Original Article

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Formulation of Gel Containing Phenylbutenoid Extract for Pain Relief

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Abstract:

Objective: A phenylbutenoid extract (PE) was obtained from *Zingiber cassumunar* rhizomes. Phenylbutenoids; namely DMPBD, compound D, and compound D acetate, have been identified as major anti-inflammatory and analgesic constituents. This present study aimed to formulate a gel containing PE that could be used as an alternative ultrasound gel for acute or chronic inflammatory treatment.

Material and Methods: Gel formulations containing 0.5, 1, and 2% w/w PE were prepared using Carbopol 934 and hydroxyethylcellulose (HEC 4,000) as gelling agents. The contents of phenylbutenoids were quantified by a high-performance liquid chromatography (HPLC). PE gels were studied on physicochemical properties and accelerated stability tests. The PE gels, F2 and F5, were used to evaluate the release of phenylbutenoids using a modified Franz diffusion cell. In the skin permeation study, the 2% PE gels were applied either with or without a 0.8 w/cm^2 intensity ultrasound for 2, 5, and 10 min.

Results: Based on physicochemical properties and accelerated stability tests, F2 and F5 formulations showed good stability. The release kinetics of 0.5% and 1% and 2% w/w PE of both formulations were best fit to Higuchi's model and zero-order model, respectively. In the skin permeation study, PE gel combined with ultrasound application for 2 min exhibited higher phenylbutenoids in the skin and also a shorter lag time than PE gel application alone.

Conclusion: The gel containing 2% w/w phenylbutenoid extract was suggested as an alternative ultrasound gel containing an anti-inflammatory agent for the treatment of musculoskeletal disorders in phonophoresis.

Keywords: formulation, kinetics, permeability, phenylbutenoid, skin, transdermal

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Introduction

Musculoskeletal diseases are injuries or disorders of the muscles, nerves, tendons, joints, and cartilage. Globally, these diseases were one of the most prevalent diseases in 2019, with approximately 1.71 billion cases[1]. Ultrasound is widely used for therapeutic purposes to improve connective tissue extensibility and pain relief in musculoskeletal injuries. Additionally, the movement of drug molecules through the skin, while being exposed to ultrasound, is known as phonophoresis. These facilitate the transfer of many drug molecules through the skin, to the points of inflammatory areas, and relieve muscle pain[2]. Therefore, an analgesic, or anti-inflammatory, drug could be added to ultrasound gel to improve treatment efficacy[3]; such as a diclofenac emulgel, which has been studied in combination with ultrasound therapy to reduce treatment costs for patients. Furthermore, some herbal medicines could be substituted for diclofenac in ultrasound therapy[4].

Zingiber cassumunar Roxb. (Zingiberaceae) rhizome has been traditionally used for topical application to treat muscle inflammation. It possessed various pharmacological effects; including anti-inflammatory, antipyretic, analgesic, antimicrobial, and antioxidant activities, with the major active compounds; namely: (*E*)-1-(3,4-dimethoxyphenyl) butadiene (DMPBD), (*E*)-4-(3,4-dimethoxyphenyl) but-3-en-1-ol (compound D), and (*E*)-4-(3,4-dimethoxyphenyl) but-3-en-1-yl acetate (compound D acetate), known as phenylbutenoids have been reported[5-9]. Therefore, these phenylbutenoids were used as marker compounds for this study.

This present study aimed to incorporate a phenylbutenoid extract (PE) into a suitable gel base that can act as an efficient ultrasound coupling agent, with enhanced permeation of phenylbutenoids. Additionally, it aimed to evaluate the effect of the formulations on ultrasound application time and permeability of phenylbutenoids through cellulose membranes and porcine skin. This was to demonstrate the advantages of a combined PE gel and ultrasound application on drug permeation.

Material and Methods

Materials

Compound D, compound D acetate, and DMPBD were purified using the method previously described[8]. Carbopol 934, hydroxyethylcellulose (HEC 4,000), propylene glycol, butylene glycol, polyethylene glycol 400 (PEG 400), and glycerin (pharmaceutical grade) were purchased from S. Tong Chemicals Co., Ltd. (Nonthaburi, Thailand). Methanol (HPLC grade) and acetic acid (analytical grade) were purchased from RCI Labscan Limited (Bangkok, Thailand). Purified water was obtained from a Milli-Q system (Bedford, MA, USA).

