

Formulation and evaluation of embelin emulgel for topical delivery

Mahendran Sekar, Nur Asyikin Ismail

Department of Pharmaceutical Chemistry, Faculty of Pharmacy and Health Sciences, Universiti Kuala Lumpur Royal College of Medicine Perak, Ipoh - 30450, Perak, Malaysia

Abstract

Background: Embelin is an isolated compound from *Embelia ribes* and well known for its potent antioxidant and anti-inflammatory properties. However, so far, embelin has not been explored into a topical dosage form due to its hydrophobic nature. Emulgel is a recently developed formulation and emerged as a promising topical delivery system for the delivery of hydrophobic drugs. **Aim:** The aim of the study to formulate emulgel of embelin and tested for its antioxidant and anti-inflammatory properties. **Materials and Methods:** The emulsion was prepared and incorporated in a gel base. The formulations were evaluated for physicochemical properties and tested for *in vitro* antioxidant activity using 2, 2-Diphenyl-1-picrylhydrazyl method. The formulated emulgels were also tested for inhibition of albumin denaturation and lipoxygenase inhibition to evaluate its *in vitro* anti-inflammatory activity. **Results and Discussion:** Both the formulated Emulgels (F1 and F2) were looks a purple creamy with a smooth homogeneous texture and glossy appearance. The formulated embelin emulgel showed potent antioxidant and moderate anti-inflammatory properties that is water soluble gel. **Conclusion:** This embelin emulgel will be further developed into a commercial standard and tested for *in vivo* studies to confirm its anti-inflammatory properties.

Key words: Antiinflammatory, antioxidant, *Embelia ribes*, embelin, emulgel, topical delivery

INTRODUCTION

Embelin is a naturally occurring alkyl substituted hydroxy benzoquinone and a major constituent of *Embelia ribes* Burm. (Family: Myrsinaceae). The plant has been reported to possess antioxidant properties in diabetic animals and anti-inflammatory to relieve rheumatism and fever.^[1,2] Embelin showed antifertility,^[3] anti-implantation,^[4] antitumor,^[5] antioxidant, analgesic anti-inflammatory,^[6,7] hepatoprotective,^[8] wound healing,^[9] antibacterial,^[10] antidiabetic,^[11] and anticonvulsant activities.^[12] Embelin also reported to be used for the treatment of neurological disorders and inflammatory bowel disease.^[13,14]

Earlier studies in our laboratory reported that embelin has significant antioxidant, analgesic, and anti-inflammatory properties.^[6,15] However, so far, there is no formulation which has been done due to its hydrophobic nature. Recent studies indicated that emulgel has been emerged as a promising drug delivery system for the delivery of hydrophobic drugs. Emulgel is a semisolid dosage forms, obtained when gel and emulsion combined together.^[16] Rachit *et al.*, 2012,^[17] reported that mefenamic acid emulgel can be used as an anti-inflammatory

analgesic agent for topical drug delivery. Emulgels for topical applications have several promising properties such as being thixotropic, greaseless, easily spreadable, easily removable, emollient, nonstaining, long shelf life, bio-friendly, transparent, and pleasing appearance.^[18]

The topical analgesic and anti-inflammatory preparations are available in the market as different dosage forms including gels, creams, semisolids, emulsions, patches, aerosols, and foams that are applied on or around the painful site. These topical preparations are made up of synthetic chemicals and produce side effect on the consumers. Hence, in the present study, we are interested to formulate a natural emulgel using embelin as an active ingredient and evaluate for its *in vitro* antioxidant and anti-inflammatory properties.

Address for correspondence:

Dr. Mahendran Sekar, Department of Pharmaceutical Chemistry, Faculty of Pharmacy and Health Sciences, Universiti Kuala Lumpur Royal College of Medicine Perak, Ipoh - 30450, Perak, Malaysia.
Phone: 006-0163346653/006-05-2536634.
E-mail: mahendransekar@unikl.edu.my

Received: 19-06-2018

Revised: 21-12-2018

Accepted: 16-01-2019

MATERIALS AND METHODS

Plant Material

The berries of *Embelia ribes* were purchased from the local Market in Perak, Malaysia and authenticated.

