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### Development of high active andrographolide tablet from *Andrographis paniculata* extract

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### Introduction

*Andrographis paniculata* (Burm. f) is widely used as a traditional medicine in Southeast Asia, which the Thailand Ministry of Public Health included this plant in 'The National List of Essential Herbal Drugs A.D. 1999' as a herbal drug for the treatment of common cold symptoms (e.g. sore throat, fever) and non-infectious diarrhea. <sup>1</sup> Andrographolide is the main active diterpene lactone of *A. paniculata*, which have been found in dried leaves and stems of the herb as 1.7% and 0.8% of the weight, respectively. <sup>2</sup> For the pharmacological effect of andrographolide that have been reported such as strong anti-inflammatory and anti-cancer properties. <sup>3,4</sup>

In Thai markets, the capsule dosage form of a crude drug is mostly available. Generally, people take 3-4 capsules and 4 times per day for consecutive 3–5 days during treatment. <sup>5</sup> Therefore, the high active andrographolide tablet can increase amount of active compound and lead to reduce a daily intake. The aim of this study was to develop the high active andrographolide tablet from andrographis extract. The extract properties such as amount of andrographolide and total lactone, cytotoxicity, and lost on drying were determined. Andrographolide tablets were direct compressed and then evaluated the tablet properties (hardness, friability, disintegration time, and andrographolide content).

### Methods

#### Materials

Flow lac<sup>®</sup> (Lactose monohydrate) was purchased from MEGGLE Group Wasserburg BG Excipients & Technology, Germany. Starch 1500<sup>®</sup> (Pregelatinized starch) was obtained from Colorcon, USA. Avicel<sup>®</sup> PH 101 (Microcrystalline cellulose), and Avicel<sup>®</sup> PH 102 (Microcrystalline cellulose) were purchased from FMC Health and Nutrition, USA. Magnesium stearate was purchased from Faci Asia Pacific Pte Ltd, China.

#### Preparation of andrographolide

The 20 mg of dried *A. paniculata* powder was macerated with the 100 ml of ethanol and acetone mixture (the ratio of 2:3) for 2 h. The extraction was repeated 3 times. To remove impurities, the extract was filtered, added 10 % of activated charcoal, and then dried in water bath. The dried extract was measured andrographolide and total lactones content. Before compressed with excipients, the dried extract was dissolved in 95% ethanol and then added Avicel<sup>®</sup> PH 101 as 1.5 fold of the extract weight. The solvent was evaporated by water bath. The extract powder was completely dried in hot-air oven at 50 °C and reduced size by passing 60 mesh sieve. The andrographolide powder was stored in desiccator at room temperature.

#### Thin layer chromatography (TLC)

The amount of andrographolide was analyzed by TLC densitometer method. The 2 µL of extract was spotted onto TLC silica gel 60 GF254 (20 cm x 10 cm). Then, TLC was placed into TLC developing tank with saturated methanol. The absorbance of band was measured by using densitometer (Camag TLC scanner 4, Switzerland). The peak area and rate of flow (Rf) were used to calculate the amount of andrographolide.

### UV spectrophotometry

The 100  $\mu\text{L}$  of sample was diluted with 900  $\mu\text{L}$  of 95% ethanol. Then, the 500  $\mu\text{L}$  of 3,5-dinitrobenzoic acid (2%w/v) and 500  $\mu\text{L}$  of KOH (5.7%w/v) were added. The absorbance at 536 nm was measured by using UV spectrophotometry (Agilent Technologies, model G1103A). The total lactone content was calculated.

### Cytotoxicity evaluation

The cell viability of normal human fibroblast (NHF) cells was investigated using the MTT assay. The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% FBS at 37 °C, 5% CO<sub>2</sub>, and 95 % RH. Then, the media was replaced with andrographolide extract for 24 h. After that, the cells were rinsed by using PBS and analyzed by MTT-containing medium (1 mg/ml) for 4 h. The absorbance measurement at 550 nm was operated by a microplate reader (Fusion™ Universal Microplate Analyzer, USA). The % cell viability was calculated by comparing with non-treated cells. The IC<sub>50</sub> value was calculated by non-linear regression model from relative cell viability.

### Lost on drying

One gram of andrographolide powder was accurately weighed. The weight loss was determined by using IR moisture determination moisture balance (Sartorius® thermo control YTX01L, Germany). The percentage of lost on drying (%LOD) was evaluated.

