

Formulation and evaluation of antibacterial ointment containing embelin isolated from *Embelia ribes*

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Abstract

Background: Embelin is an active constituent isolated from *Embelia ribes* and well known for its antibacterial potential. However, so far, embelin has not been explored into a formulation and tested for antibacterial properties. **Objective:** The objective of this study was to formulate an ointment containing embelin as an active ingredient and evaluate for its antibacterial properties. **Methods:** The embelin antibacterial ointment was formulated using base and evaluated its physicochemical properties using standard parameters. The antibacterial activity of the formulated ointment in different concentration was tested against two Gram-positive bacteria (*Staphylococcus aureus* and *Streptococcus epidermidis*) and two Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) using disc diffusion method. **Results and Discussion:** The formulated ointment showed significant antibacterial activity against all the tested organisms with zone of inhibition ranges from 7.67 ± 0.58 to 12.00 ± 1.00 mm. **Conclusion:** The observed antibacterial activity of the ointment was due to the presence of embelin, and the activity of embelin was well maintained when it was transformed into an ointment. This was good sign to do further studies to make this formulation into commercial standard and used for the treatment of bacterial infections.

Key words: Antibacterial ointment, disc diffusion method, *Embelia ribes*, embelin

INTRODUCTION

Microorganisms such as bacteria can cause many types of skin-related diseases such as skin rashes, acne, eczema, psoriasis, and dermatitis. *Staphylococcus aureus* and *Escherichia coli* are the main pathogen that causes these skin infections. Topical cream and ointment containing antibacterial properties can be used in the treatment and prevention of this kind of bacteria causes the infection. Most of the available topical formulations used to treat skin-related diseases in the market are obtained by various synthetic processes which are using chemicals and have some kinds of side effect.

The necessity for developing new antimicrobial formulation has increased significantly due to growing concerns regarding multidrug-resistant bacterial strains. Therefore, attention has been devoted to safe, new, and/or alternative antimicrobial materials in the field of antimicrobial chemotherapy^[1,2].

Embelin is reported for 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] and also reported that it is to be used for the treatment of neurological disorders and inflammatory bowel disease.^[13,14]

However, so far, there is no formulation that has been done though it was reported as potential antibacterial properties. Hence, in the present study, we are interested to formulate an ointment and evaluate its antibacterial properties.

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MATERIALS AND METHODS

Plant Material

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

Extraction and Isolation of Embelin

Coarsely powdered berries of *E. ribes* (500 g) were exhaustively extracted with *n*-hexane by cold extraction method (3 ml × 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,^[15] which on crystallization with ether afforded orange plates of embelin (2,5-dihydroxy-3-undecyl-1,4-benzoquinone, Figure 1, yield 12.5 g, 2.5%). It was found to be homogenous by high-performance thin-layer chromatography (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 mass spectrometry data with literature values.^[15,16]

Formulation of Antibacterial Ointment

The ingredients of base were shown in Table 1. All the ingredients were weighed and melted in a beaker at 70 using heating mantle. The ingredients were stirred gently for 5 min by maintaining the temperature at 70°C. Then, embelin was added and stirred well until homogenous mass is formed and transferred in the bottle. The ointment is prepared for 100 g [Table 1 and Figure 2].

Evaluation of Formulated Antibacterial Ointment

The formulated antibacterial ointment was evaluated using the following parameters.

Colour and Odour

Color and odor of prepared ointment were examined by visual examination.

Loss on Drying

1 g of ointment was placed in the Petri dish and heated in the water bath at 105°C every 30 min until it gets constant weight.

pH

The pH of ointment was determined by digital pH meter. 1 g of ointment was dissolved in 50 ml of distilled water and the pH was measured.

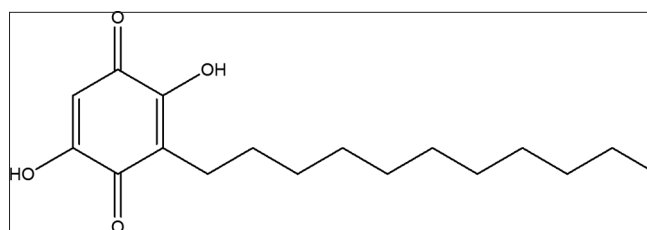


Figure 1: Structure of embelin

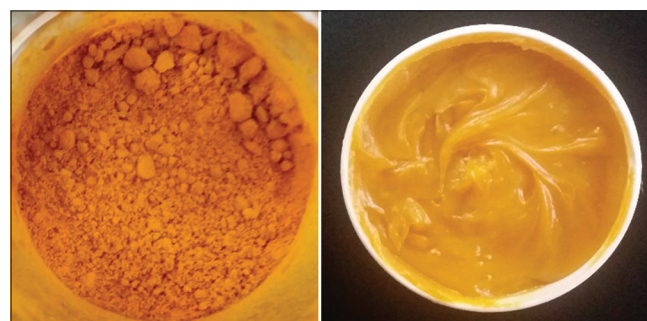


Figure 2: Pure form of embelin powder and its ointment

Table 1: Composition of the formulated embelin antibacterial ointment

Ingredients	Amount (%)
Embelin	3
Emulsifying wax	27
White soft paraffin	50
Liquid paraffin	20

Homogeneity

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

Stability Study

The stability study was carried out for the formulated ointment in different temperature and kept it for 2 months. The color change was observed in their physical appearance and other physiochemical properties.