Extraction and Isolation of Embelin

Coarsely powdered berries of *Embelia ribes* (500 g) were exhaustively extracted with *n*-hexane by cold extraction method (3 × 500 ml). After 72 h, the extracts were concentrated to dryness in a rotary evaporator under reduced pressure and controlled temperature (40–50°C). The residue so obtained was subjected to column chromatography over silica gel (100–200 mesh), and elution with benzene yielded an orange colored powder,^[6] which on crystallization with ether afforded orange plates of embelin (2,5-dihydroxy-3-undecyl-1,4-benzoquinone, yield 12.5 g, 2.5%). It was found to be homogenous by high-performance thin-layer chromatographic (HPTLC) when separated using the solvent system ethyl acetate:benzene (70:30). It was characterized by comparing its melting point, infrared (IR), nuclear magnetic resonance (NMR), and MS data with literature values.^[6]

Formulation of Embelin Emulgel^[19-21]

The emulgel was made in two steps. The first step was by making of oil in water emulsion and base of gel. The second step was by mixing the emulsion and gel base together. The oil phase emulsion made by dissolving Span 20, embelin and butylated hydroxytoluene in olive oil. Meanwhile, the water phase made by dissolving Tween 60 in distilled water. The menthol was dissolved in 96% ethanol and then blended into propylene glycol. After that, the oil phase was added to the water phase and followed by the addition of a mixture of mentholethanol-propylene glycol. Then, the mixture was stirred vigorously until emulsion formed. The gel was made by mixing the carbomer in distilled water and stirred until completely dispersed. Next, sodium hydroxide was dissolved in distilled water and added to the gel base carbomer then stirred until a thick gel base is formed. After that, the emulsion was mixed into the base gel by adding gradually and stirred vigorously until a homogeneous mass of emulgel is formed [Figure 1]. The composition of two different formulations has been shown in Table 1.

Evaluation of Formulated Embelin Emulgel

Physical examination

The formulated embelin emulgel was evaluated visually for its color, appearance, and consistency. Along with that organoleptic test, homogeneity, and pH also tested.



Figure 1: Embelin emulgel

Table 1: Composition of embelin emulgel formulations

Ingredients	Concentration (%) (w/w)	
	Formulation 1 (F1)	Formulation (F2)
Embelin	1.0	3.0
Olive oil	5.0	5.0
Propylene glycol	5.0	5.0
Menthol	1.0	1.0
96% Ethanol	3.0	3.0
NaOH	0.6	0.6
Carbomer	2.0	2.0
Span 20	1.4	1.4
Tween 40	3.6	3.6
BHT	0.03	0.03
Distilled water up to	q.s	q.s

BHT: Butylated hydroxytoluene

Organoleptic test

The embelin emulgel was kept for 8 weeks at low temperature, room temperature, and high temperature. The appearance such as color, pearlescence, and roughness was observed and graded.

Homogeneity

The formulation was tested for homogeneity by visual appearance and touch.

pH test

The pH value of 1% aqueous solution of embelin emulgel was measured using digital pH meter.

In vitro antioxidant activity by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method

A 100 µl aliquot of the different concentrations of embelin and emulgel formulations along with standards were added to 2 ml of DPPH in methanol solution (100 µM) and incubated at 37°C for 20 min. After that, the absorbance of each solution was determined at 490 nm using UV-visible spectrophotometer.^[22] The percentage inhibition was calculated as follows:

Percentage inhibition = $[(\text{Abs control} - \text{Abs sample}) \times 100] / (\text{Abs control})$.

In vitro Anti-inflammatory Activity of the Formulated Embelin emulgel

Inhibition of albumin denaturation

The anti-inflammatory activity of the formulated embelin emulgel has been evaluated by albumin denaturation method. The reaction mixture consisted of test formulations and 1% aqueous solution of bovine serum albumin fraction, pH of the reaction mixture was adjusted using a small amount of 1N HCl. The samples then incubated at 37°C for 20 min and then heated to 51°C for 20 min, after cooling the samples the turbidity were measured at 660 nm using a UV-visible spectrophotometer.^[23] The percentage inhibition of protein denaturation was calculated as follows:

Percentage inhibition = $[(\text{Abs control} - \text{Abs Sample}) \times 100] / (\text{Abs control})$.

Lipoxygenase Inhibitory Action

Anti-lipoxygenase activity was studied using linoleic acid as a substrate and lipoxygenase as an enzyme. Test samples were

dissolved in 0.25 ml of 2M borate buffer pH 9.0 and added 0.25 ml of lipoxygenase enzyme solution and then incubated for 5 min at 25°C. After that, 1.0 ml of linoleic acid solution was added, mixed well and absorbance were measured at 324 nm using UV-visible spectrophotometer.^[24] Indomethacin was used as reference standard. The percentage inhibition of protein denaturation was calculated as follows:

Percentage inhibition = $[(\text{Abs control} - \text{Abs sample}) \times 100] / (\text{Abs control})$.