### Preparation of tablet

Each tablet containing 185 mg of andrographolide powder, 100.75 mg of diluents (Flow lac®, Avicel® PH 102, or Starch 1500®), and 2.25 mg of magnesium stearate as a lubricant were direct compressed by using a Hydraulic press machine (Specac® TBit225, USA) equipped with a 9-mm flat face punch. The compression pressures were applied at 1 ton.

### Tablet properties

Tablet hardness was measured by using tablet hardness tester (Erweka® TBH225TD, Germany). The mean hardness of ten tablets was determined.

The tablet friability was determined using friabilator (Erweka, Germany). The drum was rotated at 25 rpm for 5 min. Loss of tablet weight with respect to the initial value was then calculated as percent friability.

$$\% \text{Friability} = [(\text{Weight}_{\text{initial}} - \text{Weight}_{\text{final}}) / \text{Weight}_{\text{initial}}] \times 100$$

The disintegration time was evaluated using disintegration apparatus (Erweka® ZT323, Germany) following the USP 30-NF25. One tablet was placed in each basket and then operated the apparatus with maintaining the temperature at 37  $\pm$  2 °C. The mean disintegration time of six tablets was measured.

The amount of andrographolide in tablet was also determined by TLC-densitometry. Each tablet should contain 20 mg  $\pm$  5 % of andrographolide.

### Data analysis

All data were analyzed by using one-way analysis of variance (ANOVA) with least significant difference (LSD). The p-value of 0.05 was used to judge the significant difference.

## Results

### Andrographolide powder

The amount of andrographolides in extract powder was 28.27  $\pm$  3.40%, while total lactone was 66.13  $\pm$  1.27%. After mixing the extract powder with Avicel® RH101, the appearance of powder was yellow color and have 11.01%w/w of andrographolides. The LOD of andrographolide powder was 3.41 %w/w, representing a good powder property (lower than 10%). For cytotoxicity test, the cell viability of andrographolide powder was more than 90% at 10  $\mu\text{g}/\text{mL}$  (Figure 1) and the IC<sub>50</sub> was 600  $\mu\text{g}/\text{mL}$ .

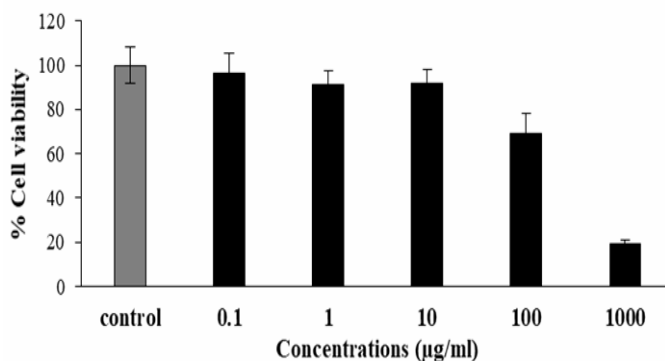


Figure 1. The percentage of cell viability in NHF cells of andrographolide extract.

### Tablet properties

All formulations were successfully formulated, which the average weight was 300 mg per tablet and the mean tablet diameter was 9 mm (Figure 1). As shown in Table 1, the hardness values of Flow lac<sup>®</sup> tablet and Avicel<sup>®</sup> PH 102 tablet were higher than Starch 1500<sup>®</sup> tablet. The percentage friability of all formulations were lower than 1%, representing a good friability. For disintegrating time, Flow lac<sup>®</sup> tablet exhibited the longest disintegration time, following Avicel<sup>®</sup> PH 102 tablet and Starch 1500<sup>®</sup> tablet, respectively. For the percentage amount of andrographolide in tablets, all formulations were more than 100% of andrographolide content in tablets.



**Figure 2.** Appearance of tablet: (A) Flow lac<sup>®</sup> tablets, (B) Avicel<sup>®</sup> PH 102 tablets and (C) Starch 1500<sup>®</sup> tablet.

