC1 also possessed the largest permeability coefficients for both resveratrol and emodin. In addition, resveratrol exhibited the most competitive advantage in the microemulsion formula for the control-release process by comparison of the transmembrane permeability of several polyphenols-encapsulated microemulsions. Taken together, it can be concluded that the matrix, which is not removed from solid-phase extraction in PC-1 extract, not only possesses antioxidant activity but also acts as an enhancer in transmembrane permeation. The structure specificity of the polyphenols plays an important role in the mechanism of the transmembrane permeation process.

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9141

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