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CHARACTERIZATION OF ANDROGRAPHOLIDE IN INCLUSION COMPLEX USING BETA CYCLODEXTRIN

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ABSTRACT: Andrographolide (AG) is a purified isolate of chemical synthesis resulted from sambiloto herbs (*Andrographis paniculata* Nees.), which has various pharmacological actions such as anti-inflammatory, hepatoprotector, antidiabetic, inhibition of replication of the HIV virus, antimalarial, anti-arthritic, anti-hyperlipidemic, anticancer, antimicrobial, immunomodulator and antiparasitic. To enhance the ability of AG to penetrate the membrane on transdermal administration, it is important to conduct the formation of inclusion complexes using beta cyclodextrin (BCD) because it has benefit such as enhancing drug release and/or permeation and stabilizing drugs in the formulation or at the absorptive site. The inclusion complexes of AG with BCD was prepared by a solvent evaporation method with mole ratio of 1:1, 1:2 and 2:1. The solids of AG inclusion complex resulted in the BCD were characterized using physical characterization of X-ray diffraction, differential scanning calorimetry, scanning electron microscope, compared to the single compound of AG, BCD, and a physical mixture of AG-BCD. Based on the results of physical characterization, the ratio that indicates the formation of inclusion complexes AG-BCD is the mole ratio of 1:2.

INTRODUCTION: The inclusion complex is a complex formed by a drug molecule acted as a guest inside the cavity of the host molecule, where the host molecules usually derived from the class of cyclodextrin derivative. The hollow structure of cyclodextrin has a lipophilic group at the inside of the cavity and a hydrophilic group on its outer surface. This structure allows the cyclodextrin to form an inclusion complexes with various organic molecules through host-guest interaction with the interior cavity that provides hydrophobic environment [1].

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Of the cyclodextrin groups, beta cyclodextrin (BCD) is most widely used in the formula development and drug delivery systems. Several studies suggested that cyclodextrins play an important role in optimizing local and systemic dermal drug delivery by enhancing drug release and/or permeation, stabilizing drugs in the formulation or at the absorptive site, enhancing solubilization of lipophilic drugs, alleviating drug-induced local irritation, providing sustained release of drugs from vehicle and altering drug bio-conversion in the viable skin [2].

Andrographolide (AG) is a purified isolate of chemical synthesis resulted from sambilo to herbs (*Andrographis paniculata* Nees.), in the form of colorless needle-shaped crystals, has a very bitter taste with the molecular formula of $C_{20}H_{30}O_5$ and molecular weight of 350.455 g/mol[3]. The melting point of AG is 228 - 230 ^{o}C and has the ultraviolet

spectrum in ethanol at maximum wavelength of 223 nm [4]. It has various pharmacological actions such as anti-inflammatory, hepatoprotector, antidiabetic, inhibition of replication of the HIV virus, antimalarial, anti-arthritic, anti-hyperlipidemic, anticancer, antimicrobial, immunomodulator and antiparasitic [5, 6, 7].

However, the solubility of AG is poor in water which caused a low ability to dissolve, penetrate the membrane and distribution of the drug when it is used on the skin or transdermal. Thus, it is necessary to modify the solubility properties of AG to increase the ability to penetrate the membrane through the formation of inclusion complexes with BCD.

The success of the formation of AG's inclusion complexes in BCD could be identified by physical characterization method such as powder X-ray diffraction (PXRD) to analyze the diffraction pattern of a solid and its crystallinity degree, differential scanning calorimetry (DSC) to analyze the thermal profile of a solid, and scanning electron microscope (SEM) to analyze the morphological properties of the solid.

MATERIALS AND METHODS:

Research Tools: Analytical balance (Mettler Toledo AG204), vortex mixer (JEIO Tech), orbital stirrer (GFL 1092), 20 mesh sieve, desiccator, X-ray diffraction powder (Bruker D8 Advance), DSC-TGA (STA PT1600, Linseis Thermal Analysis), and Scanning Electron Microscope (JSM-6510LV, JEOL USA Inc.).

Material: Andrographolide (Plamed, China), Beta-cyclodextrin (Ex PT. Sanbe Farma), methanol pro analysis and distilled water.

