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Original article

Preparation, characterization, and antibacterial activity of plaunotol and plaunoi extracts complexed with hydroxypropyl-β-cyclodextrin

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ABSTRACT

Croton stellatopilosus (Plaunoi) leaves accumulate several diterpenes and possess various pharmacological activities. The present study aimed to prepare, characterize and assess the antibacterial activity of inclusion complexes prepared by mixing plaunotol (PL) or plaunoi extract (PE) with cyclodextrins (CD), including α-CD, β-CD, γ-CD, and hydroxypropyl-β-cyclodextrin (HP-β-CD). The inclusion complexes were characterized using SEM, XRD, DSC, and FT-IR and evaluated for aqueous solubility and thermal stability. The PL and PE lyophilized complexes with HP-β-CD were further evaluated for their antibacterial activity against acne-causing bacteria. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of PL, PE, and the inclusion complexes evaluated using the agar dilution method revealed that the MIC and MBC values of the inclusion complexes were lower than those of PL or PE alone. Interestingly, the complexes had a synergistic activity with clindamycin after testing with checkerboard assay. The hydrogel containing the inclusion complex and clindamycin were assessed for antibacterial activity using the agar well diffusion method. The results indicated that the hydrogels showed significant inhibition of bacterial growth. In conclusion, the prepared solid dispersion of PL or PE with HP-β-CD could enhance antibacterial activity by increasing the drug solubility. The hydrogels containing PL or PE complex and clindamycin could be considered as a candidate for the treatment of acne vulgaris.

1. Introduction

Plaunotol (PL) is an acyclic diterpene found abundantly in plaunoi (Thai; *Croton stellatopilosus* Ohba), a tropical plant species in Euphorbiaceae Family. Extensive pharmacological investigations have revealed that plaunoi possesses wide-ranging pharmacological activities, including anti-peptic ulcer (Ogiso et al., 1978; Koga et al., 1996), gastro-protective (Fu et al., 2005), wound healing (Khovidhunkit et al., 2011), anti-inflammatory (Premprasert et al., 2013) and anti-cancer activity (Chatatikun et al., 2018). In particular, the antibacterial property of plaunotol has been demonstrated in many investigations (Koga et al., 1998; Matsumoto et al., 1998; Sasaki et al., 2007; Hada et al., 2001; Songkro et al., 2011). It has been suggested that plaunotol increases membrane fluidity and permeability of gram-positive bacteria and subsequent cell death (Koga et al., 1998). The dose of plaunotol required to cause 50% bacterial growth inhibition is in the range of 2.5 to 16 μg/mL for methicillin-resistant *Staphylococcus aureus* and 2.5 to 7.0 μg/mL for methicillin-sensitive *S. aureus* isolated from the skin of

Abbreviations: CD, cyclodextrin; DSC, Differential scanning calorimetry; FICI, fractional inhibitory concentration index; FT-IR, Fourier-transformed infrared spectrometry; HP-β-CD, hydroxypropyl-β-cyclodextrin; KM, kneading method; LM, lyophilized method; MBC, minimal bactericidal concentration; MIC, minimal inhibitory concentration; PE, plaunoi extract; PE:HP-β-CD, inclusion complex of plaunoi extract and hydroxypropyl-β-cyclodextrin; PL, plaunotol; PL:HP-β-CD, inclusion complex of plaunotol and hydroxypropyl-β-cyclodextrin; PM, physical mixture; SEM, scanning electron microscope; XRD, X-ray diffraction.

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patients suffering from atopic dermatitis (Matsumoto et al., 1998). Plaunotol also demonstrated synergistic antibacterial effects *in vitro* when combined with clarithromycin against 15 of 21 different strains or clinical isolates of clarithromycin-resistant *Helicobacter pylori*. Combination therapy of plaunotol and clarithromycin was reported to significantly lower *H. pylori* infection in Mongolian gerbils inoculated with clarithromycin-resistant *H. pylori* (Sasaki et al., 2007). Plaunotol may increase the permeability of the bacterial membrane, facilitating the entry of clarithromycin and enhancing its activity.

The American Academy of Dermatology has recommended that oral or topical antibiotics should not be applied as monotherapy. Instead, combination therapy based on topical retinoids and antimicrobials is recommended by the Global Alliance to improve outcomes in acne treatment (Gollnick et al., 2003). Guideline of care for acne vulgaris has been classified based on treatment effectiveness and the potential side effect. Among the systemic antibacterial agents, topical clindamycin (1% w/w) is usually prescribed for acne vulgaris (Zaenglein et al., 2016). Although combination therapy involving herbal drug products and antibiotics has been proposed as a strategy to improve healing and combat antibiotic resistance (Martin and Ernst, 2003; Chanda and Baravalia, 2010, Aelenei et al., 2020). The hydrogel containing aloe gel, mangosteen rind, and green tea leaf extract reportedly improved total acne lesion and severity better than the commercial 1% clindamycin gel (Waranuch et al., 2019).

Water-rich hydrogels are advantageous for application to acne-prone skin due to a favorable property balance of biocompatibility, retention on the skin surface, water-washability, and ease of handling (Aswathy et al., 2020). Hydrogel is one of the popular formulations for antiacne preparation. It can be easily prepared and lacks irritable components such as surfactants. Previously, plaunoi extract formulated as an emulsion exhibited antibacterial activity against *S. aureus*, *S. epidermidis*, and *P. acnes*. Nevertheless, it had a reversible irritation effect caused by tween in the recipe (Songkro et al., 2011).

The application of pristine plaunotol (Fig. 1) has been limited by several factors, including difficulty to synthesize via chemical route making preparation only feasible via isolation from natural sources (Tago et al., 2000), short shelf life, and poor stability caused photo-oxidation (Ogiso et al., 1978). To circumvent with these challenges, the protection of plaunotol by host molecules such as natural polymers to extend the stability and enhance aqueous solubility has been suggested. Cyclodextrins (CDs) are water-soluble. The cyclic oligosaccharides are composed of a-1,4-linked a-D-glucopyranose units (Fig. 1) that have been widely exploited to enhance drug solubility and stability (Wüpper et al., 2021). The applications include the conversion of liquid drug formulations to solid dosage forms, reducing irritation in the gastrointestinal tract or the eye, and taste and odor masking (Loftsson and Brewster, 1996). The central cavity of CD is lipophilic, and the outer surface is hydrophilic. We proposed that the lipophilic molecules of plaunotol and other diterpenes could enter the cavity and hence increase solubility and protect molecules from the surrounding environment.

The purpose of the present study was to prepare solid dispersion inclusion complex as well as antibacterial hydrogels of plaunotol (PL) and plaunoi extract (PE) with cyclodextrins to improve the solubility of PL and PE. PL and PE, and their complexes with HP-β-CD in the form of hydrogel were assessed for antibacterial activity against acne-causing bacteria *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Propionibacterium acnes*. The synergistic activity of PL or PE, when complexed with HP-β-CD and combined with clindamycin, was also investigated. The hydrogels' pH, morphology, viscosity, PL release behavior, storage stability, and antibacterial properties were determined.

2. Materials and methods

2.1. Materials

Cellulase enzyme from *Aspergillus niger* was obtained from Sigma Life Science, Hokkaido, Japan. Cyclodextrins (α-CD, β-CD and γ-CD) were procured from Wacker Chemie AG, Burghausen, Germany. HP-β-CD was purchased from Shandong Binzhou Zhiyuan Biotechnology Co., Ltd., Binzhou, China. Diaion HP-20 resin was purchased from Mitsubishi Chemical Co., Ltd., Tokyo, Japan. Carbopol® Ultrez20 was obtained from Lubrizol Corporation, Ohio, USA. Müller Hinton broth (Difco™) and brain heart infusion (Bacto™) were purchased from Le Pont de Claix, France. Anhy-

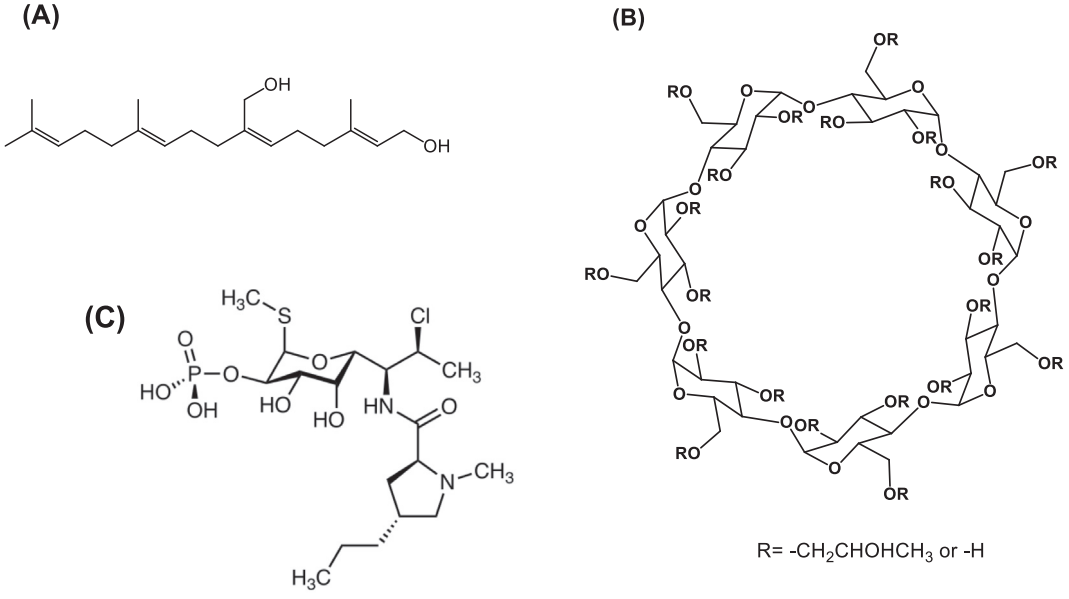

Fig. 1. Chemical structures: A) plaunotol (PL), B) hydroxypropyl-β-cyclodextrin (HP-β-CD) and C) clindamycin phosphate.

drous dextrose was purchased from HiMedia Laboratories, Mumbai, India. Resazurin sodium salt was purchased from Sigma-Aldrich Chemie GmbH, Steinheim, Germany. Bacteria used in our study were *S. aureus* ATCC 25923, *S. epidermidis* TISTR 517, and *P. acnes* DMSC 14,916 purchased from the Institute of Scientific and Technological Research, Thailand. Clindamycin phosphate was purchased from MacroPhar Co.Ltd, Thailand. All other chemicals were of analytical grade.

2.2. Preparation of plaunotol (PL) and plaunoi extract (PE)

PL was isolated from the concentrated hexane extract of plaunoi leaves, kindly provided by Tipco® Biotech, Co.Ltd as described in Aung et al. (2021). Briefly, the extract (20 g) was loaded onto silica gel column (7 × 20 cm; SiliaFlash® P60, Quebec, Canada). The column was eluted with chloroform:*n*-propanol (24:0.5 v/v). The plaunotol-containing fractions were pooled based on thin-layer chromatography analysis. Six grams of PL was obtained.