ANTIBACTERIAL SCREENING

Test Microorganisms

A panel of common pathogenic microorganisms was used in the study, which includes two Gram-positive bacteria (*S. aureus* and *S. epidermidis*) and two Gram-negative bacteria (*E. coli* and *P. aeruginosa*).

Disc diffusion Method

The antibacterial activity of the ointment and extract was evaluated using disc diffusion method. A suspension of

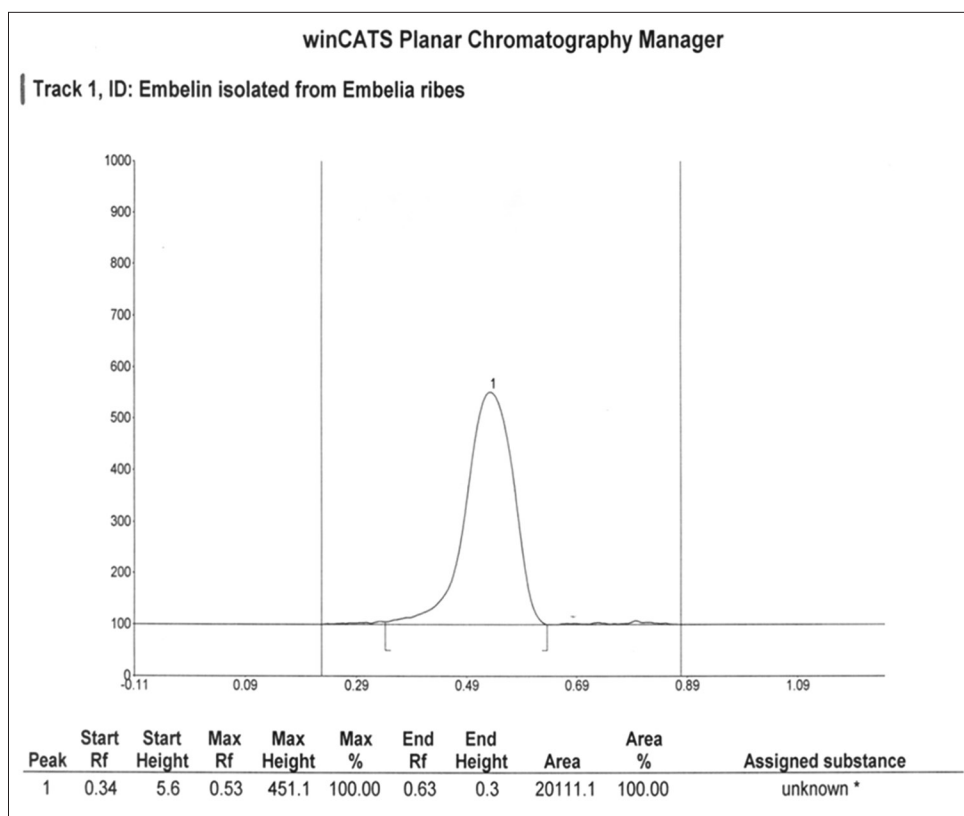


Figure 3: High-performance thin-layer chromatography spectrum of embelin (1)

the tested microorganisms was uniformly swabbed on agar plates using sterile cotton swabs. Sterile blank discs were individually impregnated to the different concentrations of formulated ointment (50, 25, and 12.5 mg/ml) that was placed onto the inoculated agar plates. The plates were inverted and incubated at 37°C for 24 h for bacteria. The antibacterial activity was measured by measuring diameter of the resulting zone of inhibition against the tested organisms.^[17]

RESULTS

Embelin was isolated from the berries of *E. Ribes* and found to be homogenous by HPTLC [Figure 3] 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: 3309 (O-H), 2920, 2849 (C-H), 1746 (α , β -unsaturated C=O), 1615 (C=C) [Figure 4]; ^1H 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 $[\text{M-H}]^{-1}$.

