Investigation of plaunoi-loaded micro/nanoemulsions for the treatment of dermatitis: formulation, evaluation and skin irritation studies

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The main objective of this work was to formulate microemulsions and nanoemulsions as delivery vehicles for plaunoi extract (Croton stellatopilosus Ohba) to treat dermatitis. Plaunotol, a constituent of plaunoi, has been shown to possess antimicrobial activity. In this work, 2 % w/w plaunoi-loaded formulations were prepared and investigated for their physical property (size and viscosity), efficacy and safety. The plaunoi-loaded microemulsions (ME1-P (w/o), ME2-P (o/w)) and nanoemulsion (NE-P (o/w)) had different average sizes and apparent viscosities. Using modified Franz diffusion cell and a synthetic membrane, the release rate of plaunotol from NE-P was found to be the highest. With regard to the in vitro skin penetration, only o/w microemulsion (ME2-P) could deliver plaunotol through the newborn pig skin into the receptor fluid. Unlike ME1-P, the accumulation of plaunotol within the excised newborn pig skin was observed in ME2-P and NE-P. The antimicrobial property of the formulations was further investigated using a cylinder- plate method. Staphylococcus aureus, Staphylococcus epidermidis and Propionibacterium acnes were the tested gram microorganisms. It was found that the plaunoi-loaded formulations exhibited somewhat improved antibacterial activity for S. aureus and S. epidermidis when compared with the blank formulations. The skin irritation was not observed in all treated rabbits for the nanoemulsion NE-P. Both ME1-P and ME2-P showed slight erythema. These results suggest a potential use of nanoemulsions for topical delivery of plaunoi extract.

Key words: Plaunotol – Microemulsion – Nanoemulsion – Antimicrobial activity – Skin irritation.

Dermatitis, also known as eczema, is a general term used to describe an inflammation of the skin. With respect to the cause of condition, dermatitis can be classified into several types such as contact dermatitis, atopic dermatitis, and nummular dermatitis. The general symptoms of dermatitis involve skin erythema, vesiculation, itching and skin lesions [1]. The pathological changes may occur in the epidermis and dermis layers. Both endogenous factors (e.g. genetics) and exogenous factors (e.g. exotoxins of certain bacteria and chemical agents) can trigger dermatitis.

Up until now, corticosteroids have been primarily used for the treatment of dermatitis. However, topical corticosteroids may elicit several local and systemic side effects, such as thin skin, ulceration, glaucoma, proximal myopathy and adrenal insufficiency, after long-term use [2, 3]. These drawbacks have led to the search for an alternative treatment with natural medicines. A number of plants have been explored for their potential therapies [4]. These cover chamomile (*Matricaria chamomilla* L.), St. John's wort (*Hypericum perforatum* L.) and Oolong tea [4, 5].

Croton stellatopilosus Ohba (Euphorbiaceae), commonly known as plaunoi in Thailand, is a source of several diterpenes, in particular plaunotol, an acyclic diterpene alcohol [6, 7]. Plaunotol is a yellow viscous liquid with a distinctive odor. It is soluble in many organic solvents but insoluble in water. It has a molecular formula of $C_{20}H_{34}O_2$ and its chemical structure is shown in *Figure 1* [8].

Given orally, plaunotol has been used for the treatment of peptic ulcer and gastritis [2]. A commercial product of plaunotol for this indication is Kelnec (DaiichiSankyo, Japan). Plaunotol has been shown to have antibacterial activity against *Helicobacter pylori* that causes peptic ulcers [9]. In addition, plaunotol is reported to increase the gastric mucosa blood flow [8] and possesses cytoprotective properties [2].

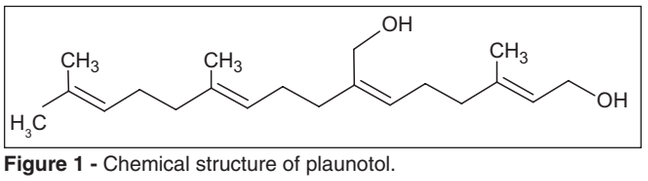

Figure 1 - Chemical structure of plaunotol.

In the case of topical application, plaunotol has been found to exhibit antimicrobial activity against *Staphylococcus aureus*, a frequent cause of infection in atopic dermatitis [10, 11]. These results have indicated the possibility that plaunoi extract could prevent or reduce bacterial infections in dermatitis, in particular atopic dermatitis which is caused by exotoxins of *Staphylococcus aureus*.