The PE was prepared according to previous report (Aung et al., 2021). The cellulase- and ultrasonic-assisted extraction afforded the crude ethanol extract, partially purified on diaion HP-20 resin pre-equilibrated with 40% v/v ethanol. After loading the extract, the column was washed thoroughly with 40% v/v ethanol. Finally, the column was eluted with about 4-column volumes of absolute ethanol. The fractions containing PL were pooled and evaporated to dryness. The quantification of PL in the extract was done using the validated HPLC method described previously (Aung et al., 2021). The PL content in the PE was 8.81 % w/w.

2.3. HPLC analysis of plaunotol

The HPLC method for determining PL content in the extract and the inclusion complex was performed as described previously (Aung et al., 2021). HPLC (Prominence *i* 2030C, Shimadzu Corporation, Shimadzu, Kyoto, Japan) equipped with a diode array detector was employed. The HPLC column was a VertiSep™ UPS C18 HPLC column (4.6 × 250 mm, 5 µm). The mobile phase was set in gradient mode using acetonitrile and water: (a) initial mobile phase, 20% acetonitrile for 5 min, (b) linear increase from 20% to 90% acetonitrile, 5 to 25 min, (c) 90% acetonitrile, 25 to 30 min, and (d) return to 20% acetonitrile, 30 to 35 min. The total running time was 35 min. The injection volume was 20 µL, and the detection wavelength was 220 nm. The content of PL was quantified and expressed as % w/w.

2.4. Phase solubility study

Phase solubility study was performed with PL mixing with CDs including α-CD, β-CD, γ-CD, and HP-β-CD, respectively. PL (10 mg) was added to 10 mL of CD solution and had the final concentration of 3.27 mM. The CD solution was prepared in distilled water in 3, 6, 9, 12, 15 mM, which afforded 1:1–1:5 ratios, respectively. The mixtures were shaken at 32 °C, 100 rpm for 48 h. The aliquot was filtered through a 0.22 µm membrane filter to remove any undissolved PL. The filtrate was measured using a UV–Visible spectrophotometer (GENESYS 6, Thermo Scientific, Massachusetts, USA). The wavelength was set at 220 nm. The amount of PL (mM) was calculated. Solubility profiles were presented as phase solubility diagrams by plotting PL concentration (mM) against CD concentration (mM). The binding constant (*K*s) was calculated from the slope of the diagram and the intrinsic solubility of PL (S_0) according to the following equation:

$$K_s = \frac{\text{Slope}}{[S_0 x(1 - \text{Slope})]}$$

2.5. Complexation of plaunotol and plaunoi extract with HP-β-CD

HP-β-CD was selected as the host polymer, suggested by phase solubility study. Inclusion complex preparation including physical mixing (PM), kneading method (KM) and lyophilization method (LM) were studied at 1:1 and 1:2 M ratio of PL or PE, and HP-β-CD. HP-β-CD of 10.14 g and 20.27 g were required to prepare 1:1 and 1:2 M ratios, respectively. While PL of 2.14 g was used, afforded PL: HP-β-CD complex and PE of 24.31 g (equivalent to PL 2.14 g) was used, afforded PE: HP-β-CD complex.

For the physical mixture, dry blending between drug and HP-β-CD was vigorously triturated in a ceramic mortar until a homogenous mixture was obtained. HP-β-CD was blended with a small amount of distilled water for the kneading method, and then PL or PE was added. The mixture was triturated to obtain a slurry-like component. The damp mass was passed through sieve No.60. The mixture was dried at 40 °C to constant weight.

For the lyophilization method, the weights of HP-β-CD, PL, or PE were dissolved in distilled water (100 mL) in a conical flask. The mixture was shaken at 32 °C, 100 rpm for 48 h. The mixture was then filtered and lyophilized (DELTA2-24 LSC Plus, Martin Christ, Osterode, Germany). Dry powders of PL: HP-β-CD and PE: HP-β-CD were collected and kept in an airtight container until used.

The inclusion complex tested for antibacterial activity and hydrogel formulation was obtained from the lyophilization method at 1:2 ratio of HP-β-CD. The PL content in the complexes was determined using HPLC. The PL content in PL:HP-β-CD complex and PE:HP-β-CD were 4.6 % w/w and 1.1 % w/w, respectively.

2.6. Physico-chemical characterization of the inclusion complex

2.6.1. Scanning electron microscope (SEM)

The surface morphology of the complexes were determined using a scanning electron microscope (Quanta 400®, FEI Company, Brno, Czech Republic). Gold was used as the coating material and the acceleration voltage was set at 15.00 kV. The horizontal field width (HFW) to access the images were1.28 mm, 0.25 mm, and 0.08 mm for magnification of 100×, 500×, and 1500×, respectively.

2.6.2. Fourier-transformed infrared spectrometry

FT-IR analysis was performed on Spectrum-One® FTIR spectrometer (PerkinElmer, Inc., Massachusetts, USA) using the KBr pellet method. Ten milligrams of the sample was mixed with 100 mg of KBr using mortar and pestle. The mixture was made into a thin film. The film was transferred onto the sample holder. The percent transmittance (%T) was measured in the range of 4000 to 450 cm^{-1}, with a spectrum resolution of 1 cm^{-1}, and 16 scans.

2.6.3. X-ray diffraction (XRD)

X-ray diffraction patterns of the samples were recorded using a diffractometer (X'Pert MPD, Malvern Instruments Ltd., Philips, Netherlands). One gram of each sample was made into the pressed pellet. A copper tube was used as an X-ray tube. The wavelength was 0.154 nm (CuKα). The scan range (2θ) was 5° to 90° and a scan speed of 3°/min.

2.6.4. Differential scanning calorimetry (DSC)

The thermal behavior of the complexes was investigated using a DSC calorimeter (DSC7, PerkinElmer, Massachusetts, USA). One milligram of the sample was heated from 80 °C to 500 °C at a heating rate of 10 °C/min. Nitrogen was used as the purge gas.

2.6.5. Aqueous solubility test

The inclusion complexes were evaluated for aqueous solubility. The complex (1 g) was transferred into 5 mL of distilled water. The suspension was shaken at 37 °C, 100 rpm for 48 h. Then, the mix-

ture was filtered through a 0.22 μm membrane filter. The contents of plaunotol and plaunolide were determined by HPLC. The percent dissolved plaunotol was calculated. The samples were performed in triplicate.

2.6.6. Thermal stability test

The thermal stability of the complexes was determined according to ASEAN guidelines on stability study of drug products. The aluminum-capped vials containing 100 mg of the complex were kept in a climatic chamber (Newtronic Lifecare Equipment Pvt Ltd., Mumbai, India) at 40 °C ± 1 °C with 75% relative humidity for six months. The physical appearance of samples were recorded. The amount of PL was determined by HPLC. After each study period, samples were reconstituted with methanol and adjusted to volume (5 mL). One milliliter was aliquot, centrifuged, and the supernatant was subjected to HPLC. Samples were prepared in triplicate.

2.7. Determination of minimal inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The MIC was determined by broth microdilution assay (Rankin, 2005) using 96-well plate (Nunclon™, Thermo Scientific, Denmark). *S. aureus* and *S. epidermidis* were prepared in Müller Hinton Broth, grown in a shaking incubator (120 rpm, 37 °C, 18 h) to activate bacteria. *P. acnes* was inoculated in brain heart infusion broth, grown in an anaerobic jar, and shaking incubator (120 rpm, 37 °C, 96 h). Before the antibacterial assay, cells were harvested (3,000 rpm, 10 min) and suspended in 0.9 % w/w normal saline to get the concentration equivalent to 0.5 McFarland standard in UV–visible (625 nm). 100 μL of bacterial suspension was mixed with 9.9 mL of double-strength media in a 96-well plate.

Eight serial dilutions of PL, PL:HP-β-CD, PE, PE:HP-β-CD, clindamycin, and HP-β-CD were prepared for antibacterial activity. PL (15.6 μg/mL to 2000 μg/mL), PL:HP-β-CD, PE and PE:HP-β-CD with equivalent amounts of PL were added into the 96-well plate as well as HP-β-CD (0.32 mg/mL to 41.16 mg/mL) and clindamycin (0.45 μg/mL to 62.5 μg/mL). Clindamycin was used as positive control, and the bacterial suspension without any treatment served as negative control. Each well in 96-well plate in the sample panel was filled with 50 μL of sample solution and 50 μL of the bacterial suspension. *S. aureus* and *S. epidermidis* were incubated at 37 °C for 18 h. And plate for *P. acnes* was incubated in an anaerobic jar at 37 °C for 96 h. After the incubation period, 5 μL of resazurin solution (2 mg/mL) was added to the wells and incubated at 37 °C for 3 h. The lowest sample concentration prior to the color change was taken as the MIC value.

For the MBC determination, after the MIC test, the solution from the 96-well plate was taken, using sterile cotton bud and streaked on agar plate. The culture medium of Müller Hinton agar plate was used for *S. aureus* and *S. epidermidis*, while brain heart infusion was used for *P. acnes* for the MBC determination. After incubation, the lowest sample concentration resulting in microbial death was considered as the MBC value.

2.8. Synergy checkerboard assay

Synergistic activities of PL: HP-β-CD, PE: HP-β-CD, and clindamycin were studied using the checkerboard method (Sopirala et al., 2010). The tested bacteria were *S. aureus*, *S. epidermidis*, and *P. acnes*. The concentration range of PL: HP-β-CD and PE: HP-β-CD was from 0.11 μg/mL to 15.6 μg/mL. The concentration range of clindamycin was 0.22 μg/mL to 7.8 μg/mL. In each well of 96-well plate, 25 μL of sample solution, 25 μL of clindamycin, and 50 μL of bacterial suspension were loaded. The study had two groups: PL:HP-β-CD with clindamycin and PE:HP-β-CD with clindamycin. The MIC values were recorded after incubation. Synergistic action of the drugs was denoted as fractional inhibitory concentration index (FICI), and it was calculated as follows:

$$FICI = \frac{[MIC(A)\ in\ combination]}{MIC(A)\ alone} + \frac{[MIC(B)\ in\ combination]}{MIC(B)\ alone}$$

MIC (A) = MIC of PL:HP-β-CD or PE:HP-β-CD.
MIC (B) = MIC of clindamycin.

2.9. Formulation of hydrogels and characterization

Carbopol® Utrez20 was used as the gelling agent in the presence of 10% w/v NaOH. Five kinds of hydrogels were formulated: PL:HP-β-CD, PL:HP-β-CD and clindamycin, PE:HP-β-CD, PE:HP-β-CD and clindamycin, and clindamycin. Control was blank hydrogel. Hydrogels based on PL or PE complexed with HP-β-CD contained the equivalent amount of 50 mg of PL. The composition of hydrogels is shown in Table 1.

2.9.1. Physical appearance, pH, and morphology of the hydrogels

The physical appearance of the hydrogels was determined by visual inspection. The pH of gel samples was measured at 25 °C using a calibrated pH meter (Eutech Instruments Pte Ltd., Singapore) following mixing of 1 g of material with 25 mL of deionized water. Hydrogel samples were lyophilized before investigating the surface morphology using scanning electron microscopy (SEM) (Quanta 400®, FEI Company, Brno, Czech Republic). Samples were coated with gold prior to examination in the SEM. Pore size and size distribution were obtained from the micrographs.

