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Liquid and solid self-microemulsifying drug delivery systems for improving the oral bioavailability of andrographolide from a crude extract of *Andrographis paniculata*

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ABSTRACT

The purpose of this study was to develop self-microemulsifying formulations of an *Andrographis paniculata* extract in liquid and pellet forms for an improved oral delivery of andrographolide. The optimized liquid self-microemulsifying drug delivery system (SMEDDS) was composed of *A. paniculata* extract (11.1%), Capryol 90 (40%), Cremophor RH 40 (40%) and Labrasol (8.9%). This liquid SMEDDS was further adsorbed onto colloidal silicon dioxide and microcrystalline cellulose, and converted to SMEDDS pellets by the extrusion/spheronization technique. The microemulsion droplet sizes of the liquid and pellet formulations after dilution with water were in the range of 23.4 and 30.3 nm. The *in vitro* release of andrographolide from the liquid SMEDDS and SMEDDS pellets was 97.64% (SD 1.97%) and 97.74% (SD 3.36%) within 15 min, respectively while the release from the initial extract was only 10%. The oral absorption of andrographolide was determined in rabbits. The C_{max} value of andrographolide from the *A. paniculata* extract liquid SMEDDS and SMEDDS pellet formulations (equivalent to 17.5 mg/kg of andrographolide) was 6-fold and 5-fold greater than the value from the initial extract in aqueous suspension (equivalent to 35 mg/kg of andrographolide), respectively. In addition, the AUC_{0-12h} was increased 15-fold by the liquid SMEDDS and 13-fold by the SMEDDS pellets compared to the extract in aqueous suspension, respectively. The results clearly indicated that the liquid and solid SMEDDS could be effectively used to improve the dissolution and oral bioavailability that would also enable a reduction in the dose of the poorly water soluble *A. paniculata* extract.

1. Introduction

Andrographis paniculata (Burm.f.) Nees, also known as "King of Bitters", is a herbaceous plant in the family of Acanthaceae. This herb has a long history of medicinal uses in Asian countries. The aerial parts (stems and leaves) of *A. paniculata* have been traditionally used for relief from fever, common cold, non-infectious diarrhea, inflammation, herpes, sore throat and a variety of other chronic infectious diseases. The pharmacological activities of this plant include its hepatoprotectivity (Singha et al., 2007), anti-inflammatory (Chandrasekaran et al., 2010), anti-oxidative (Sheeja et al., 2006) and hypoglycemic activities (Reyes et al., 2006). The primary bioactive constituent of this plant is andrographolide. It is mainly concentrated in the leaves (>2%). Andrographolide has a very bitter taste, is a colorless and crystalline bicyclic compound, sparingly soluble in water but soluble in acetone, methanol, chloroform and ether. It has an experimental log *P* value of 2.632 (SD 0.135) and an aqueous solubility of 3.29 (SD 0.73) μg/mL at 25 °C (Bothiraja et al., 2009). The therapeutic use of andrographolide, the active component, is restricted by its poor solubility in water which results in low bioavailability after oral administration. At present, there are several methods used to improve the solubility and oral absorption of andrographolide including complexing with hydroxypropyl-beta-cyclodextrin (Bothiraja et al., 2009) and solid dispersion with polyvinylpyrrolidone (Ren et al., 2009).

In recent years, much attention has been focused on lipid-based systems with particular emphasis on self-emulsifying drug delivery systems (SEDDSs) to improve oral bioavailability of poorly water-soluble compounds. SEDDS are isotropic mixtures of oils, surfactants, co-solvents or co-surfactants and drug substances. These systems form oil-in-water (o/w) emulsions when diluted in aqueous media such as GI fluid under mild agitation provided by gastrointestinal motion, which is necessary for self-emulsification *in vivo*. The size of the droplet formed is between 100 and 300 nm while self-microemulsifying drug delivery systems

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(SMEDDS) form transparent microemulsions with a droplet size of less than 50 nm (Gursoy and Benita, 2004). The SEDDS/SMEDDS are physically stable, easy to manufacture, and can be filled in soft gelatin capsules. A higher bioavailability of low aqueous solubility drugs incorporated in SMEDDS was earlier reported (Hong et al., 2006; Balakrishnan et al., 2009). The already dissolved form of the drug in a SMEDDS formulation was beneficial for enhancing drug absorption (Cui et al., 2009). The oily solution that forms microemulsions increased the amount of hydrophobic drugs transported via the intestinal lymphatic system (Hoim et al., 2002). An alternative dosage form of SMEDDS has been recently investigated. In this case, the SMEDDS were formulated in solid dosage forms including tablets (Nazzal et al., 2002), pellets (Abdalla and Mäder, 2007; Setthacheewakul et al., 2011) and powders (Yi et al., 2008). Formulation of solid SMEDDS provides a better opportunity for the industrial manufacture of drug products. Iosio et al. (2011) showed that self-emulsifying pellets of silymarin prepared by the extrusion/spheronization technique improved the *in vivo* oral bioavailability of the main components of the extract by more than 10-fold. In another study, the SMEDDS of curcumin in liquid and pellet forms provided a 14- and 10-fold greater absorption, respectively compared to the same oral dose of unformulated curcumin (Setthacheewakul et al., 2010).

The objective of this study was to develop the SMEDDS formulations that enhanced the solubility, dissolution and oral absorption of andrographolide, the active component of *A. paniculata* extract. Some studies have demonstrated that the pharmacological activities of andrographolide are similar to those of the *A. paniculata* extract (Sheeja et al., 2007). Based on the ease of a manufacturing process and the possibility to produce the formulation in a large scale, an *A. paniculata* extract was used in this study.