Fresh *Z. cassumunar* rhizomes were purchased from Hat-Yai market, Thailand, in October 2018. The voucher specimen (specimen No. SKP 206 26 03 01) was deposited at the Faculty of Pharmaceutical Science, Prince of Songkla University, Thailand. The rhizomes were washed and sliced into small pieces, then dried in a hot air oven at 60±2°C, for 48 h. The dried rhizomes were ground into powder and passed through a sieve (No. 45).

Methods

Preparation of PE

The dried powder (10 kg) was extracted with PEG 400 (28 kg) using a microwave extractor (Baan Innov Co, Ltd, Nakorn Si Thammarat, Thailand), at 4,000 Watts and 90±5°C, for 16 min. The extract was then filtered and standardized to contain total phenylbutenoids content of 1.42% w/w (0.28% w/w compound D, 0.21% w/w compound D acetate, and 0.93% w/w DMPBD) using an HPLC method.

Formulation of gel containing PE

Five gel formulations (Table 1) were prepared using PE and various humectant ingredients. Briefly; Carbopol

934 was added to purified water and stirred until swelling. The mixture was then placed on a hot plate (70°C) and mixed with HEC 4,000, then stirred until a gel solution was formed. A solution of PE, methylparaben, propylparaben, glycerin, PEG 400, and butylene glycol was prepared in propylene glycol. This was then added to the gel solution. Triethanolamine was added to adjust the pH (5.5–6.0): the volume was adjusted by adding purified water[10].

Evaluation of the phenylbutenoid gels

Physicochemical properties

The physical appearance, pH and viscosity of the formulations was evaluated. The pH of the test samples was determined using the potentiometric technique (ORION model 410, USA). The viscosity was measured using a viscometer with spindle TE. (Model DV–III Ultra, Brookfield, USA), at various speeds ranging from 0 to 10 rpm.

Analytical method

HPLC analysis was carried out using the Shimadzu LC–20A Series coupled to an SPD–M20A photodiode array detector and SIL–20AHT autosampler. The HPLC conditions composed of an ACE® C18–PFP column (5 µm, 4.6×150 mm i.d.) eluted with a solution of methanol and 2% acetic acid (55:45% v/v), at a flow rate of 1.0 mL/min. The analytes were detected at a wavelength of 254 nm. These HPLC conditions produced a good resolution of compound D, compound D acetate, and DMPBD; with retention times of 4.62±0.01, 12.64±0.04, and 22.03±0.08 min, respectively. A working solution of the standard phenylbutenoids was prepared at a concentration of 0.1 mg/mL in methanol and diluted to six concentrations in the ranges of 1.6–50.0 µg/mL, for the establishment of the calibration curves. The calibration curves produced good linearity having (r^2) values of ≥0.9997.

PE (0.1 g) was dissolved in methanol and adjusted to a volume of 5 mL. PE gel (1 g) was dissolved in methanol and its volume was adjusted to 10 mL. This was then vortexed for 5 min, sonicated for 15 min, and centrifuged at 4°C, 4,000 rpm for 30 min. Sample solutions were filtered through a 0.45 µm membrane filter, and subjected to quantitative HPLC analysis for phenylbutenoid content.

Stability study

PE gels (2% w/w PE) were stored in properly enclosed containers, protected from light, and the freeze–thaw stress stability test was performed by placing them at 4°C for 24 h and then at 45°C for 24 h, for 6 cycles[10]. Physical appearance, pH, viscosity, and the content of the phenylbutenoids were then evaluated. The criteria for consideration of their stability was when the remaining content of PE was over 90%, the gel appearance must be clear, and the viscosity of the gel should not increase. Formulations that were within these criteria, after the freeze–thaw stability test, were selected for further study.

In vitro release study

The PE gels, F2 and F5, containing 3 concentrations of PE (0.5%, 1%, and 2% w/w), were used to evaluate the release of phenylbutenoids using a modified Franz diffusion cell (Model Hanson 57–6M, California, USA). The PE gels (1 g) were applied on the synthetic cellulose acetate membrane (Spectra/Por 3, Rancho Dominguez, CA, USA) and placed on the receptor cell, of which the effective area for diffusion was 1.77 cm^2. The receptor compartment was filled with a 12 mL of isotonic phosphate buffer (PBS) pH 7.4 and propylene glycol (80:20% v/v) solution. The diffusion cell was thermoregulated with a water jacket at 37°C±0.5°C, and the receptor compartment was stirred using a magnetic stirrer. Samples (1 mL) were withdrawn at 0.5, 1, 2, 3, 4, 6, 8, and 12 h. After each sampling, an equal volume of fresh medium was immediately added to the receptor cell. The samples were analyzed for phenylbutenoid content using an HPLC method. The data were analyzed to determine the coefficient of determination (r^2) and release kinetics models[11].