2.9.2. Viscosity

The viscosity of the hydrogels was determined using a Brookfield dial reading viscometer (Brookfield Engineering Laboratories Inc., Stoughton, USA). Ten grams of each hydrogel was used for this determination, and the tests were performed in triplicate at 25 °C. The rotational speeds of the spindle were 0.5, 1, 2.5, 5, 10, and 20 rpm. The rheogram for the hydrogels was constructed as shear rate (the rotational speed) against shear stress (cP).

2.9.3. Franz cell diffusion testing

Release studies were carried out using a modified Franz diffusion cell fitted with a cellulose dialysis membrane (Spectra/Por®, Cole-Parmer, USA) as reported by Songkro et al. (2011). The length of the membrane was 4 cm. The membrane was cleaned with distilled water and hydrated by soaking in 50% v/v ethanol for 30 min. The receptor fluid (12 mL) was a degassed 50% v/v ethanol. The receptor compartment of the Franz cell was connected with to a circulating water-bath (37 °C), providing a membrane temperature of 32 °C. The diffusional surface area was 1.767 cm^2. The donor compartment was set, and one gram of hydrogel was loaded. The sample (500 μL) was withdrawn at the time intervals of 0.5, 1, 2, 3, 4, 6, 8, 10, 12, and 24 h and subjected to HPLC analysis. Four replicates of each hydrogel were analyzed for the release study. The cumulative amount of PL released from the hydrogel was determined using the equation below.

Release profiles were constructed as the cumulative amount of plaunotol in the receptor fluid per unit area of membrane (A = 1.767 cm^2) against time. The release kinetics were compared with the zero-order, Higuchi, and first-order drug release models described in Table 2.

$$Qt = VrCt + \sum_{i=0}^{t-1} VsCi$$

Q_t = drug release from sample.
V_r = volume of the receptor fluid.

Table 1
Composition of the hydrogel formulations.

Active and Excipient	PL:HP-β-CD	PL:HP-β-CD plus clindamycin	PE:HP-β-CD	PE:HP-β-CD plus clindamycin	Clindamycin	Blank
Carbopol® Ultrez 20	1.00 g	1.00 g	1.00 g	1.00 g	1.00 g	1.00 g
10% w/v NaOH	4.16 g	4.16 g	4.16 g	4.16 g	4.16 g	4.16 g
PL:HP-β-CD	1.00 g [a]	1.00 g [a]	–	–	–	–
PE: HP-β-CD	–	–	4.54 g [b]	4.54 g [b]	–	–
Clindamycin	–	0.10 g	–	0.10 g	0.10 g	–
Distilled water q.s. to	100 g	100 g	100 g	100 g	100 g	100 g

[a] PL:HP-β-CD 1:2 LM (1 g) contains equivalent of 50 mg of plaunotol.
[b] PE:HP-β-CD 1:2 LM (4.54 g) contains equivalent of 50 mg of plaunotol.

Table 2
Drug release models and their release kinetic equations.

Release model	Release kinetic equation
Zero order	$Q_t = k_0 t$
Higuchi	$Q_t = k_h t^{1/2}$
First order	$\text{Ln } Q_t = k_f t$

Q_t = drug release through the membrane (μg/cm^2).
t = time.
k_0 = rate constant for zero order model.
k_h = rate constant for Higuchi model.
k_f = rate constant for first order model.

C_t = plaunotol concentration in the receptor fluid at each sampling time.
V_s = volume of the sampling solution.
C_i = drug concentration of the i^{th} sample.

2.9.4. Assay of antibacterial activity of hydrogel

Antibacterial activity of the formulated hydrogel was determined against *S. aureus*, *S. epidermidis*, and *P. acnes* using the agar well diffusion method. The bacterial suspension was prepared as mentioned in 2.7. Bacterial suspension, which was equivalent to 0.5 McFarland standard was spread over the surface of the agar plate using sterile cotton. The agar plate was punched with the sterile stainless steel cylinder to get 300 μL capacity wells. The hydrogel was loaded into the well and incubated at 37 °C for 24 h for *S. aureus* and *S. epidermidis*. *P. acnes* was incubated under anaerobic condition at 37 °C for 96 h. The inhibition zones were measured using a Vernier caliper and recorded in millimeters.

2.9.5. Storage stability study

The storage stability of the gel formulations was evaluated according to ASEAN guidelines (2013) on the stability of drug products. One gram of hydrogel was kept in the aluminum capped 20 mL vial and put in a climatic chamber at 40 ± 1 °C, 75 %RH for six months. Aliquot from the vial was withdrawn at 0, 1, 2, 4, and 6 months intervals and was subject to HPLC analysis. PL was extracted from the hydrogel by dissolving in methanol, centrifuged, and adjusted to volume with acetonitrile. The experiment was performed in triplicate. The result was presented as a percentage (w/w) of the remaining PL in the hydrogel compared to the original PL content at time zero.

2.10. Statistical analysis

Data were reported as mean ± standard deviation (Mean ± SD). Statistical analysis was performed using analysis of variance (one-way ANOVA) with SPSS software (17.0 version), and a *p*-value less than 0.05 was set as the significance level.

3. Results

3.1. Phase solubility study

According to Higuchi and Connors phase solubility diagram, HP-β-CD showed A_L type while α-CD, β-CD, and γ-CD displayed B_S, B_S, and B_i type, respectively (Fig. 2). The phase solubility diagram suggests that HP-β-CD was the most appropriate CD to make a complex with PL. The binding constant (K_S value) of PL and HP-β-CD was calculated from the slope of the A_L type isotherm, and the K_S was 266.68 M^{-1}.

3.2. Physico-chemical properties of the complexes

3.2.1. Scanning electron microscope (SEM)

The resulting complexes obtained from different methods were found to have different surface appearance. HP-β-CD itself and PL:HP-β-CD 1:1 PM and PL:HP-β-CD 1:2 PM showed hollow spherical shape. Optical microscopy revealed the adherence of plaunotol oil droplets to the surface of porous, spherical particulates of HP-β-CD when complexes were prepared by physical mixing. However, the complexes obtained from the kneading method were of different morphology, and agglomerations of irregular shape particles were observed. Complex formation by kneading of slurries resulted in physical breakdown of the original particulate of HP-β-CD. Lyophilized complexes of PL had smooth sheet-type or platelet structures (Fig. S1).

In the SEM images of PE complexes, both ratios of PM and KM complexes did not show the original morphology of HP-β-CD. The adhesive nature of the PE completely masked the morphology of HP-β-CD particulates in complexes prepared by PM or KM and made the agglomeration and deformation of HP-β-CD. The lyophilized complexes of PE displayed smooth surfaces with flat shapes, but with fragmented form compared to those of PL complexes (Fig. S2).

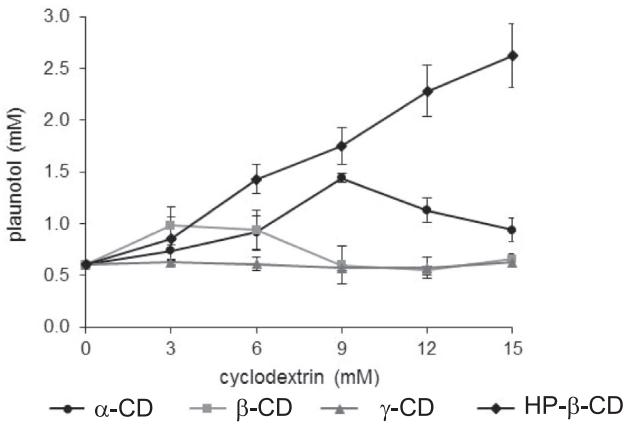

Fig. 2. Phase-solubility diagram of plaunotol in aqueous solution of cyclodextrins.

3.2.2. Fourier-transformed infrared spectrometry (FT-IR)

The FT-IR spectra of PL, PE, HP-β-CD, and complexes are depicted in Fig. 3. An acyclic structure of PL presented a dominant sharp peak at 1668 cm^{-1} and broad band at 3333 cm^{-1} consistent with C = C stretching and O-H stretching, respectively. Complexations between PL and HP-β-CD in all types of preparation showed complete superimposition bands of PL and HP-β-CD spectra, and the sharp C = C stretching of PL was disappeared (Fig. 3A). The spectra obtained from PE complexes prepared by lyophilization were similar to the HP-β-CD spectrum. The appearance of a band at 1650 cm^{-1} and 1738 cm^{-1} is attributed to PE. These bands were attenuated in the spectra of complexes prepared by lyophilization (Fig. 3B).

3.2.3. X-ray diffraction (XRD)

Samples of PL and PE were oily viscous substances and unsuitable for analysis by XRD. Diffractograms of all plaunotol complexes displayed an amorphous pattern similar to the one observed for HP-β-CD (Fig. 4A). In contrast, crystalline peaks were recorded for PE complexes at 2θ values of 15° and 26° for physical mixtures, 2θ values of 15° only for complexes prepared by kneading (Fig. 4B). The diffractograms of PE complexes prepared by lyophilization showed only an amorphous character.

3.2.4. Differential scanning calorimetry (DSC)

DSC thermograms revealed a small exothermic PL peak at 361 °C (Fig. 5A). Endothermic transitions were observed at 104 °C and 355 °C for HP-β-CD. The major endotherm relating to the glass to rubber transition temperature (Tg) was replicated in all plaunotol complexes at a broadly similar temperature at 340 °C to 356 °C. Small endothermic peaks were detected at 98 °C, 262 °C, and 282 °C in thermograms obtained for PE, indicating the presence of crystalline material (Fig. 5B). The glass to rubber transition of HP-β-CD measured at approximately 350 °C was not clear in PE complexes prepared by physical mixing or kneading, compared with plaunotol complexes. However, the endothermic transition was detected between 347 °C and 351 °C in complexes prepared by lyophilization.

3.3. Aqueous solubility and thermal stability of the complexes

Non-complexed PL exhibited poor aqueous solubility in distilled water. Only about 16 % of PL was dissolved in water. Among PL complexes, the highest percentage of PL dissolved in distilled water was observed in the complexes prepared by lyophilization method (LM). The mixing method of PM and KM at ratios of 1:1 and 1:2 yielded PL water solubility of about 20% and 40%, respectively (Fig. 6A). The PL content of PE dissolved in water was about 1%, which was less than that of pure PL. The solubility of PL in PE:HP-β-CD prepared by PM or KM resulted in a minor improvement in solubility while complexation by LM dramatically increased the solubility to almost 100%. (Fig. 6B).

PL, PE, HP-β-CD, and complexes were investigated for their stability under the accelerated condition of 40 °C with 75% RH for 6 months. In the case of PL:HP-β-CD, the content of PL left in the PM, KM, and LM complexes were about 65, 80, and 90%, respectively. Pure PL showed only 39% remaining after storage for 6 months (Fig. 7A). In contrast, for PE:HP-β-CD, PL that remained in the KM and LM complexes was about 97% and 100%, respectively (Fig. 7B). It should be noted that the appearance of PL- and PE-complexes prepared from PM and KM changed from a powdery to partially melted state, while the LM complex retained its powdery form (Fig. S3-Fig. S4).

3.4. Antibacterial activity of plaunotol, plaunoi extract and their complexes

The MIC values measured against *S. aureus*, *S. epidermidis*, and *P. acnes* are presented in Table 3. Complexation of PL or PE with HP-β-CD indicated a significant improvement in bacteriostatic activity, except PL and PL-HP-β-CD against *S. aureus*. The MBC values presented in Table 3 confirm the superior bacteria-killing activity of PL or PE complexes and clindamycin.