The formulated ointment was evaluated for physicochemical parameters such as color, odor, and pH, the results were shown in Table 2. There are no color changes and pH variation was observed during the stability study of the formulated ointment. This was good indication for ointment and it was acceptable. The antibacterial potential of the formulated

Table 2: Physiochemical properties of formulated embelin ointment

Parameters	Formulated ointment
pH	7.2
Homogeneity	Moderate
Appearance	Moderate
Odor	Good
Spreadability	Good
After feel	Good
Type of smear	Non-greasy
Removal	Easy
Stability	Stable in tested temperatures for 2 months

ointment was studied with three different concentrations. The ointment also showed significant antibacterial activity against all the tested organisms with zone of inhibition ranges from 7.67 ± 0.58 to 12.00 ± 1.00 mm [Table 3]. However, the standard ciprofloxacin (5 μg) showed significant ($P < 0.05$) antibacterial activity with low concentration when compared to tested ointment containing embelin.

DISCUSSION

Embelin is reported for antifertility,^[3] anti-implantation,^[4] antitumor,^[5] antioxidant, analgesic, anti-inflammatory,^[6,7]

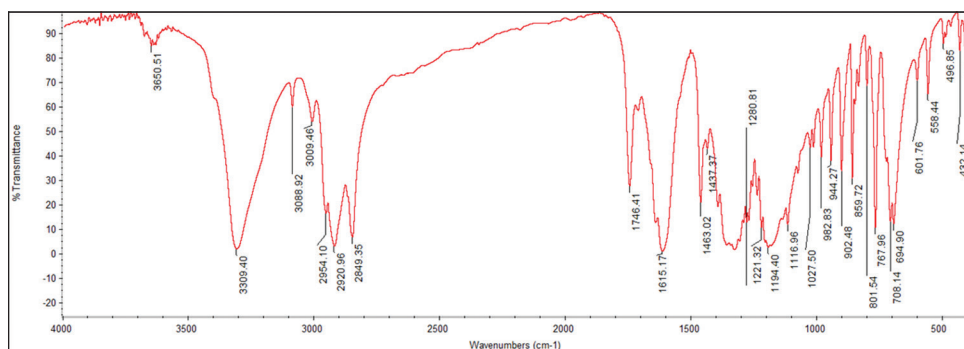


Figure 4: Infrared spectrum of embelin

Table 3: Antibacterial activity of embelin ointment

Concentration of ointment mg/ml	<i>Staphylococcus aureus</i>	<i>Streptococcus epidermidis</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>
12.5	7.67±0.58	8.00±0.00	7.67±0.58	8.33±0.33
25	8.33±0.58	8.33±1.16	8.67±0.58	10.00±1.00
50	9.33±0.58	8.67±0.58	9.67±1.16	12.00±1.00
Positive control ciprofloxacin (5 µg)	28.67±0.58	37.33±1.16	31.00±1.00	24.67±0.58
Negative control	No inhibition			

Values are expressed as Mean±SD (n=3). $P<0.05$ between negative control disc and embelin ointment-treated disc. $P<0.05$ between negative control disc and standard treated disc. SD: Standard deviation

hepatoprotective,^[8] wound healing,^[9] antibacterial,^[10] antidiabetic,^[11] and anticonvulsant activities^[12] and also reported that it is to be used for the treatment of neurological disorders and inflammatory bowel disease.^[13,14]

With reference to the antibacterial activity, Chitra *et al.*, 2003,^[10] reported that embelin (100 µg) exhibited significant antibacterial activity against *S. aureus*, *Streptococcus pyogenes*, *Shigella flexneri*, *Shigella sonnei*, and *P. aeruginosa*; however, it showed moderate zone of lysis against *Salmonella typhi*, *Shigella boydii*, and *Proteus mirabilis*. Feresin *et al.*, 2003,^[18] reported that embelin inhibited both methicillin-sensitive and methicillin-resistant strains of *S. aureus* and *E. coli*.

In the present study, embelin was used as a main ingredient for an ointment formulation and tested for its antibacterial potential using disc diffusion method against *S. aureus*, *S. epidermidis*, *E. coli*, and *P. aeruginosa*.

From the result, the antibacterial activity of embelin ointment showed slightly higher zone of inhibition toward Gram-negative bacteria compare to Gram-positive bacteria. These results were supported by the earlier research was conducted on antibacterial activity of embelin.^[10,18,19]

Hence, the observed antibacterial activity of the ointment was due to the presence of embelin and the activity also well maintained when it was converted into an ointment. This was good sign to do further studies to make this formulation into

commercial standard and used for the treatment of bacterial infections.

CONCLUSION

The formulated embelin ointment showed moderate antibacterial activity toward *S. aureus*, *S. epidermidis*, *E. coli*, and *P. aeruginosa*. This indicates that antibacterial activity of embelin was well maintained when it was converted into ointment. The formulated ointment can be used in the treatment of common skin infections caused by bacteria. The ointment was stable after 2 months. In the future, the formulated antibacterial ointment can be tested for other microorganisms to confirm its effectiveness.

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