In vitro skin permeation

The F2 gel, containing 2% w/w PE (1 g), was used to determine the effect of ultrasound application times. The experiments were carried out on a modified Franz diffusion cell (Model Hanson 57-6M, California, USA) using porcine ear skin, which was positioned between the donor and receptor compartments to avoid bubble formation[12]. The gel was placed on the skin and covered by the upper compartment of a Frantz-type cell. The gel was applied with ultrasound at 1 MHz, continuous mode, and 0.8 W/cm^2 for 2, 5, and 10 min (US PRO 2000 DU3035, Ohio, USA). PE gel without ultrasound application was used as a control. Furthermore, F2 and F5 gels with and without ultrasound application for 2 min were used to determine the effect of formulation on skin permeation. The samples (1 mL) were withdrawn at 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 h from the receptor chamber and immediately replaced with an equal volume of fresh receptor medium. The content of three phenylbutenoids was determined and the permeation profile was calculated.

Statistical analysis

The data were expressed as mean±S.D. The statistical analyses were calculated using one-way ANOVA and Turkey's multiple comparison post-test. The statistical differences between values showing p-value<0.05 were considered significant.

Results

Physical properties

The gel-forming agent and other ingredients are shown in Table 1. PE gels (F1-F5) were analyzed for their physicochemical properties. All gels were homogenous, yellow in color with a clear appearance; except F1, which was turbid. The pH values of all formulations were between 5.2 to 5.7.

Freeze-thaw stress cycles

Based on the freeze-thaw cycle assay, all formulations (F1-F5) had an appearance of a smooth texture, without signs of phase separation. All formulations were clear and yellow in appearance; although F5 had the clearest formulation. The remaining drugs of F1, F2, F3, and F5 were over 90%, while that of F4 was lower than 90%.

In vitro drug release study

The *in vitro* release profiles of phenylbutenoids through the cellulose membrane are shown in Figure 1. The release of phenylbutenoids was found to be in the order of: 2% >1% >0.5%, for both formulations (F2 and F5). At concentrations of 0.5% and 1% PE, the releases of phenylbutenoids from both formulations were not significantly different. However, at 2% PE, the release of phenylbutenoids from F2 was higher than that of F5.

In vitro skin permeation and skin retention studies

Skin permeation parameters of the phenylbutenoids from the F2 formulation, with various ultrasound application times, are shown in Table 2. Skin permeation evaluation of F2 with an ultrasound application time of 2 min throughout the assay period of 24 h showed that the steady-state flux (J_{ss}), the permeability coefficient (K_p), and the accumulative amount (Q_{24}) in the receptor compartment of compound D and DMPBD were significantly higher than F2, with an ultrasound application time of 5 and 10 min (p-value< 0.05). Although J_{ss}, K_p, and Q_{24} of compound D acetate in F2 with ultrasound application of 2 min were lower than other ultrasound application times, the total phenylbutenoid concentration was still the highest when ultrasound was applied for 2 min. Furthermore, 2 min of ultrasound application had the shortest lag time of skin permeation in all compounds. From these results, the application time of ultrasound for 2 min was suitable for stimulating skin

permeation of phenylbutenoids for transdermal purposes. In the receptor compartment, the amount of compound D was the highest (Table 3). This was because it had more hydrophilicity, with a partition coefficient of 1.9 and low molecular weight (208.25 g/mole), compared to the other two compounds.