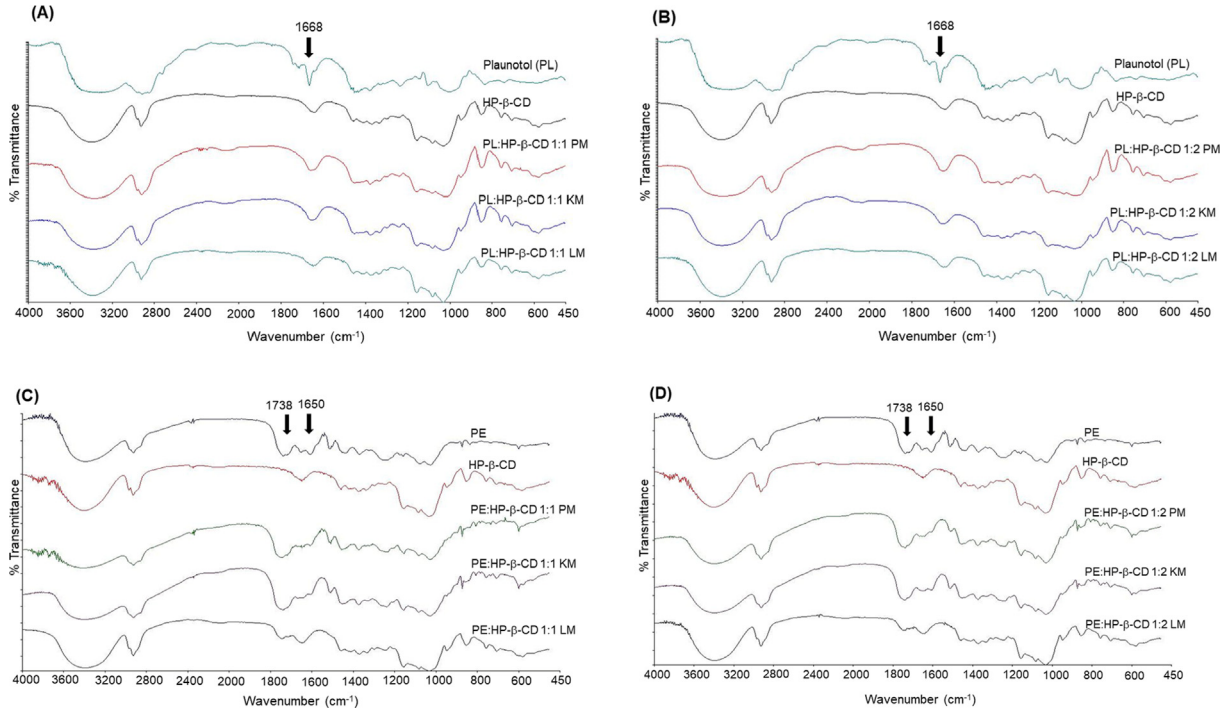

Fig. 3. FT-IR spectra of the complexes: A) PL: HP-β-CD 1:1, B) PL:HP-β-CD 1:2, C) PE:HP-β-CD 1:1 and D) PE:HP-β-CD 1:2.

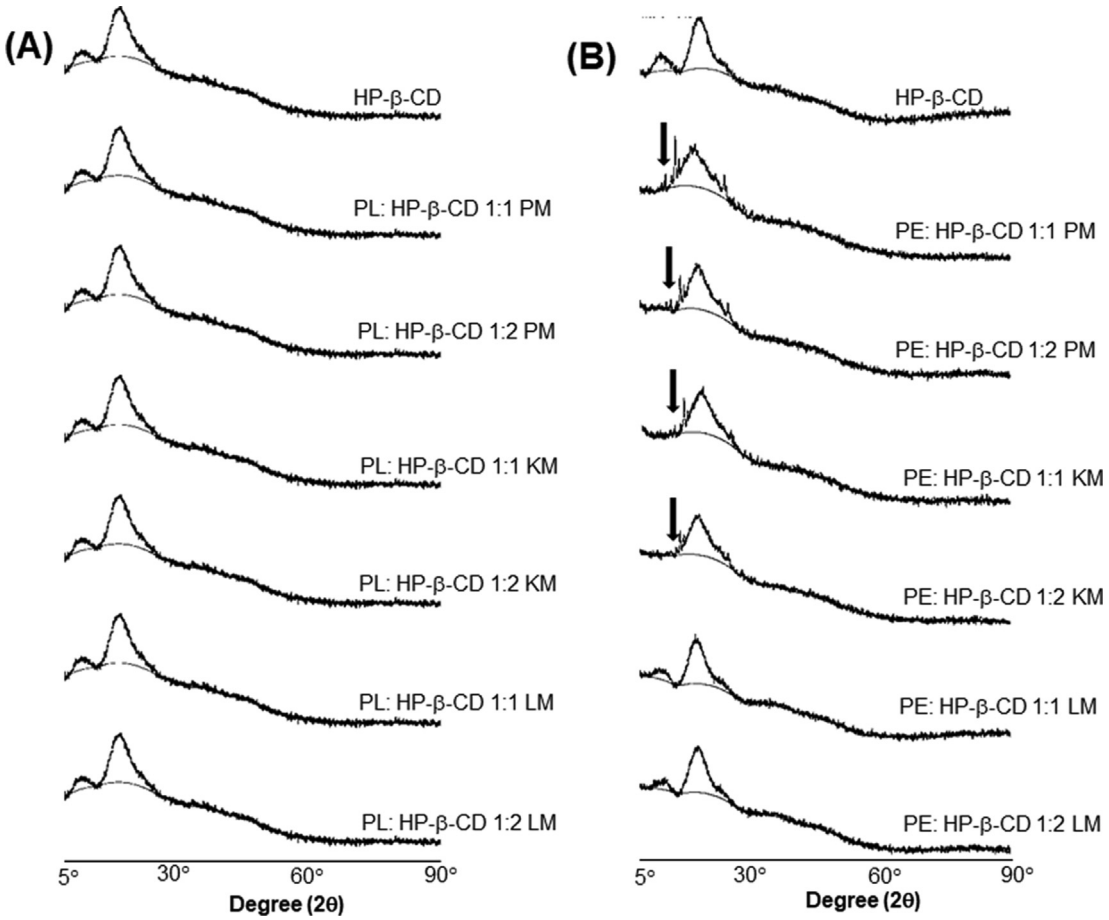

Fig. 4. Diffractograms of the complexes: A) PL:HP-β-CD complexes and B) PE::HP-β-CD complexes.

3.4.1. Synergistic activity of the inclusion complex and clindamycin

Considering that PL:HP-β-CD and PE:HP-β-CD had antibacterial activities as revealed in the previous paragraph, in this work, we also sought to understand whether the prepared inclusion complexes had synergistic antibacterial activity with clindamycin using a checkerboard assay. The MIC values of single components are shown in Table 3. The MIC values of the combinations were parallel studies recorded and calculated for the FICI (Table 4). The FICI values of PL:HP-β-CD with clindamycin were in the range of 0.03 to 0.15, and it showed an excellent synergistic action between PL:HP-β-CD and clindamycin. FICI value of PE:HP-β-CD and clindamycin against *S. aureus* was 1.5, and against *S. epidermidis* and *P. acnes* was 0.5.

3.5. Hydrogel formulations

3.5.1. Physical appearance, pH, and morphology of hydrogels

Most of the hydrogel samples exhibited translucent/transparent appearance following preparation and after storage for six months at 40 °C ± 1 °C, 75 % RH (Table 5). The yellow coloration of gels containing PE:HP-β-CD changed to a brownish shade on storage (Fig. S5). The pH of freshly prepared gel samples ranged from 6.4 to 7.3 and was essentially unchanged (6.2–6.9) following storage at 40 °C, 75% RH for six months (Table 5).

Morphological examination of lyophilized hydrogels using SEM revealed similar highly porous structures of interconnected, irregular-shaped pores (Fig. 8). The range of pore sizes in hydrogels containing PL or PE complexes and clindamycin were approximately 25–100 μm and tended to be smaller than blank gels (80–100 μm)(Table 6).

3.5.2. Viscosity of the hydrogels

All hydrogels exhibited similar rheograms. In freshly prepared formulations, the viscosity of PE complex was slightly lower than that of PL complex. The rank order of viscosity was clindamycin hydrogel > blank hydrogel > PL:HP-β-CD with clindamycin > PL:HP-β-CD hydrogel > PE:HP-β-CD with clindamycin hydrogel > PE:HP-β-CD hydrogel (Fig. 9).

3.5.3. Plaunotol release behavior

The amount of plaunotol released from the hydrogels was monitored throughout a period of 24 h. All hydrogels displayed gradual plaunotol release over 24 h. However, hydrogels containing PE:HP-β-CD complex showed an initial lag phase extending for approximately 4 h and the lowest rate of PL release. Hydrogels containing a combination of PE:HP-β-CD complex and clindamycin resulted in a small burst effect over the first hour, prior to gradual PL release over the remaining test period (Fig. 10).

The release parameters obtained on fitting the plaunotol release data to the zero-order, Higuchi, and first-order kinetic models are listed in Table 7. All hydrogel formulations showed best fit to the Higuchi release kinetic model (coefficient of correlation (r^2) >=0.9728). In comparison, the release rate constant order was PL hydrogel > PL: HP-β-CD hydrogel > PL: HP-β-CD with clindamycin hydrogel > PE: HP-β-CD hydrogel > PE: HP-β-CD with clindamycin hydrogel.