**Tablet 1** Tablet properties of andrographolide tablets. \* indicates significant difference between formulations.

Formulations	Hardness (N)	% Friability	Disintegration time (min)	% amount of andrographolide
Flow lac <sup>®</sup> tablet	130.67 ± 4.04	0.16	22.65 ± 0.34	103.21 ± 1.53
Avicel <sup>®</sup> PH 102 tablet	113.33 ± 1.15	0.10	10.85 ± 0.20*	102.67 ± 2.99
Starch 1500 <sup>®</sup> tablet	33.67 ± 0.58*	0.45	8.83 ± 0.31*	101.62 ± 1.24

### Discussion

In this study, andrographolide was successfully extracted and powdered with Avicel<sup>®</sup> PH 101. The high active andrographolide powder exhibited a good physicochemical properties such as high amount of andrographolide and total lactone, low humidity that might reduce the microbial growth, and low cytotoxicity on normal cells. For tablet properties, the different kinds of diluents (Flow lac<sup>®</sup>, Avicel<sup>®</sup> PH 102, and Starch 1500<sup>®</sup>) exhibited the different tablet properties. Although all formulations represented a good friability, the disintegration time less than 30 min, and high amount of andrographolide content in tablets, the hardness values of Flow lac<sup>®</sup> tablet and Avicel<sup>®</sup> PH 102 tablet were higher than Starch 1500<sup>®</sup> tablet. This result related to the longest disintegration time of Flow lac<sup>®</sup> tablet, following Avicel<sup>®</sup> PH 102 tablet and Starch 1500<sup>®</sup> tablet, respectively. Therefore, Starch 1500<sup>®</sup> tablet represented a suitable hardness and fast disintegration time, indicating that Starch 1500<sup>®</sup>, also known as Pregelatinized starch, has employed as a binder, diluent, and disintegrant.<sup>6</sup> A suitable tablets containing 20 mg andrographolides have been formulated. Generally, the usual daily dose of andrographolides for common cold, sinusitis, and tonsillitis is 60 mg.<sup>7</sup> Therefore, the high active andrographolide tablet might be an alternative treatment of crude drug capsules, leading to decrease daily drug intake.

### Conclusion

This study demonstrated that tablets containing 20 mg andrographolide, Starch 1500<sup>®</sup> as a diluent, and magnesium stearate as a lubricant provided a suitable tablet properties. High andrographolide content, suitable hardness, and fast disintegration time of tablet might be an alternative treatment instant of crude *A. paniculata* capsules.

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## References

1. Akbar S. *Andrographis paniculata*: a review of pharmacological activities and clinical effects. *Alternative Medicine Review*. 2011;16:66-77.
2. Pholphana N, Rangkadilok N, Thongnest S, Ruchirawat S, Ruchirawat M, Satayavivad J. Determination and variation of three active diterpenoids in *Andrographis paniculata* (Burm.f.) Nees. *Phytochemical Analysis*. 2004;15(6):365–371.
3. Lee KC, Chang HH, Chung YH, Lee TY. Andrographolide acts as an anti-inflammatory agent in LPS-stimulated RAW264.7 macrophages by inhibiting STAT3-mediated suppression of the NF-kappa B pathway. *Journal of Ethnopharmacol*. 2011;135:678-684.
4. Suriyo T, Pholphana N, Rangkadilok N, Thiantanawat A, Watcharasit P, Satayavivad J. *Andrographis paniculata* extracts and major constituent diterpenoids inhibit growth of intrahepatic cholangiocarcinoma cells by inducing cell cycle arrest and apoptosis. *Planta Med*. 2014;80:533-543.
5. Pholphana N, Panomvana D, Rangkadiloka N, Suriyo T, Puranajoti P, Ungtrakul T, Pongpun W, Thaeopattha S, Songvut P, Satayaviva J. *Andrographis paniculata*: Dissolution investigation and pharmacokinetic studies of four major active diterpenoids after multiple oral dose administration in healthy Thai volunteers. *Journal of Ethnopharmacology*. 2016;194(24):513-521.
6. Manek RV, Builders PF, Kolling WM, Emeje M, Kunle OO. Physicochemical and Binder Properties of Starch Obtained from *Cyperus esculentus*. *AAPS PharmSciTech*. 2012;13(2):379–388.
7. Mkrtychyan A, Panosyan V, Panossian A, Wikman G, Wagner H. A phase I clinical study of *Andrographis paniculata* fixed combination Kan Jang versus ginseng and valerian on the semen quality of healthy male subjects. *Phytomedicine*. 2005;12(6-7):403-409.