In the past decade, microemulsions and nanoemulsions have received much attention for the delivery of several compounds for dermal applications [12-15]. Microemulsions are thermodynamically stable dispersions of oil and water stabilized by an interfacial film of surfactant/cosurfactant. Unlike ordinary emulsions, microemulsions are optically transparent, with an average size range of about 10-140 nm [16]. To prepare pharmaceutically accepted microemulsions, nonionic surfactants are preferred because of their low risks of irritating the skin with a balanced hydrophilic and lipophilic property [17, 18]. It has been reported that Tweens (nonionic surfactant) possess minimal toxicity. Furthermore, some Tweens, for example, Tween 20 and Tween 80 have also been employed for parenteral and oral administration [12]. Both synthetic and natural oils can be employed as the oil phase. The construction of a ternary or psuedoternary phase diagram is a practical approach to characterize the microemulsion regions [12, 19]. Microemulsions can be easily prepared by mixing appropriate amounts of oil, water, surfactant/cosurfactant by simple agitation with or without heating. Three types of microemulsions have been classified namely

oil-in-water (o/w) microemulsions, water- in-oil (w/o) microemulsions and bicontinuous microemulsions. Nowadays, applications of microemulsions in pharmaceutical and cosmetics fields are generally recognized [14, 20-24]. Microemulsions have been shown to increase systemic or local delivery of certain drugs [23, 24]. The advantages of microemulsions include simple technology of preparation, attractiveness (optically transparent), high drug solubilizing power and excellent thermodynamic stability [25]. Although microemulsions possess numerous benefits as previously mentioned, the risk of skin irritation following topical application of microemulsions is quite high. This is due to high concentrations of surfactants and cosurfactants required in the system.

Nanoemulsions are defined as fine oil-in-water dispersions with the droplet size under 1,000 nm [26]. In another publication, the nanoemulsion droplets can range in size from 100-600 nm [15]. Nanoemulsions are composed of a water phase, oil phase and stabilizers (surfactants). Unlike microemulsions, nanoemulsions are metastable systems and can be prepared by several techniques. These include spontaneous emulsification and the use of high shear device [15, 18]. For large scale production, nanoemulsions can be easily manufactured by high pressure homogenizer. As with microemulsions, nanoemulsions have been widely applied in pharmaceutics, medicines and cosmetics [15, 18, 27]. The advantages of nanoemulsions include kinetic stability, relatively long-term physical stability, high drug-loading capacity and low skin irritation for topical delivery [28].

For the treatment of dermatitis, the goal of the dosage form design is to localize the drug in the skin (local-skin targeting). In this case, percutaneous absorption of the particular drug is not required in order to avoid systemic side effects. It has been reported that microemulsions and nanoemulsions are capable of enhancing the skin accumulation of certain drugs without increasing the systemic side effects [24, 29]. In this regard, the use of micro/nanoemulsion formulations of plaunoi extract (plaunotol) is expected to serve this purpose.

In light of the above, the main objective of this work was to formulate the dermal preparations of plaunoi extract using microemulsions and nanoemulsions as topical carriers. To our knowledge, microemulsions and nanoemulsions as vehicles for plaunoi extract (*Croton stellatopilosus* Ohba) have not yet been investigated. The prepared plaunoi-loaded micro/nanoemulsions were evaluated by means of particle size measurement, transmission electron microscope and viscosity determinations.

The effectiveness of these formulations was tested by *in vitro* release, skin penetration and retention studies. In addition, the antimicrobial activity of plaunoi-loaded micro/nanoemulsions against tested microorganisms was investigated using a cylinder-plate technique. The skin irritation potential of the formulations containing plaunoi extract was further evaluated using an *in vivo* acute dermal irritation test in rabbits.