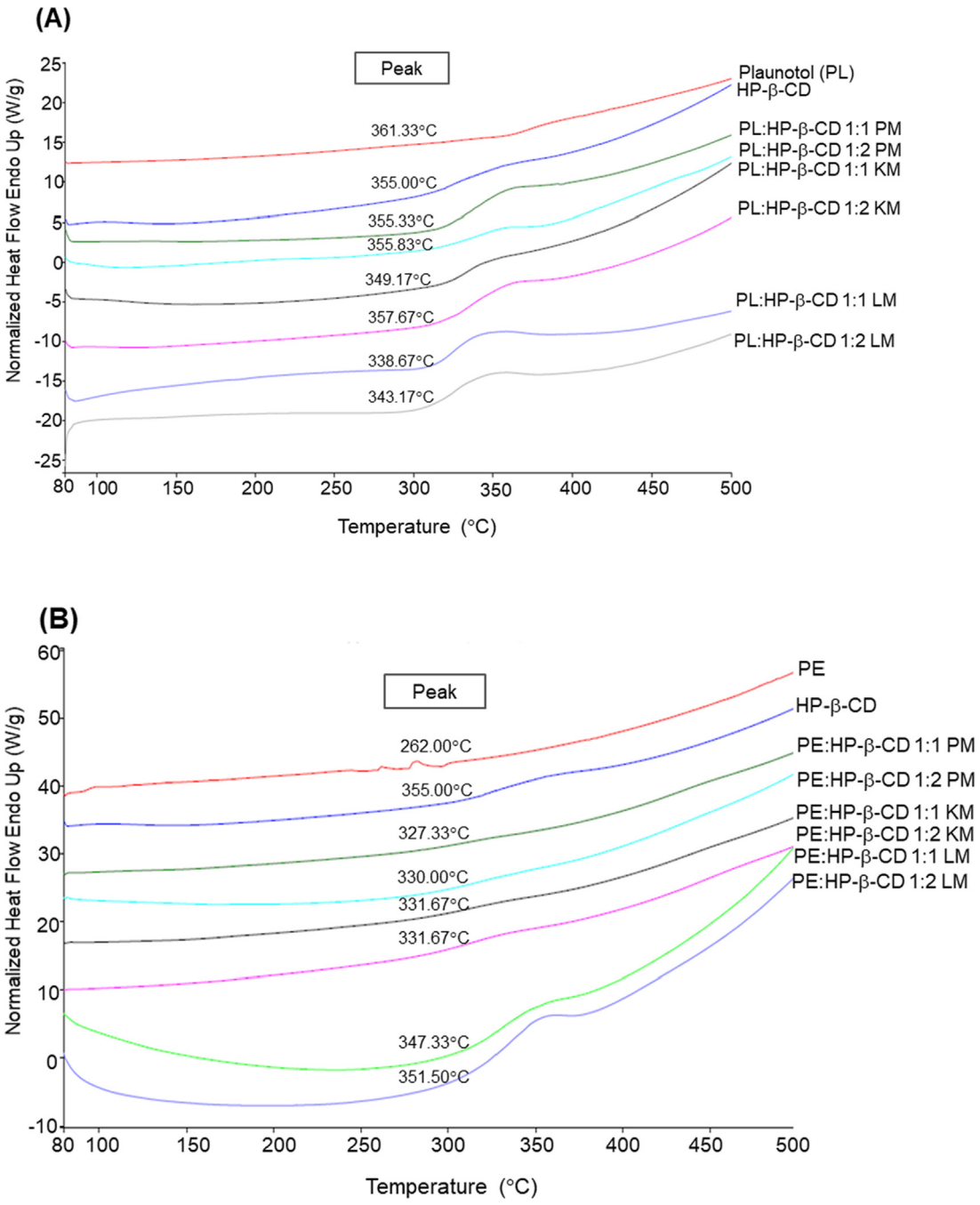

Fig. 5. Thermograms of the complexes: A) PL:HP-β-CD complexes and B) PE::HP-β-CD complexes.

3.5.4. Antibacterial activity of hydrogels

Antibacterial activity of the hydrogels was determined using the agar well diffusion method. The PL:HP-β-CD, PE:HP-β-CD, and blank gel did not result in a prominent inhibition zone (Table 8). PL:HP-β-CD combined with clindamycin in the hydrogel showed significant increasing antibacterial activity. Both formulations resulted in significantly higher activity than both clindamycin hydrogels.

3.5.5. Storage stability of hydrogels

The physical appearances of hydrogels were evaluated via visual perception (Table 5, Fig. S5). The pH of six kinds of hydrogel formulations are summarized in Table 5. The amount of intact plaunotol in hydrogel samples that had been stored for six months at 40 °C, 75% RH was>94% (Fig. 11).

4. Discussion

Cyclodextrins are widely used as smart pharmaceutical excipients to improve the solubility and stability of drug substances. By virtue of their hydrophobic internal cavity and hydrophilic outer surface, they are capable of improving the aqueous solubility of poorly water-soluble drugs (Duchêne, 2011). Based on the results from the phase solubility studies, HP-β-CD was found to be the most appropriate host polymer among the various cyclodextrins considered for the preparation of the inclusion complexes with PL.

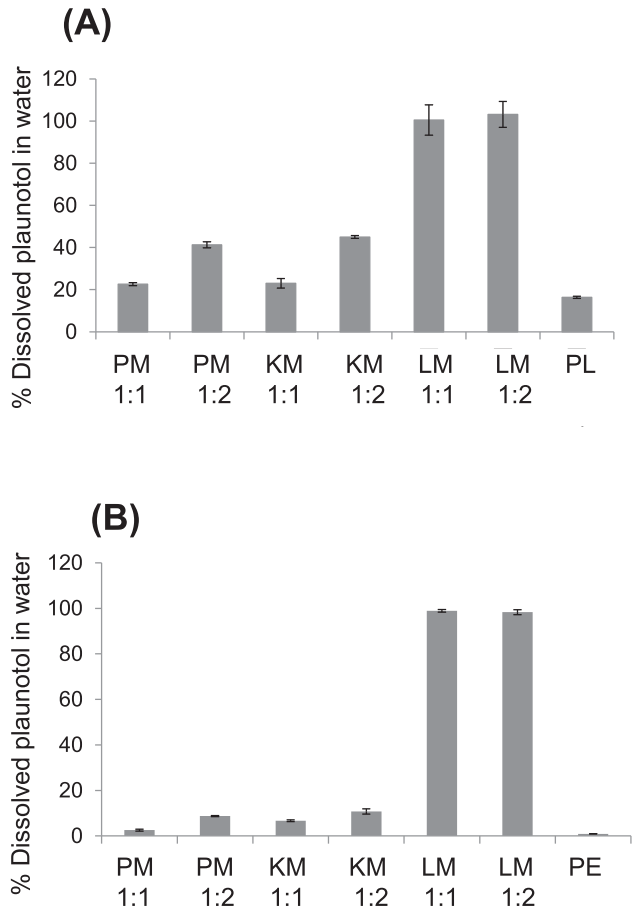

Fig. 6. Aqueous solubility study: Percent plaunotol dissolved in water A) in PL:HP-β-CD complexes and B) in PE: PE:HP-β-CD complexes. Each point represents mean ± SD, n = 3 where n is the number of samples.

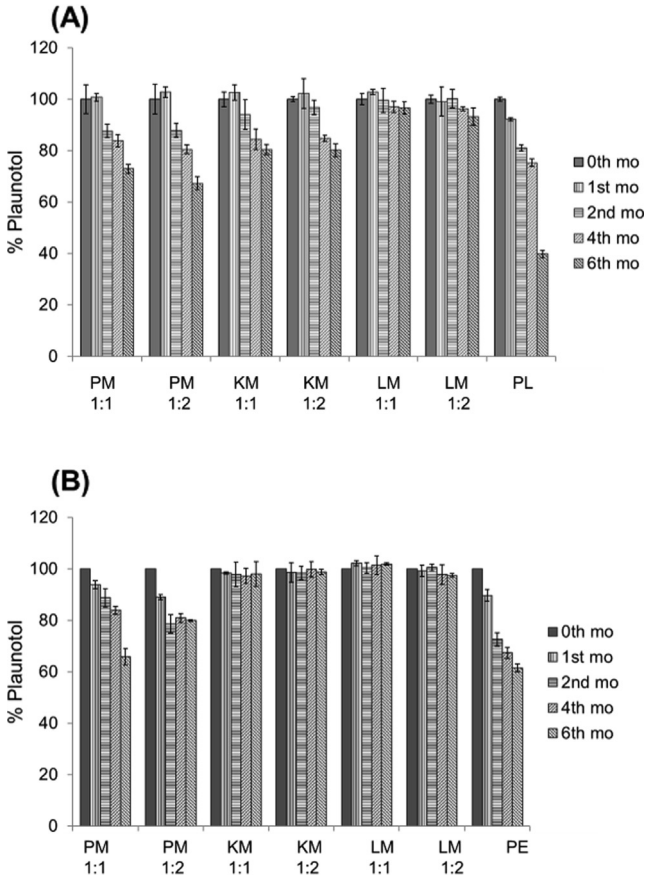

Fig. 7. Thermal stability of plaunotol in the complexes: A) PL:HP-β-CD complexes and B) PE::HP-β-CD complexes. Each point represents mean ± SD, n = 3 where n is the number of samples.

In this study, inclusion complexes of PL or PE with HP-β-CD were successfully prepared by the lyophilization method. The linear structure of PL presumably facilitated the entry of the molecule into the hydrophobic cavity of HP-β-CD. The ratio 1:1 of HP-β-CD was sufficient to properly accommodate PL into the inner cavity. In contrast to PL, PE was obtained from partially purified ethanol crude extract by adsorbing on a hydrophobic resin. The PE contained 8.81% w/w PL. Besides, it also contained other components such as diterpenes, phytosterols, and fatty acids (Kitazawa and Ogiso, 1981, Kitazawa et al., 1982, Aung et al., 2021). As shown in Fig. 3C and 3D, PM and KM preparations could not incorporate all the components of PE into the inner cavity of the excipient. The additional components from PE that were not harbored within the polymer were probably adsorbed unto its surface.

Notably, the complexes prepared by LM were found to display different characteristics. This was quite apparent as evidenced by the XRD results. All the inclusion complexes containing PL were amorphous. For the inclusion complexes containing PE, only the complexes prepared by LM were completely amorphous. Those prepared by PM and KM indicated the presence of some crystalline character. In other words, some portion of PE remained crystalline after preparation of the complexes. The amorphous nature attributed to the LM approach can lead to enhanced aqueous solubility of the prepared complexes.

Active pharmaceutical ingredients (APIs) often encounter difficulties associated with low aqueous solubility and stability. This does not only limit the bioavailable of the drug but also constitutes a challenge during formulation development (Chaudhary and Patel, 2012). Insights from the physicochemical properties of the substances are essential for selecting a suitable polymer and encapsulation method to obviate some of these limitations (Saokham et al., 2018). The inclusion complexes prepared using HP-β-CD, i.e., PL: HP-β-CD and PE:HP-β-CD improved the aqueous solubility and thermal stability. Similarly, increased water solubility was previously reported from encapsulation of APIs with β-CD using spray drying and kneading methods (Borghetti et al., 2009). Combining naringenin with HP-β-CD prepared by the freeze-drying method improved its solubility (Wen et al., 2010). Many studies confirmed that enhancing the solubility of active components also improved their pharmacological activity and bioavailability. The inclusion complex of garlic oil/β-CD displayed higher antibacterial activity than garlic alone (Piletti et al., 2019). The inclusion complex of umbelliferone with α-CD showed good bioavailability and minimized the therapeutic dose (Roy et al., 2020). On the basis of these findings, we suspected that the PL:HP-β-CD and PE: HP-β-CD prepared in the present study could exhibit better antibacterial activity and may be used in combination with a conventional antibiotic.

Complexation of PL or PE with HP-β-CD generally improved the antibacterial activity of the compounds, which is explained by enhancing PL and PE solubility. In a related study, the complex formation of lavender essential oil and HP-β-CD improved the active compound's aqueous solubility. The complex enhanced the antibacterial activity 3-fold by increasing the drug accession at the membrane and inside the cytoplasm of the bacteria (Yuan et al., 2019). Likewise, a cyclodextrin-based carrier was developed to improve drug solubility, stability, sustained drug release, and

Table 3
Minimum inhibitory concentration (MIC) values and minimum bactericidal concentration (MBC) values for PL, PE and their complexes with HP-β-CD against acne-associated bacteria *S. aureus*, *S. epidermidis* and *P. acnes*.

Sample	MIC (μg/mL)			MBC (μg/mL)		
	S. aureus	*S. epidermidis*	*P. acnes*	*S. aureus*	*S. epidermidis*	*P. acnes*
PL	500	1000	500	500	2000	500
PL:HP-β-CD	500	250	250	500	500	500
PE	80	80	80	160	160	80
PE:HP-β-CD	25	25	25	100	100	25
Clindamycin	31	15	31	31	31	31
HP-β-CD	>40000	>40000	>20000	>40000	>40000	>40000

Table 4
Minimum inhibitory concentration (MIC) values of PL:HP-β-CD, PE:HP-β-CD in combination with clindamycin and fractional inhibitory concentration index (FICI) of the combination the complex with clindamycin.