Statistical Analysis

Each experiment was carried out in three replicates; the mean of variable \pm standard deviation was calculated.

RESULTS

Embelin was isolated from the berries of *Embelia ribes*, found to be homogenous by HPTLC [Figure 2] when separated using the solvent system ethyl acetate:benzene (70:30, $R_f = 0.53$). Obtained as orange plates mp 141–143°C; yield 12.5 g, 2.5%; IR ν_{max} (KBr) cm^{-1} : 3309 (O-H), 2920, 2849 (C-H), 1746 (α , β -unsaturated C=O), 1615 (C=C) [Figure 3]; $^1\text{H NMR}$ (400 MHz, CDCl_3) δ : 7.68 (s, 2H, -OH), 6.00 (s, 1H, H-6), 2.44 (t, 2H, H-1'), 1.47 (m, 2H, H-2'), 1.25–1.30 (m, 16H, H-3' to 10'), 0.88 (t, 3H, H-11'); Negative ESI-MS: m/z calculated for 294.18, Found: 293 $[\text{M-H}]^{-1}$.

Physical Appearance

Emulgel formulations (F1 and F2) were looks a purple creamy with a smooth homogeneous texture and glossy appearance. The results have been shown in Table 2.

In vitro Antioxidant Activity

Embelin showed potent antioxidant activity with a percentage of inhibition value 91.66 ± 3.68 at 125 $\mu\text{g/ml}$ in the DPPH method. The formulations 1 and 2 showed potent antioxidant activity with a percentage of inhibition value 50.12 ± 2.00

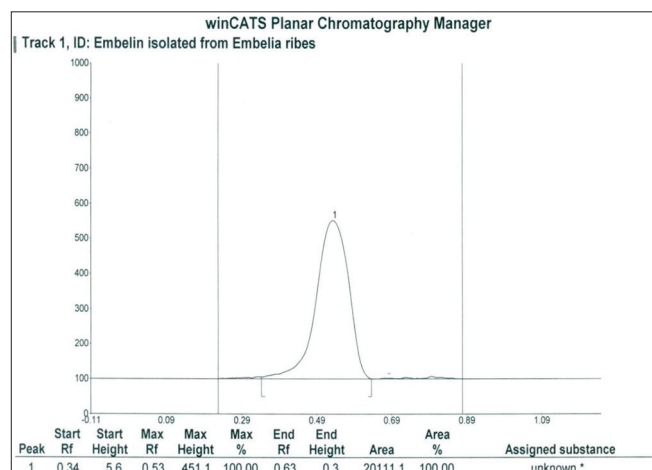


Figure 2: High-performance thin-layer chromatographic spectrum of embelin (1)

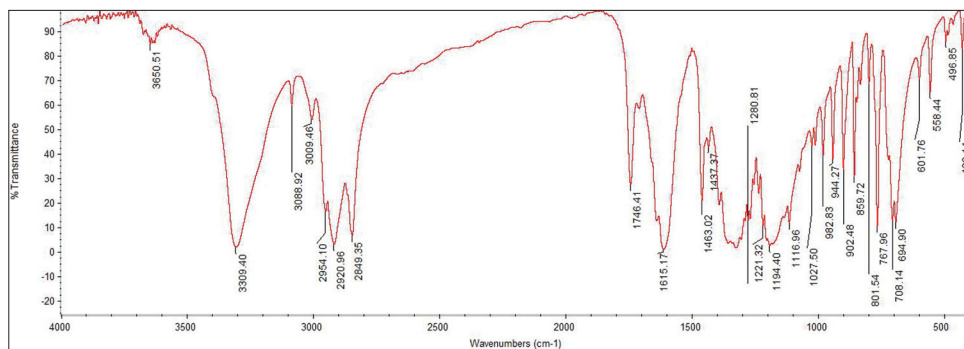


Figure 3: Infrared spectrum of embelin

Table 2: Physicochemical properties evaluation on formulated Emulgel

Parameters	Formulation 1	Formulation 2
Color	Light purple	Dark purple
pH	7.8	7.6
Homogeneity	Good	Good
Appearance	No color changes	No color changes
Consistency	Good	Good
Phase separation	None	None
Odor	Good	Good
Spreadability	Good	Good
After feel	Good	Good
Removal	Easy	Easy
Stability	Stable in tested temperature	