I. MATERIALS AND METHODS

1. Materials

The hexane extract of mature leaves of *Croton stellatopilosus* Ohba was a gift from TipCo Food Public Co. Ltd. (Prachuabkirikhan, Thailand). Analysis by GC technique in our lab showed that the plaunoi extract contained 30.87 ± 0.99 % w/w plaunotol. Kelnac was obtained from DaiichiSankyo, Japan. Virgin coconut oil produced by centrifugation method was purchased from Tropical Nutrition Co. Ltd. (Prachuabkirikhan, Thailand). Tween 20, Tween 80, Span 20, Span 80 and propylene glycol were supplied by Fluka (Buchs, Switzerland). Glycerin was obtained from Ajax Finechem Pty Ltd (Australia). Ethanol (absolute) was purchased from Merck (Germany). *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* TISTR 517 and *Propionibacterium acnes* DMSC 14916 were obtained from the American Type Culture Collection, Thailand Institute of Scientific and Technological Research and Department of Medical Sciences, respectively. Difco Mueller-Hinton agar and Bacto brain heart infusion agar were supplied by Becton Dickinson and Co. Cellulose acetate membrane (Spectra/Por3) was purchased from Spectrum Laboratories, Inc. (United States, Canada). All chemicals and solvents were pharmaceutical grade or analytical grade.

2. Preparation of microemulsions and plaunoi-loaded microemulsions

2.1. Construction of ternary phase diagram

To identify microemulsion regions, a preliminary study was carried out using Tween 80, Tween 20, Span 80, Span 20, glycerin and propylene glycol as surfactants/cosurfactants, purified water as water phase and virgin coconut oil as oil phase [30]. The safety, attractiveness and benefits of virgin coconut oil for topical application have been published elsewhere [31]. Samples were prepared by adding the appropriate amount of oil, surfactant/cosurfactant and water into the individual screwcap glass vials then mixing with a vortex mixer until homogenous. To find the most suitable proportions of surfactant and cosurfactant, the weight ratios between surfactants and cosurfactants were also varied. The samples were stored at room temperature for at least 24 h in order to reach equilibrium before further investigation. The samples were designated as microemulsions when transparent, single-phase mixtures were obtained. Afterwards, ternary phase diagrams of the systems were constructed.

It was found that Tween 80/Span 80 and Tween 20/Span 20 with weight ratios of 1:1, 1:2 and 2:1 could provide microemulsion regions. However, no microemulsion region was obtained when glycerin was used as cosurfactant. In the case of propylene glycol, only 2:1 ratio of Tween 80/propylene glycol exhibited microemulsion region. In this current investigation, the surfactant (Tween 80)/cosurfactant (Span 80) at a weight ratio of 1:1 was selected since it produced a substantial microemulsion region. The ternary phase diagram of Tween 80/Span 80 (1:1), virgin coconut oil and water, which was constructed to identify the microemulsion region, is shown in *Figure 2*. All microemulsion samples remained transparent for over 6 months. Two formulations of microemulsions containing 70 or 80 % w/w surfactant mixture were selected to represent w/o microemulsion (ME1) and o/w microemulsion (ME2), respectively.

2.2. Plaunoi-loaded microemulsion preparation

The lipophilic plaunoi extract at 2 % w/w (0.62 % w/w plaunotol) was selected to formulate the plaunoi-loaded microemulsions. The formulations ME1 and ME2 which contained plaunoi extract were

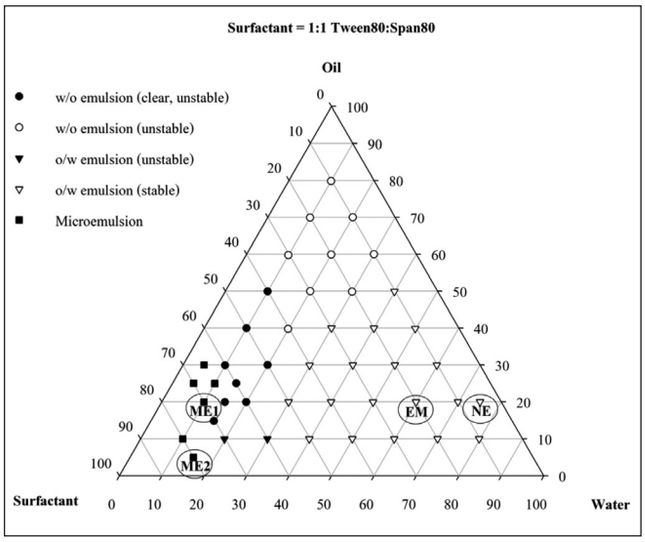

Figure 2 - Representative phase diagram of the system of virgin coconut oil/water/Tween 80:Span 80 (1:1).