Bacteria	MIC (μg/mL) in combination		FICI[a] PL: HP-β-CD and clindamycin	MIC (μg/mL) in combination		FICI[a] PE: HP-β-CD and clindamycin
	PL: HP-β-CD	clindamycin		PE: HP-β-CD	clindamycin	
S. aureus	7.8	0.5	0.15	6.2	7.8	1.5
S. epidermidis	7.8	1.9	0.03	12.5	15.6	0.5
P. acnes	7.8	3.9	0.15	6.2	7.8	0.5

[a] FICI value was calculated from the MIC values of single drug from Table 2 and of in combination.

Table 5
Physical appearance and pH of the hydrogels.

Hydrogel	Physical appearance		pH (mean ± SD, n = 3)	
	freshly prepared	after 6 months	freshly prepared	after 6 months
PL:HP-β-CD	Clear and transparent	Clear and transparent	6.67 ± 0.01	6.67 ± 0.02
PL:HP-β-CD with clindamycin	Clear and transparent	Clear and transparent	7.33 ± 0.01	6.67 ± 0.01
PE:HP-β-CD	Yellowish and translucent	Brownish and translucent	6.37 ± 0.01	6.27 ± 0.02
PE: HP-β-CD with clindamycin	Yellowish and translucent	Brownish and translucent	6.43 ± 0.04	6.24 ± 0.07
Clindamycin	Clear and transparent	Clear and transparent	6.73 ± 0.01	6.80 ± 0.02
Blank	Clear and transparent	Clear and transparent	6.53 ± 0.02	6.87 ± 0.02

n = 3, where n is number of sample replicates.

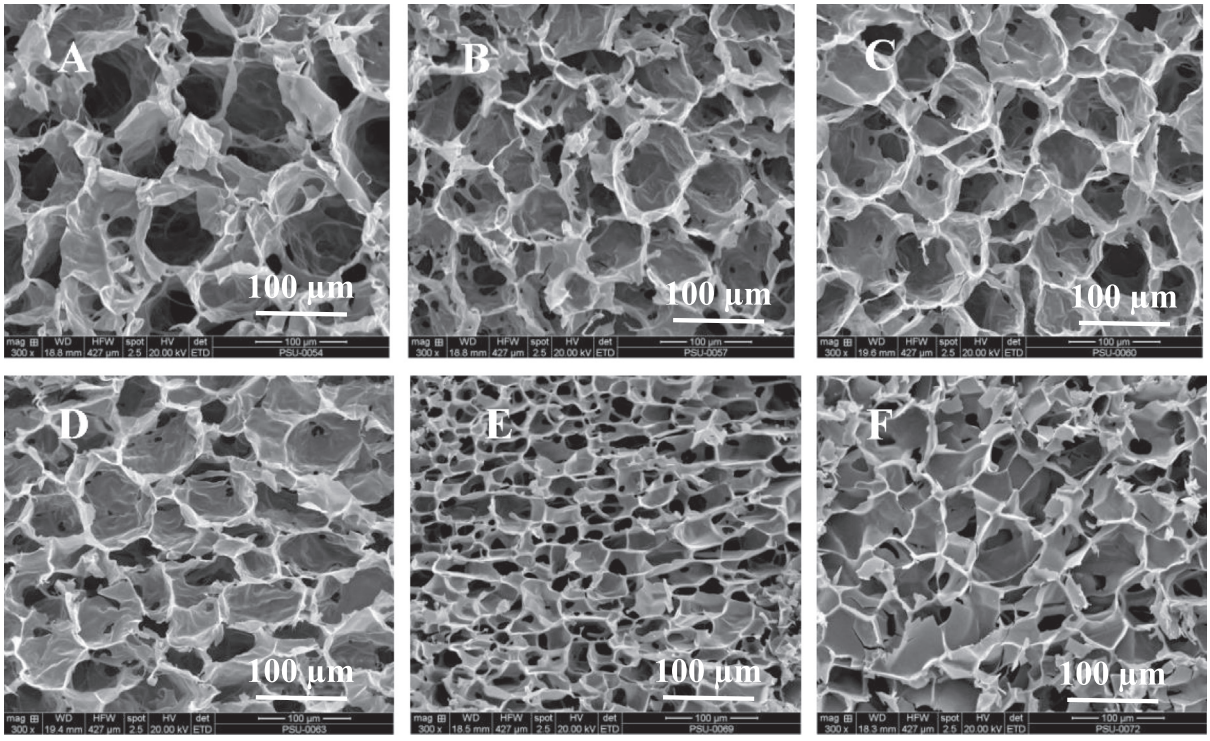

Fig. 8. Photomicrographs (300 ×) of hydrogels: A) blank, B) clindamycin, C) PL:HP-β-CD, D) PL:HP-β-CD with clindamycin, E) PE:HP-β-CD and F) PE:HP-β-CD with clindamycin.

Table 6
Ranges of the pore sizes of the various hydrogels.

Hydrogel	Pore size (μm)
Blank gel	78–101
Clindamycin	43–100
PL:HP-β-CD	54–96
PL:HP-β-CD with clindamycin	54–89
PE:HP-β-CD	24–68
PE:HP-β-CD with clindamycin	28–83

limit photodegradation, resulting in better treatment of acne vulgaris (Vyas et al., 2014). Avoiding the side effects or developing resistance to antibiotics, topical antibiotics are recommended for control of acne vulgaris, according to the American Academy of Dermatology. For instance, 1% w/w clindamycin is prescribed as a systemic antibacterial agent for adult acne (Zaenglein et al., 2016).

In this study, PE exhibited antibacterial activity against acne-causing bacteria better than PL and nearly equivalent to the ability of clindamycin at tested concentration. Complexation of the agents with HP-β-CD slightly increased their antibacterial activity. A combination of antibacterial agents is recommended, as suggested in the guideline. We combined PL: HP-β-CD or PE: HP-β-CD with clindamycin and performed a checkerboard assay. The result showed that the inclusion complexes exhibited synergistic and additive effects with clindamycin. Hydrogels were then prepared in the presence of 0.1% w/w clindamycin in combination with the inclusion complex. The hydrogels were evaluated for their physicochemical properties.

Hydrogels containing PL and PE complexes were subsequently prepared using Carbopol® Ultrez20 as the gel-forming polymer. The polyacrylic acid polymers display sol–gel transition in aqueous solution at pKa values above approximately 5.5 and have been investigated extensively as topical delivery systems for application to the skin and eye (Bukhari et al., 2015; Calixto et al., 2015; Karolewicz, 2015). The antibacterial hydrogels produced in the present study contained either PL:HP-β-CD complex or PE:HP-β-CD complex alone or each complex in combination with clindamycin. The appearance of PL:HP-β-CD hydrogel looked better than PE:HP-β-CD hydrogel throughout the stability testing period. The pH of freshly prepared gel samples and those stored at 40 °C, 75% RH for 6 months ranged from 6.2 to 7.3 and are within acceptable limits (4.0–7.0) for topical application (Lambers et al., 2006; Józsa et al., 2020). Ostensibly, the pore sizes of PE:HP-β-CD hydrogels were smaller than PL:HP-β-CD hydrogels. The pore size correlates with the viscosity of the hydrogels. The porous hydrogel network controlled the friction or hydrodynamic flow and was inversely proportional to the pore size (Cuccia et al., 2020). The PE hydrogel showed low viscosity at a slow shear rate compared to blank and clindamycin hydrogel. All hydrogel formulations showed non-Newtonian, pseudoplastic flow behavior or 'shear thinning', which is generally regarded as advantageous for application to the skin. >94 % w/w of the plaunotol content of hydrogels remained intact after prolonged storage at 40 °C, 75% RH for six months.

The kinetic release profiles of PL from hydrogels showed the best fit with the Higuchi model (Table 7), suggesting that diffusion of PL molecules controls the release mechanism through fluid-filled pores of the hydrogel matrix. The highly interconnected pore structure revealed in the dried hydrogels (Fig. 8) is expected to facilitate the diffusion process. The release rates of the formulations containing PL:HP-β-CD, PL:HP-β-CD with clindamycin, and PE:HP-β-CD with clindamycin were not significantly different ($p > 0.05$). Comparatively, lower release rate was found in hydrogels containing PE:HP-β-CD and clindamycin. The reason for this behavior is unclear at present. Other components in PE may interact with clindamycin or obstruct the release of PL from the hydrogel.

In contrast to PL and PE complexes with HP-β-CD, hydrogels of the active ingredients did not give rise to prominent antibacterial activity. This behavior suggests that the release over 24 h of PL or PL associated with other antibacterial compounds from PE was insufficient to attain the MIC. The absence of activity for PE:HP-β-CD hydrogels compared with PL:HP-β-CD hydrogels (Table 8) may correlate with the reduced rate of release of PL in the former system. However, PL:HP-β-CD or PE:HP-β-CD combined with clindamycin in the hydrogel showed significant increases in antibacterial effect. This evidence suggests that PL released from the hydrogels is acting to enhance the activity of clindamycin. The present study indicated that combining the complexes with clindamycin synergized the antibacterial activity. Moreover, only 1/10th of the recommended concentration of clindamycin was used in the formulation. The benefit of utilizing lower amounts antibiotics is that it can reduce the side effect and avoid the incidence of resistance to antibacterial agents (Zaenglein et al., 2016).

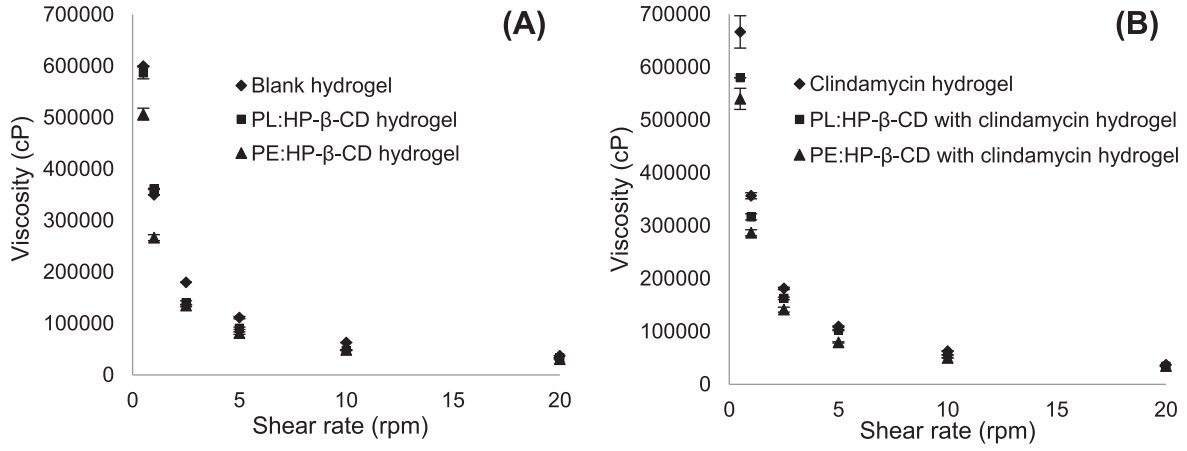

Fig. 9. Rheograms of freshly prepared hydrogels: A) blank, PL:HP-β-CD, PE:HP-β-CD and B) clindamycin, PL:HP-β-CD with clindamycin, PE:HP-β-CD with clindamycin. Each point represents mean ± SD, n = 3 where n is number of samples.