2. Materials and methods

2.1. Materials

A. paniculata (3 months old) was collected from U-thong hospital, Suphanburi province, Thailand. A voucher specimen (specimen no. SKP 001 01 16 01) was deposited in the herbarium of the Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand. Andrographolide (98%) was from Sigma–Aldrich (Saint Louis, MO, USA). Capryol® 90 (propylene glycol monocaprylate), Labrafac® PG (propylene glycol caprylate/caprate) and Labrasol® (caprylocaproyl macrogol-8 glycerides) were from Gattefossé (Saint-Priest, France). Cremophor® RH 40 and Cremophor® EL were from BASF (Ludwigshafen, Germany). Maisin® 35-1, Labrafac® CC, Lauroglycol® FCC, Lauroglycol® 90, Labrafil® M2125 CS and Plurol oleique were from Gattefossé (Saint-Priest, France). Polyethylene glycol 400 (PEG 400) was from the PC Drug Center Co., Ltd. (Bangkok, Thailand). Ethyl oleate was from Sigma-Aldrich (Buchs, Switzerland). Oleic acid was from Srichand United Dispersary Co., Ltd. (Bangkok, Thailand). Flocel® 101 (microcrystalline cellulose) was purchased from Gujarat Microwax Private Limited (Mehsana, India), Aerosil® 200 (colloidal silicon dioxide) was from Degussa-Hüls AG (Hanau, Germany). Starch® 1500 (pregelatinized maize starch) was from Colorcon (Indiana, USA). Primellose® (croscarmellose sodium) was from DMV-Fonterra Excipients B.V. (Foxhol, The Netherlands). Hard gelatin capsules (size 00) were from Capsugel (Bangkok, Thailand). Methanol (HPLC grade) was from RCI Labscan (Bangkok, Thailand). All other chemicals were of analytical grade.

2.2. Preparation of A. paniculata extract

The ground dried leaf part of *A. paniculata* weighing 700 g was extracted with 2 L of 95% ethanol by reflux apparatus for 1 h (Chien et al., 2010). The liquid extract was removed and the remaining material was re-extracted by the same procedure. The obtained liquid extracts were combined and concentrated using a rotary evaporator and dried under vacuum at room temperature. The extract was then milled and sieved to obtain a uniform powdered extract of *A. paniculata*. The crude extract of *A. paniculata* contained 17.84% (SD 2.30%) of andrographolide. The obtained extract was stored in a well-closed glass container at room temperature.

2.3. Development of the liquid SMEDDS of A. paniculata extract

2.3.1. Solubility studies

The solubility of an *A. paniculata* extract in different oils, surfactants and co-surfactants was determined. A 500 mg amount of *A. paniculata* extract was added to each microtube containing 1 mL of each vehicle. These mixtures were vortexed using a mixer (Vortex-gene 2, Becthai, Bangkok Equipment & Chemical, Bangkok, Thailand) then shaken in a shaking bath (Comfort Heto Master Shake, Heto Lab Equipment) at 37 °C for 72 h (Bachhav and Patravale, 2009). The tubes were centrifuged at 6000 rpm for 10 min at room temperature. The supernatant was suitably diluted with methanol. Andrographolide in the supernatant was quantified by HPLC. The vehicles that provided the maximum andrographolide solubility were selected for further experiments.

2.3.2. Construction of ternary phase diagrams

Ternary phase diagrams of the selected compositions in SMEDDS from solubility studies were constructed using SigmaPlot 11.0 software. The ternary phase diagrams were plotted to identify the self-microemulsifying regions and to find the optimal component concentrations of the SMEDDS formulations. A series of mixtures of oil, surfactant and co-surfactant or the mixtures of oil and the combination of 2 surfactants were prepared. The compositions were weighed into glass test tubes and mixed using a vortex mixer. The concentration range of each component was 10–50% oil, 25–90% surfactant and 0–25% co-surfactant. One gram of each mixture was dispersed in 20 mL of distilled water. The efficiency of the self-microemulsification was observed visually and scored according to the grading system described by Singh et al. (2009). Grade I, denoted a rapid forming microemulsion which is clear or slightly bluish in appearance (less than 1 min); Grade II denoted a rapid forming, but slightly less clear emulsion that had a bluish white appearance (less than 2 min); Grade III denoted a bright white emulsion (similar to milk in appearance) (less than 3 min); Grade IV denoted a dull, grayish white emulsion with a slightly oily appearance that was slow to emulsify (longer than 3 min) and Grade V denoted a poor or minimal emulsification with large oil droplets present on the surface (longer than 3 min).

2.3.3. Preparation of liquid SMEDDS of A. paniculata extract

According to the ternary phase diagram studies, some SMEDDS at desired component ratios were selected for the incorporation of *A. paniculata* extract. The extract was added to the SMEDDS mixture and the dispersion was stirred continuously until a homogenous solution was formed. The SMEDDS formulations were left for 48 h at room temperature. Hard gelatin capsules (size 00) were manually filled with the liquid SMEDDS of *A. paniculata* extract and stored in a tightly sealed glass bottle at room temperature until used.

2.3.4. Emulsion droplet size measurements

One gram of each formulation was introduced into 20 mL of distilled water (20-fold dilution) at room temperature. The content was gently stirred by a magnetic stirrer for 5 min. The droplet size and polydispersity index of the resultant microemulsion were determined using a ZetaPALS, Zeta potential and particle size ana-