Table 3: *In vitro* antioxidant activity by DPPH method

Concentration (µg/ml)	% Inhibition		
	Embelin	Formulation 1	Formulation 2
1000		50.12±2.00	84.88±2.49
500		33.97±2.93	75.68±4.58
250		16.81±1.94	57.26±3.65
125	91.66±3.68	--	44.72±3.30
62.5	73.15±4.81	--	30.58±2.16
31.25	50.25±2.06	--	--
15.60	21.69±1.53	--	--
7.80	--	--	--
3.90	--	--	--
1.95	--	--	--
Ascorbic acid (10 µg/ml)	92.45±3.82		
Rutin (10 µg/ml)	48.25±2.50		

--: No inhibition. Values are expressed as mean±SD (n=3).
DPPH: 2, 2-Diphenyl-1-picrylhydrazyl

and 84.88 ± 2.49 at 1000 µg/ml, respectively. Formulation 2 showed a higher percentage of inhibition when compared to formulation 1 in all the tested concentrations. However, standard ascorbic acid and rutin showed higher inhibition at low concentration when compared to embelin and its emulgel formulations. The results were shown in Table 3.

In vitro Anti-inflammatory Activity

As part of the investigation on anti-inflammation activity, the ability of embelin and formulated emulgels (F1 and F2) for inhibition of protein denaturation was studied. It was effective in inhibiting heat-induced albumin denaturation. Maximum inhibition of 47.65 ± 4.49, 10.34 ± 4.58, and 31.08 ± 4.61 was observed at 1000 µg/ml for embelin, F1 and F2, respectively [Table 4]. The lower concentrations did not show

Table 4: Effect of embelin and its emulgel on albumin denaturation

Concentration (µg/ml)	% Inhibition		
	Embelin	Formulation 1	Formulation 2
1000	47.65±4.49	10.34±4.58	31.08±4.61
500	40.87±1.40	--	24.94±3.61
250	34.93±2.37	--	--
125	27.95±4.22	--	--
Aspirin (100 µg/ml)	64.58±4.25		

--: No inhibition. Values are expressed as mean±SD (n=3).

Table 5: Effect of embelin and its emulgel on lipoxygenase inhibitory action

Concentration (µg/ml)	% inhibition		
	Embelin	Formulation 1	Formulation 2
1000	60.28±2.85	--	30.65±1.40
500	43.58±2.55	--	17.07±1.50
250	17.63±2.05	--	--
Indomethacin (100 µg/ml)	80.25±4.86		

--: No inhibition. Values are expressed as mean±SD (n=3)

significant inhibition. Aspirin, a standard anti-inflammatory drug, showed the maximum inhibition of 64.58 ± 4.25 at the concentration of 100 µg/ml when compared with control.

In lipoxygenase inhibitory action, embelin and its emulgel formulations (F1 and F2) have been checked at 250, 500, and 1000 µg/ml concentrations. Maximum inhibition of 60.28 ± 2.85 and 30.65 ± 1.40% was observed at 1000 µg/ml for embelin and F2, respectively [Table 5]. Formulation 1 does not show any inhibition up to 1000 µg/ml concentration. The standard indomethacin showed 80.25 ± 4.86 of percentage inhibition at a concentration of 100 µg/ml.

Stability Test

Both the formulated emulgels (F1 and F2) were found to be stable upon storage for 3 months, no color change was observed in their physical appearance and other physicochemical properties.

DISCUSSION

Embelin isolated from *Embelia ribes* is known for its potent biological properties.^[1-13] Our earlier studies reported that it has potent antioxidant, analgesic, and anti-inflammatory properties.^[6,15] In the present study, we are formulated embelin emulgel and tested for its *in vitro* antioxidant and anti-inflammatory properties. The embelin showed potent antioxidant activity and incorporated when it was converted into emulgel formulations. The results also well correlate with our earlier studies of antioxidant activity of embelin.^[14,15]

Based on these results, both the formulations (F1 and F2) along with embelin were tested for *in vitro* anti-inflammatory activity. In the present study, anti-inflammatory activity of embelin and its emulgel formulations was evaluated by determination of inhibition of albumin denaturation and lipoxygenase inhibition. Embelin and its formulated emulgels exhibited a moderate effect in both the tested methods. The observed anti-inflammatory properties of embelin and its formulations may be due to their potent antioxidant nature.

CONCLUSION

Embelin is a hydrophobic compound with potent antioxidant and anti-inflammatory properties. Since emulgel is helpful in enhancing spreadability, adhesion, viscosity, and extrusion, this novel drug delivery becomes popular. The formulated embelin emulgel showed potent antioxidant and moderate anti-inflammatory properties which is water soluble gel. This embelin emulgel will be further developed into a commercial standard for topical delivery and tested for *in vivo* studies to confirm its anti-inflammatory properties.

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Source of Support: Nil. **Conflict of Interest:** None declared.