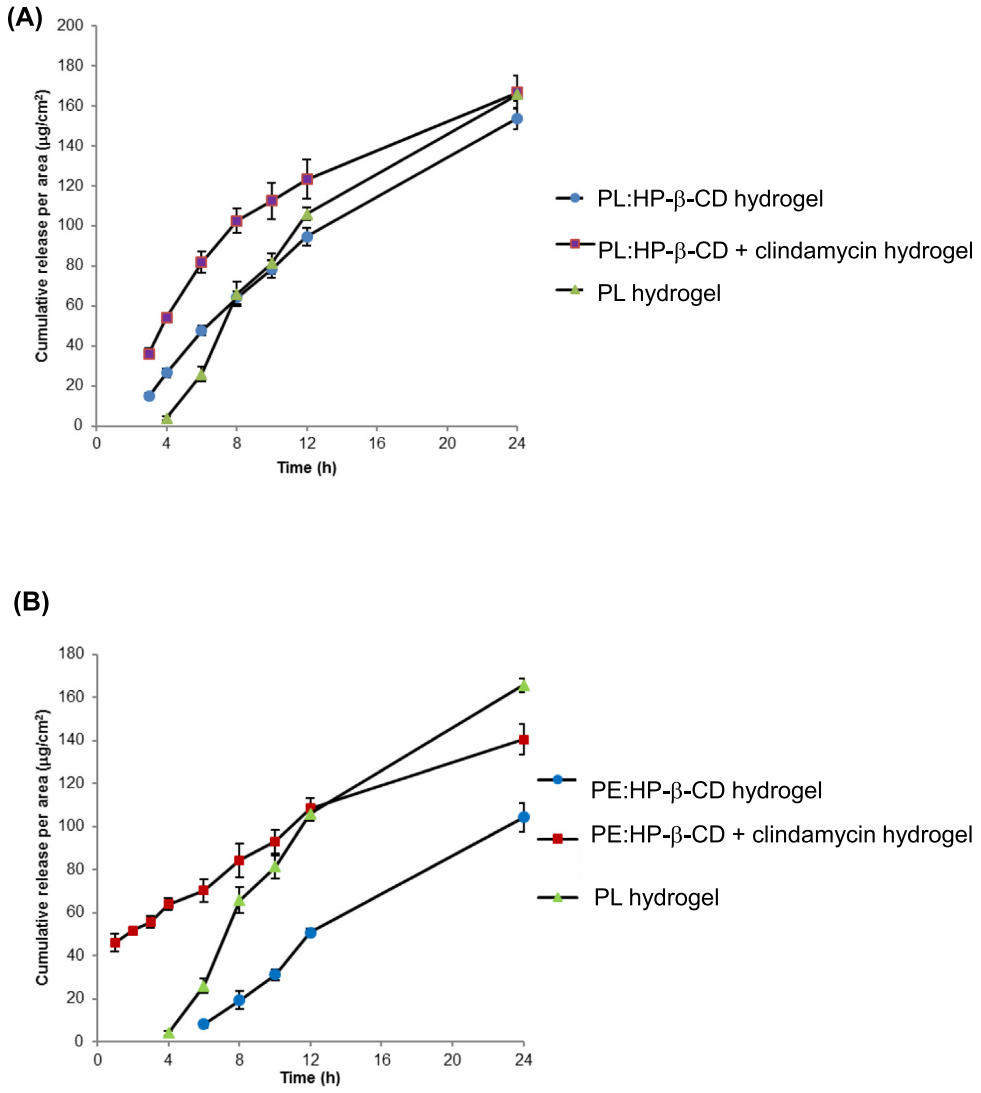

Fig. 10. *In vitro* plaunotol release from hydrogels in the presence of clindamycin compared with PL hydrogel: A) PL:HP-β-CD complexes and B) PE::HP-β-CD complexes. Each point represents mean ± SD, n = 4 where n is the number of replicates.

Table 7
Release parameters of plaunotol from different hydrogels.

Gel formulation	Zero order		Higuchi model		First order	
	r^2	k_0 (µg/cm²/h) (n = 4, mean ± SD)	r^2	k_h (µg/cm²/h$^{1/2}$) (n = 4, mean ± SD)	r^2	k_f (1/h) (n = 4, mean ± SD)
PL:HP-β-CD	0.9679	6.4691 ± 0.2411	0.9991	*44.0645 ± 1.4812	0.7484	0.0962 ± 0.0029
PL:HP-β-CD with clindamycin	0.8805	5.7919 ± 0.4455	0.9614	*40.574 ± 2.7970	0.7051	0.0616 ± 0.0036
PE: HP-β-CD	0.9858	5.3233 ± 0.4314	0.9938	*40.231 ± 3.1850	0.802	0.1232 ± 0.0118
PE: HP-β-CD with clindamycin	0.9648	4.2449 ± 0.3492	0.9728	**25.4550 ± 2.0185	0.8944	0.0491 ± 0.0034

Each point represents mean ± SD, n = 4 where n is the number of replications.
* The release rates of PL:HP-β-CD, PL:HP-β-CD with clindamycin and PE:HP-β-CD hydrogels displayed no significant difference among these three formulations ($p > 0.05$).
** PE:HP-β-CD with clindamycin exhibited the lowest release rate among all hydrogels (p less than 0.05).

Table 8
Antibacterial activity of hydrogels using agar well diffusion methods.

Hydrogel	Inhibition zone (mm), (mean ± SD, n* = 3)		
	S. aureus	*S. epidermidis*	*P. acnes*
PL:HP-β-CD	9.33 ± 0.25	9.20 ± 0.34	8.73 ± 0.25
PL:HP-β-CD with clindamycin	28.26 ± 0.23	28.13 ± 0.57	28.07 ± 0.25
PE:HP-β-CD	8.00 ± 0.00	8.00 ± 0.00	8.00 ± 0.00
PE:HP-β-CD with clindamycin	26.80 ± 0.17	22.07 ± 0.87	21.36 ± 0.21
Clindamycin	22.83 ± 0.31	20.36 ± 0.40	17.53 ± 0.68
Blank	8.00 ± 0.00	8.00 ± 0.00	8.00 ± 0.00

n* is the number of replicates.

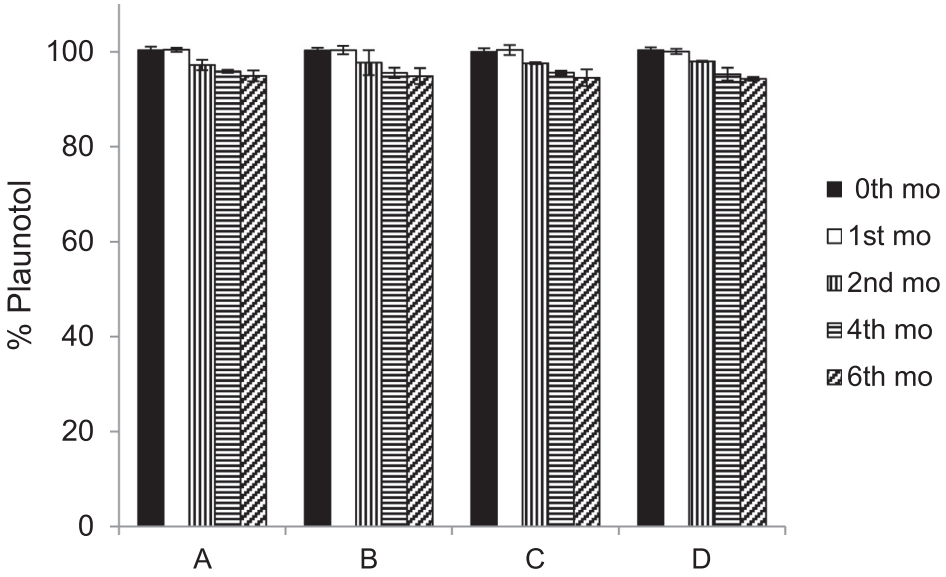

Fig. 11. Stability study of hydrogels: A) PL:HP-β-CD, B) PL:HP-β-CD with clindamycin, C) PE:HP-β-CD and D) PE:HP-β-CD with clindamycin. Each point represents mean ± SD, n = 3 where n is the number of samples.

5. Conclusion

The lyophilization method was used for the successful preparation of inclusion complexes of PL or PE with HP-β-CD. The oily state of PL and PE changed to powder form. Additionally, it enhanced the aqueous solubility and thermal stability of the APIs and the hydrogel was easy to formulate. Assessment of antibacterial and synergistic activities against acne-causing bacteria led to conclusive results indicating that the inclusion complexes and clindamycin have synergistic interaction. Hydrogels containing clindamycin and PL or PE complexes prepared using Carbopol as a gel-forming polymer showed substantial promise as topical antibacterial agents for treating acne.

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Declaration of Competing 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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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jsps.2022.04.004.

References

Aelenei, P., Rimbu, C.M., Horhogea, C.E., Lobiuc, A., Neagu, A.N., Dunca, S.I., Motrescu, I., Dimitriu, G., Aprotosoaie, A.C., Miron, A., 2020. Prenylated phenolics as promising candidates for combination antibacterial therapy: morusin and kuwanon G. Saudi Pharm. J. 28, 1172–1181. https://doi.org/10.1016/j.jsps.2020.08.006.

Aswathy, S.H., Narendrakumar, U., Manjubala, I., 2020. Commercial hydrogels for biomedical applications. Heliyon. 6, e03719. https://doi.org/10.1016/j.heliyon.2020.e03719.

Aung, W.M., Limsuwanchote, S., Wungsintaweekul, J., 2021. Preparation of diterpenes-enriched extract from *Croton stellatopilosus* ohba leaves using enzyme- and ultrasonic-assisted extraction. Phcog Res 13 (1), 22.

Borghetti, G.S., Lula, I.S., Sinisterra, R.D., Bassani, V.L., 2009. Quercetin/β-cyclodextrin solid complexes prepared in aqueous solution followed by spray-drying or by physical mixture. AAPS Pharm Sci Tech. 10 (1), 235–242. https://doi.org/10.1208/s12249-009-9196-3.

Bukhari, S.M.H., Khan, S., Rehanullah, M., Ranjha, N.M., 2015. Synthesis and characterization of chemically cross-linked acrylic acid/gelatin hydrogels: effect of pH and composition on swelling and drug release. Int. J. Polym. Sci. 2015, 187961. https://doi.org/10.1155/2015/187961.

Calixto, G., Yoshii, A.C., Silva, H.R., Cury, B.S.F., Chorilli, M., 2015. Polyacrylic acid polymers hydrogels intended to topical drug delivery: preparation and characterization. Pharm. Dev. Technol. 20 (4), 490–496. https://doi.org/10.3109/10837450.2014.882941.

Chanda, S., Baravalia, Y., 2010. Novel leads from herbal drugs for infectious skin diseases. In: Mendez-Vilas, A. (Ed.), Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology. Formatex Research Center, Spain, pp. 451–456.