Furthermore, the effect of F2 and F5 formulations on skin permeation was determined either without or with ultrasound, for 2 min. F2, in combination with ultrasound, showed significantly higher J_{ss}, K_p, and Q_{24} of compound D than those without ultrasound. However, the ultrasound application did not affect the skin permeation of compound D acetate and DMPBD. On the other hand, the application of ultrasound could not increase J_{ss}, K_p, and Q_{24} of all compounds when applied with F5 (Table 4). Interestingly, ultrasound was effective in reducing the lag time of all phenylbutenoids to facilitate a constant rate of skin permeation in both F2 and F5. The percentage recoveries of compound D acetate and DMPBD in pig ear skin of F2 with ultrasound application were significantly higher than those without ultrasound, while compound D concentration in the receptor compartment was significantly higher in ultrasound application than that without ultrasound (Table 5). The application of ultrasound was effective for F5, as it significantly enhanced all phenylbutenoids accumulation in the skin when compared to without ultrasound (Table 5). F2 showed higher permeation values of phenylbutenoids than F5.

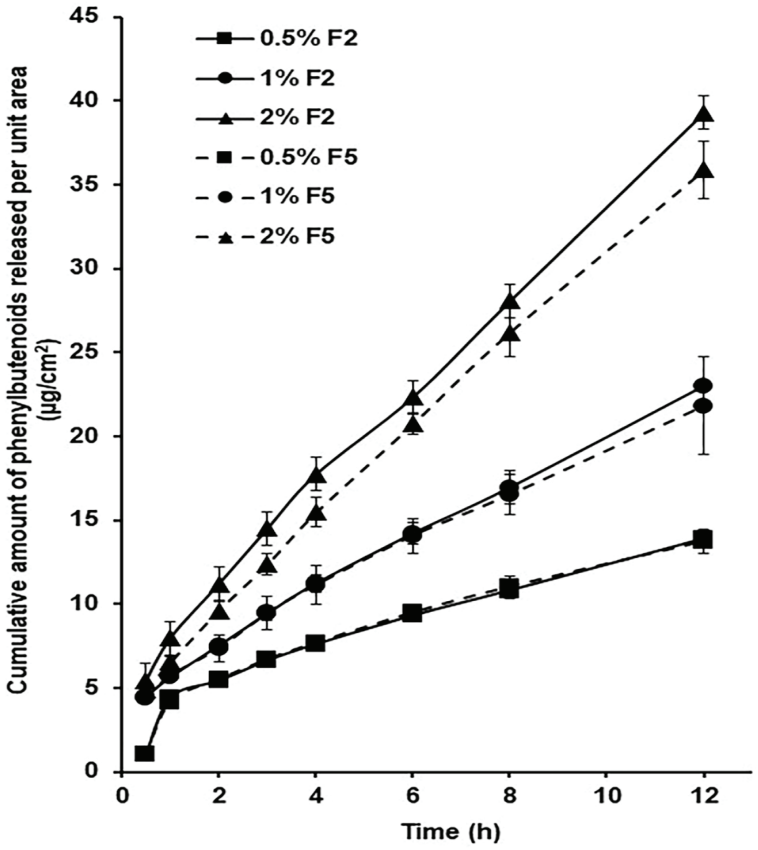

Figure 1 The cumulative amount of phenylbutenoids released from the F2 and F5 formulations (Mean±S.D., n=4)

Table 1 Types and content of ingredients used in PE gel formulations F1–F5

Components	Formulations (% w/w)					Functions
	F1	F2	F3	F4	F5	
Phenylbutenoid extract	Amount of the extract equivalent to 2% phenylbutenoids					Active ingredient
Carbopol 934	0.5	0.5	0.5	0.5	0.5	Gelling agent
HEC 4,000	1	1	1	1	1	Gelling agent
Propylene glycol	15	15	15	15	15	Humectant
Glycerin	20	15	10	5	5	Humectant
Polyethylene glycol 400	5	10	15	20	15	Humectant
Butylene glycol	–	–	–	–	5	Humectant
Methylparaben	0.15	0.15	0.15	0.15	0.15	Preservative
Propylparaben	0.02	0.02	0.02	0.02	0.02	Preservative
Triethanolamine	0.2	0.2	0.2	0.2	0.2	Neutralized agent
Water q.s.	100	100	100	100	100	Vehicle

PE=phenylbutenoid extract, HEC 4,000=hydroxyethylcellulose

Table 2 Skin permeation parameters of compound D, compound D acetate, and DMPBD from F2 formulation, with various ultrasound application times

Parameters	Compounds	F2 formulation+US		
		2 min	5 min	10 min
J_{ss} (μg/cm^2/h)	Compound D	0.81±0.04^b	0.54±0.04^a	0.44±0.12^a
	Compound D acetate	0.20±0.02^a	0.31±0.04^b	0.37±0.08^b
		0.43±0.03^b	0.15±0.03^a	0.18±0.04^a
T_{lag} (h)	Compound D	0.28±0.08^a	0.25±0.03^a	0.65±0.04^b
	Compound D acetate	0.57±0.07^a	3.50±0.13^b	3.62±0.15^b
	DMPBD	1.18±0.14^a	3.35±0.02^b	3.37±0.06^b
K_p (x10^{-3} cm/h)	Compound D	13.79±0.59^b	9.10±0.62^a	7.51±2.00^a
	Compound D acetate	4.45±0.51^a	7.07±0.92^b	8.29±1.71^b
	DMPBD	3.03±0.20^b	1.03±0.22^a	1.31±0.26^a
Q_{24} (μg/cm^2)	Compound D	19.17±0.81^b	12.84±0.55^a	12.13±2.05^a
	Compound D acetate	4.76±0.54^a	7.20±1.12^{a,b}	8.64±1.97^b
	DMPBD	9.57±0.64^b	3.12±0.74^a	4.05±0.92^a

Data are expressed as mean±S.D. (n=4). Significance values (p-value<0.05) are shown in different letters in the same row. US=ultrasound, Q_{24}=amount of drug permeated into receptor medium at 24 h (skin permeation study), J_{ss}=steady-state flux, T_{lag}=the lag-time, K_p=permeability coefficient, DMPBD=phenylbutenoids

Table 3 The percent recovery of compound D, compound D acetate, and DMPBD from F2 formulation in three compartments, with various ultrasound application times

% Amount recovery	Compounds	F2 formulation+US		
		2 min	5 min	10 min
Donor compartment	Compound D	40.54±3.79[b]	34.01±0.16[a]	42.57±0.90[b]
	Compound D acetate	58.03±3.97[b]	42.29±6.99[a]	41.88±2.15[a]
	DMPBD	54.81±8.71[a]	63.27±1.55[a, b]	68.63±2.21[b]
Pig ear skin	Compound D	12.87±0.54	14.04±1.81	13.72±2.36
	Compound D acetate	25.32±2.84	22.08±3.85	25.32±2.84
	DMPBD	17.08±4.82	15.41±2.24	13.77±3.80
Receptor compartment	Compound D	43.39±3.63[b]	36.89±0.97[b]	28.00±4.41[a]
	Compound D acetate	16.05±1.03	18.78±4.57	16.05±1.03
	DMPBD	12.24±1.90[b]	3.97±0.95[a]	4.32±0.38[a]
Total	Compound D	96.79±2.31[b]	84.93±0.75[a]	84.30±3.06[a]
	Compound D acetate	99.40±6.57[b]	83.15±2.01[a]	83.26±3.22[a]
	DMPBD	84.13±3.34	82.66±1.52	86.72±2.55

Data are expressed as % of each compound in the applied dose (mean±S.D., n=4). Significance values (p-value<0.05) are shown in different letters in each row, US=ultrasound, DMPBD=phenylbutenoids

Table 4 Skin permeation parameters of compound D, compound D acetate, and DMPBD from two formulations (F2 and F5) with and without ultrasound application

Parameters	Compounds	Applications			
		F2-gel	F2-gel+US	F5-gel	F5-gel+US
J_{ss} (μg/cm^2/h)	Compound D	0.5±0.10[a]	0.81±0.04[b]	0.69±0.09[b]	0.65±0.04[b]
	Compound D acetate	0.4±0.03[c]	0.20±0.02[a]	0.28±0.03[b]	0.19±0.03[a]
	DMPBD	0.53±0.04[b]	0.43±0.03[a, b]	0.32±0.13[a]	0.29±0.05[a]
T_{lag} (h)	Compound D	0.65±0.04[b]	0.28±0.08[a]	0.33±0.13[a]	0.31±0.05[a]
	Compound D acetate	1.72±0.09[b]	0.57±0.07[a]	1.62±0.09[b]	0.53±0.08[a]
	DMPBD	2.04±0.26[b]	1.18±0.14[a]	2.40±0.04[b]	1.57±0.18[a]
K_p (x10^{-3} cm/h)	Compound D	7.74±1.70[a]	13.79±0.59[b]	11.72±1.53[b]	11.00±0.71[b]
	Compound D acetate	7.87±0.73[c]	4.45±0.51[a]	6.32±0.69[b]	4.14±0.64[a]
	DMPBD	3.72±0.30[b]	3.03±0.20[b]	1.78±0.72[a]	1.61±0.26[a]
Q_{24} (μg/cm^2)	Compound D	10.37±1.78[a]	19.17±0.81[c]	16.37±1.87[b, c]	15.56±1.02[b]
	Compound D acetate	7.38±0.69[b]	4.76±0.54[a]	5.83±0.64[a]	4.43±0.69[a]
	DMPBD	11.78±1.11[b]	9.57±0.64[b]	4.53±1.25[a]	6.88±0.69[a]