Chatatikun, .M, Yamauchi, T., Yamasaki, K., Aiba, S., Chiabchalard, A., 2018. Anti-melanogenic effect of *Croton roxburghii* and *Croton sublyratus* leaves in α-MSH stimulated B16F10 cells. J. Tradit. Complement Med. 9(1), 66–72. https://doi.org/10.1016/j.jtcme.2017.12.002.

Chaudhary, V.B., Patel, J.K., 2012. Cyclodextrin inclusion complex to enhance solubility of poorly water soluble drugs: a review. Int. J. Pharm. Sci. 4 (1), 68–76. https://doi.org/10.13040/IJPSR.0975-8232.4(1).68-76.

Cuccia, N., Pothineni, S., Wu, B., Harper, J.M., Burton, J.C., 2020. Pore-size dependence and slow relaxation of hydrogel friction on smooth surfaces. PNAS. 117 (21), 11247–11256. https://doi.org/10.1073/pnas.1922364117.

Duchêne, D., 2011. Cyclodextrins and their inclusion complexes. In: Bilensoy, E. (Ed.), Cyclodextrins in pharmaceutics, cosmetics, and biomedicine, current and future industrial applications. John Wiley & Sons Inc, Singapore, pp. 3–18.

Fu, H.Y., Yabe, Y., Asahi, K., Hayashi, Y., Murata, H., Eguchi, H., Tsujii, M., Tsujii, S., Kawano, S., 2005. (*2E,6Z,10E*)-7-hydroxymethyl-3,11,15-trimethyl-2,6,10,14-hexadecatetraen-1-ol (plaunotol) increases cyclooxygenase-2-expression via nuclear factor κB and cyclic AMP response element in rat gastric epithelial cells. Eur. J. Pharmacol. 524, 38–43. https://doi.org/10.1016/j.ejphar.2005.09.044.

Gollnick, H., Cunliffe, W., Berson, D., Dreno, B., Finlay, A., Leyden, J.J., Shalita, A.R., Thiboutot, D., 2003. Management of acne: A report from a global alliance to improve outcomes in acne. J. Am. Acad. Dermatol. 49 (1), S1–S37. https://doi.org/10.1067/mjd.2003.618.

Hada, T., Furuse, S., Matsumoto, Y., Hamashima, H., Masuda, K., Shiojima, K., Arai, T., Sasatsu, M., 2001. Comparison of the effects *in vitro* of tea tree oil and plaunotol on methicillin-susceptible and methicillin-resistant strains of *Staphylococcus*

aureus. Microbios. 106 (Suppl 2), 133–141 https://pubmed.ncbi.nlm.nih.gov/11548201.

Józsa, L., Ujhelyi, Z., Vasvári, G., Sinka, D., Nemes, D., Fenyvesi, F., Váradi, J., Vecsernyés, M., Szabó, J., Kalló, G., Vasas, G., Bácskay, I., Fehér, P., 2020. Formulation of creams containing *Spirulina platensis* powder with different nonionic surfactants for the treatment of acne vulgaris. Molecules. 25 (20), 4856. https://doi.org/10.3390/molecules25204856.

Karolewicz, B., 2015. A review of polymers as multifunctional excipients in drug dosage form technology. Saudi Pharm. J. 24, 525–536. https://doi.org/10.1016/j.jsps.2015.02.025.

Khovidhunkit, S.P., Yingsaman, N., Chairachvit, K., Surarit, R., Fuangtharnthip, P., Petsom, A., 2011. *In vitro* study of the effects of plaunotol on oral cell proliferation and wound healing. J. Asian Nat. Prod. Res. 13, 149–159. https://doi.org/10.1080/10286020.2010.546790.

Kitazawa, E., Kurabayashi, M., Kasuga, S., Oda, O., Ogiso, A., 1982. New esters of a diterpene alcohol from *Croton sublyratus*. Ann. Rep. Sankyo Res. Lab. 34, 39–41.

Kitazawa, E., Ogiso, A., 1981. Two diterpenes alcohols from *Croton sublyratus*. Phytochemistry. 20 (2), 287–289. https://doi.org/10.1016/0031-9422(81)85108-4.

Koga, T., Kawada, H., Utsui, Y., Domon, H., Ishii, C., Yasuda, H., 1996. *In-vitro* and *in-vivo* antibacterial activity of plaunotol, a cytoprotective antiulcer agent, against *Helicobacter pylori*. J. Antimicrob. Chemother. 37, 919–929. https://doi.org/10.1093/jac/37.5.919.

Koga, T., Watanabe, H., Kawada, H., Takahashi, K., Utsui, Y., Domon, H., Ishii, C., Narita, T., Yasuda, H., 1998. Interactions of plaunotol with bacterial membranes. J. Antimicrob. Chemother. 42, 133–140. https://doi.org/10.1093/jac/42.2.133.

Lambers, H., Piessens, S., Bloem, A., Pronk, H., Finkel, P., 2006. Natural skin surface is on average below 5, which is beneficial for its resident flora. Int. J. Cosmet. Sci. 28, 359–370. https://doi.org/10.1111/j.1467-2494.2006.00344.x.

Loftsson, T., Brewster, M.E., 1996. Pharmaceutical applications of cyclodextrins: drug solubilization and stabilization. J. Pharm. Sci. 85 (10), 1017–1025. https://doi.org/10.1021/js950534b.

Martin, K.W., Ernst, E., 2003. Herbal medicines for treatment of bacterial infections: a review of controlled clinical trials. J. Antimicrob. Chemother. 51, 241–246. https://doi.org/10.1093/jac/dkg087.

Matsumoto, Y., Hamashima, H., Masuda, K., Shiojima, K., Sasatsu, M., Arai, T., 1998. The antibacterial activity of plaunotol against *Staphylococcus aureus* isolated from the skin of patients with atopic dermatitis. Microbios. 96, 149–155 https://pubmed.ncbi.nlm.nih.gov/10399344.

Ogiso, A., Kitazawa, E., Kurabayashi, M., Sato, A., Takahashi, S., Noguchi, H., Kuwano, H., Kobayashi, S., Mishima, H., 1978. Isolation and structure of antipeptic ulcer diterpene from Thai medicinal plant. Chem. Pharm. Bull. 26 (10), 3117–3123. https://doi.org/10.1248/cpb.26.3117.

Piletti, R., Zanetti, M., Jung, G., de Mello, J.M.M., Dalcanton, F., Soares, C., Riella, H.G., Fiori, M.A., 2019. Microencapsulation of garlic oil by β cyclodextrin as a thermal protection method for antibacterial action. Mater. Sci. Eng. C 94, 139–149.

Premprasert, C., Tewtrakul, S., Plubrukarn, A., Wungsintaweekul, J., 2013. Anti-inflammatory activity of diterpenes from *Croton stellatopilosus* on LPS-induced RAW264.7 cells. J. Nat. Med. 67, 174–181. https://doi.org/10.1007/s11418-012-0668-5.

Rankin, I.D., 2005. MIC testing. In: Coyle, M.B. (Ed.), Manual of antimicrobial susceptibility testing. American Society for Microbiology, Washington, pp. 53–62.

Roy, N., Ghosh, B., Roy, D., Bhaumik, B., Roy, M.N., 2020. Exploring the inclusion complex of a drug (umbelliferone) with α-cyclodextrin optimized by molecular docking and increasing bioavailability with minimizing the doses in human body. ACS Omega. 5, 30243–30251. https://doi.org/10.1021/acsomega.0c04716.

Saokham, P., Chutimon, M., Jansook, P., Loftsson, T., 2018. Solubility of cyclodextrins and drug/cyclodextrin complexes. Molecules. 23, 1161. https://doi.org/10.3390/molecules23051161.

Sasaki, M., Mizoshita, T., Mizushima, T., Inoue, H., Kamiya, T., Kataoka, H., Ogaswara, N., Wada, T., Kubota, E., Mori, Y., Shimura, T., Hirata, H., Ando, K., Okamoto, Y., Ohara, H., Nakao, H., Joh, T., 2007. Effect of plaunotol in combination with clarithromycin against clarithromycin-resistant *Helicobacter pylori in vitro* and *in vivo*. J. Antimicrob. Chemother. 60, 1060–1063. https://doi.org/10.1093/jac/dkm329.

Songkro, S., Pichayakorn, W., Songkarak, S., Wungsintaweekul, J., 2011. Investigation of plaunoi-loaded micro/nanoemulsions for the treatment of dermatitis: formulation, evaluation and skin irritation studies. J. Drug Del. Sci. Tech. 21, 401–410. https://doi.org/10.1016/S1773-2247(11)50065-6.

Sopirala, M.M., Mangino, J.E., Gebreyes, W.A., Biller, B., Bannerman, T., Balada-Llasat, J.M., Pancholi, P., 2010. Synergy testing by Etest, microdilution checkerboard and time-kill methods for Pan-drug-resistant *Acinetobacter baumannii*. Antimicrob. Agents Chemother. 54 (11), 4678–4683. https://doi.org/10.1128/AAC.00497-10.

Tago, K., Arai, M., Kogen, H., 2000. A practical total synthesis of plaunotol via highly *Z*-selective Wittig olefination of α-acetal ketones. J. Chem. Soc. Perkin Trans. 1, 2073–2078. https://doi.org/10.1039/B001977L.

Vyas, A., Sonker, A.K., Gidwani, B., 2014. Carrier-based drug delivery system for treatment of acne. Sci. World J. 2014, 276260. https://doi.org/10.1155/2014/276260.

Waranuch, N., Phimnuan, P., Yakaew, S., Nakyai, W., Grandmottet, F., Onlom, C., Srivilai, J., Viyoch, J., 2019. Antiacne and antiblotch activities of formulated combination of *Aloe barbadensis* leaf powder, *Garcinia mangostana* peel extract, and *Camellia sinensis* leaf extract. Clin. Cosmet. Investig. Dermatol. 12, 383–391. https://doi.org/10.2147/ccid.s200564.

Wen, J., Liu, B., Yuan, E., Ma, Y., Zhu, Y., 2010. Preparation and physicochemical properties of the complex of naringenin with hydroxypropyl-β-cyclodextrin. Molecules. 15, 4401-4407. https://dx.doi.org/10.3390%2Fmolecules15064401.

Wüpper, S., Lüersen, K., Rimbach, G., 2021. Cyclodextrins, natural compounds, and plant bioactives- a nutritional perspective. Biomolecules. 11, 401. https://doi.org/10.3390/biom11030401.

Yuan, C., Wang, Y., Liu, Y., Cui, B., 2019. Physicochemical characterization and antibacterial activity assessment of lavender essential oil encapsulated in hydroxypropyl-beta-cyclodextrin. Ind. Crops Prod. 130, 104–110. https://doi.org/10.1016/j.indcrop.2018.12.067.

Zaenglein, A.I., Pathy, A.I., Schlosser, B.J., Alikhan, A., Baldwin, H.E., Berson, D.S., Bowe, W.P., Graber, E.M., Harper, J.C., Kang, S., Keri, J.E., Leyden, J.J., Reynolds, R.V., Silverberg, N.B., Gold, L.F.S., Tollefson, M.M., Weiss, J.S., Dolan, N.C., Sagan, A.A., Stern, M., Boyer, K.M., Bhushan, R., 2016. Guidelines of care for the management of acne vulgaris. J. Am. Acad. Dermatol. 74 (5), 945–973. https://doi.org/10.1016/j.jaad.2015.12.037.