Data are expressed as mean±S.D. (n=4). Significance values (p-value<0.05) are shown in different letters in the same row.
US=ultrasound, Q_{24}=amount of drug permeated into receptor medium at 24 h (skin permeation study), J_{ss}=steady-state flux, T_{lag}=the lag-time, K_p=permeability coefficient, DMPBD=phenylbutenoids

Table 5 The percent recovery of compound D, compound D acetate, and DMPBD from two formulations (F2 and F5) with and without ultrasound application

% Amount recovery	Compounds	Applications			
		F2-gel	F2-gel+US	F5-gel	F5-gel+US
Donor compartment	Compound D	40.73±0.28	40.54±3.79	38.93±2.12	37.42±1.99
	Compound D acetate	51.71±4.99	58.02±3.97	61.09±7.20	59.40±5.64
	DMPBD	63.89±7.06^{a}	54.81±8.71^{a}	71.39±2.45^{b}	58.35±5.01^{a}
Pig ear skin	Compound D	11.90±1.90^{b}	12.87±0.54^{b}	3.86±1.06^{a}	12.52±2.10^{b}
	Compound D acetate	6.04±2.50^{a}	25.32±2.84^{b}	5.21±1.61^{a}	19.94±3.85^{b}
	DMPBD	8.47±1.93$^{a, b}$	17.08±4.82^{c}	4.75±1.57^{a}	13.97±1.93$^{b, c}$
Receptor compartment	Compound D	31.13±5.43^{a}	43.39±3.63^{b}	41.06±0.83$^{a, b}$	40.75±4.98$^{a, b}$
	Compound D acetate	25.77±2.41^{b}	16.05±1.03^{a}	20.06±2.18$^{a, b}$	15.49±3.13^{a}
	DMPBD	14.38±1.01	12.24±1.90	12.22±4.18	13.11±2.80
Total	Compound D	83.76±4.44^{a}	96.79±2.31^{b}	83.85±1.30^{a}	90.69±4.57$^{a, b}$
	Compound D acetate	83.52±2.50	83.26±3.22	86.35±6.99	94.84±5.61
	DMPBD	86.74±6.07	84.13±3.34	88.36±6.95	86.33±4.98

Data are expressed as % of each compound in the applied dose (mean±S.D., n=4). Significance values (p-value<0.05) are shown in different letters in the same row, US=ultrasound, DMPBD=phenylbutenoids

Discussion

Carbopol 934 and HEC 4,000 were used as gelling agents in the formulations as they are biodegradable, bioadhesive, biocompatible, irritation-free, and are not absorbed into the body. The pH values of all formulations (5.2 to 5.7) were in the range of skin pH, which indicated that might not cause skin irritation. Hence, the PE gels were suitable to be used as a dermatological product. Furthermore, the rheological behavior of the PE gels exhibited a non-Newtonian shear thinning pseudoplastic type of flow, i.e., a decrease in viscosity at increasing shear rates[13]. This is advantageous for transdermal pharmaceutic formulations in terms of pourability, handling, and application on the skin surface[14].

After the freeze-thaw cycle study, all formulations (F1-F5) were analyzed for their physicochemical properties and drug content. A slight decrease in gel viscosity was found in four formulations (F1, F2, F4, and F5); except for F3, which had an increase in gel viscosity. Furthermore, the drug remaining in the gel formulations was over 90%; except in F4, which was lower than 90%. Moreover, the highest amount of drug content remaining was observed in F2. Therefore, F2 and F5 were selected for *in vitro* drug release and permeation study.

The release of phenylbutenoids from F2 was higher than that of F5. The higher release rate of phenylbutenoids from F2 might be due to its lower viscosity. Typically, formulations with a higher viscosity reduce the release rate of active compounds[15]. *In vitro* release profiles of gels that fitted various kinetic models were used to find out the mechanism for drug diffusion. The coefficient of determination values (r^2) was obtained from different kinetic equations (zero-order, Higuchi, and first-order). The release of 0.5% PE of F2 and F5 were best fitted to Higuchi's model, with (r^2) values of 0.9774 and 0.9819, respectively. Moreover, the 1% PE of both formulations was best fit to

the zero-order model, with (r^2) values of 0.9957 and 0.9908, respectively. Similarly, the 2% PE of both formulations was also best fitted to the zero-order model, with (r^2) values of 0.9964 and 0.9980, respectively. Zero-order kinetics is preferred for its sustained release and transdermal use, as it has a constant rate of drug release. This kinetic model is found mainly in osmotic pumps and transdermal systems, matrix tablets with low-soluble drugs, and coated forms[16]. Therefore, the skin permeation of F2 and F5 containing 2% PE was further investigated. This was because of their higher release, and because they fit the zero-order kinetic model.

The skin permeation of PE gel applied with ultrasound through porcine ear skin was evaluated. This skin was used due to its similar stratum corneum to human skin in terms of lipid position and thickness[17]. The transdermal pharmacokinetics parameters of phenylbutenoids were measured during phonophoresis *in vitro* experiments. The optimal concentration of PE gel was established as 2% w/w. F2 showed higher permeation values of phenylbutenoids than F5. This might be due to its lower viscosity, as viscosity is one of the factors that affect the diffusion of the drug[15]. Furthermore, the composition of the gel plays a critical role in permeation enhancement. The addition of glycerin in F2 increased the solubility of active ingredients and hydrated the stratum corneum.

The lag time is the time required for the drug to permeate through the skin layer before reaching the receptor chamber, and it was observed under various conditions. The lag time of PE gels with a combination of ultrasound was shorter than PE gels alone; as shown in Table 4. This result might be because ultrasound acts as a physical transdermal enhancer, which operates by disordering lipids; thereby, increasing the amount of drug entering the skin, and thus increasing permeation[18]. The total amount of phenylbutenoids recovered from three compartments for 2% w/w PE gels was calculated to evaluate the performance and reliability of the method. When PE gels were administered to the skin, all phenylbutenoids from all conditions mostly remained in the donor compartment (Table 3 and Table 5). The low permeability of all phenylbutenoids might be due to the structure of the outermost skin layer; the stratum corneum. It consists of protein-rich corneocytes embedded in a matrix of lipids. This domain is the rate-limiting step for the penetration of drugs[19]. The application of ultrasound could not produce a noticeable effect on the cumulative amount of all compounds in the receptor compartment, which acts like the blood circulation system. This phenomenon could be explained by small molecules of the phenylbutenoids, having their molecular weight being in the range of 190 to 250 g/mole, which makes it easier to pass through the skin easier than large molecules. However, ultrasound played an important role in lowering lag time by enhancing all compounds, making them pass through the skin more rapidly and giving a constant permeation rate in transdermal delivery. Furthermore, the ultrasound application enhanced the accumulation of the phenylbutenoids in the skin significantly higher than PE gel alone. These results showed the effectiveness of combining PE gel and ultrasound for pain relief, because the compounds deposited in the skin can prolong the sustained release effect. Moreover, the shorter time of drug delivery could provide more rapid pain relief. Therefore, phonophoresis is a great physical option for enhancing PE formulations by facilitating faster transdermal drug delivery and prolonging the effect of pain relief.

Conclusion

Transdermal delivery of anti-inflammatory agents is a potential alternative to oral delivery that helps to avoid gastrointestinal side effects. The physicochemical properties, freeze-thaw stability, and *in vitro* release confirmed that

F2 containing 2% w/w PE could be used as an alternative medicated ultrasound gel. Moreover, the phonophoresis using a frequency of 1 MHz, continuous mode, and 0.8 W/cm^2 for 2 min was capable of reducing the lag time of skin permeation in addition to enhancing the phenylbutenoid accumulation in the skin. Thus, 2% w/w PE gel might be used as an alternative ultrasound gel in phonophoresis for muscle pain relief.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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