Detailed Procedure: The experiment was done from December 2015 until November 2016 in Veterinary pharmacy laboratory. Physical characteristics of AG single compound and BCD single compound was examined using PXRD, DSC and SEM. Preparation of the inclusion complex compound of AG-BCD used solvent evaporation method, followed by physical characterization of the inclusion complex compound of AG-BCD using PXRD, DSC and SEM, to find a ratio which forms the compound of inclusion complexes of AG with BCD.

Solvent Evaporation Method: Each ingredient of AG and BCD was measured based on the mole ratio of 1:1, 1:2 and 2:1. AG then dissolved in methanol while BCD was dissolved in distilled water. Both were mixed with a constant stirring speed of 500 rpm for 24 hours. This mixture was allowed to stand at room temperature to evaporate the solvent to obtain a precipitate. After dry, the precipitate of inclusion complex compound formed was sieved with a 20 mesh sieve, and then stored in a desiccator.

Phisical Characterization Method:

Powder X-Ray Diffraction (PXRD): A total of 100-200 mg of sample in the sample holder was placed in the sample chamber of X-ray diffractometer. The analysis was performed in the range of 2θ 5-65° diffraction angles using radiation of CuKα (Kα1 = 1.54060 nm; Kα2 = 1.54439 nm) at 40kV and 35mA.

Differential Scanning Calorimetry (DSC): A total of 5 - 20 mg of sample was placed in the alumina crucible of DSC instrument. Thermal analysis was conducted at a temperature range of 30 - 300 °C with a heating rate of 10 °C per minute.

Scanning Electron Microscope (SEM): A few of samples was placed on the sample holder and coated with gold-palladium with auto fine coater. Samples then placed on the instrument SEM specimen chamber and observed on the computer to be photographed at an appropriate magnification.

RESULT AND DISCUSSION:

Preparation of Inclusion Complexes of AG-BCD Using Solvent Evaporation Method: Based on the solubility data, AG were soluble in methanol while BCD were soluble in distilled water. The inclusion complexes of AG-BCD was made by the mole ratio of 1:1, 1:2, and 2:1 based in the **Table 1**.

TABLE 1: THE WEIGHT OF AG AND BCD IN EACH MOLE RATIO

Molecule	Measured weight based on the mole ratio		
	1 : 1	1 : 2	2 : 1
AG	0.701 g	0.701 g	1.402 g
BCD	2.270 g	4.540 g	2.270 g

Physical Characterization Using PXRD: In **Fig. 1a**, the pattern of X-ray diffraction of AG showed a peak intensity that is high (approximately 5000)

and sharp with typical interference peaks on the 2 thetas 5.04; 9.95; 14.90; and 15.79. Results of diffractogram AG showed crystalline characteristics. Its crystalline substance was composed of a regular structure, so the distance between parallel fields can be measured and form a diffraction pattern as intense peaks.

The result of diffractogram BCD (**Fig. 1b**) showed the characteristics of amorphous. It is shown by the diffraction pattern which is spread with a low peak intensity (approximately up to 1000) and ramps, because the substance of amorphous is a substance with irregular structure and are arranged without direction. It was characterized by the typical of interference peaks on the 2 thetas 12.63; 17.87; 18.39 and 22.72.

The results of a physical mixture powder of diffractogram AG-BCD (**Fig. 1c**) shows the crystalline pattern of AG mixed with amorphous patterns of BCD. It is showing a lower and ramps peak intensity. This was proven by the presence of unique interference peaks in the two components, which also indicated the start of the physical interaction between the AG and BCD although the AG and BCD were not fused yet to form an inclusion complex compound.

However, the result of inclusion complex that showed the different diffraction pattern with the pattern of X-ray diffraction of AG is in the ratio of 1: 2 (**Fig 1e**). It is shown by the diffraction pattern of amorphous with no typical interference peaks on AG, but the typical interference peaks was clearly seen on BCD that is at 2 theta 12.07 with a high peak intensity. It is because the ratio of the number of BCD's moles doubled from moles AG. The Amorphous may show the interaction between the AG and the BCD and indicate that the molecules of AG are included to BCD.

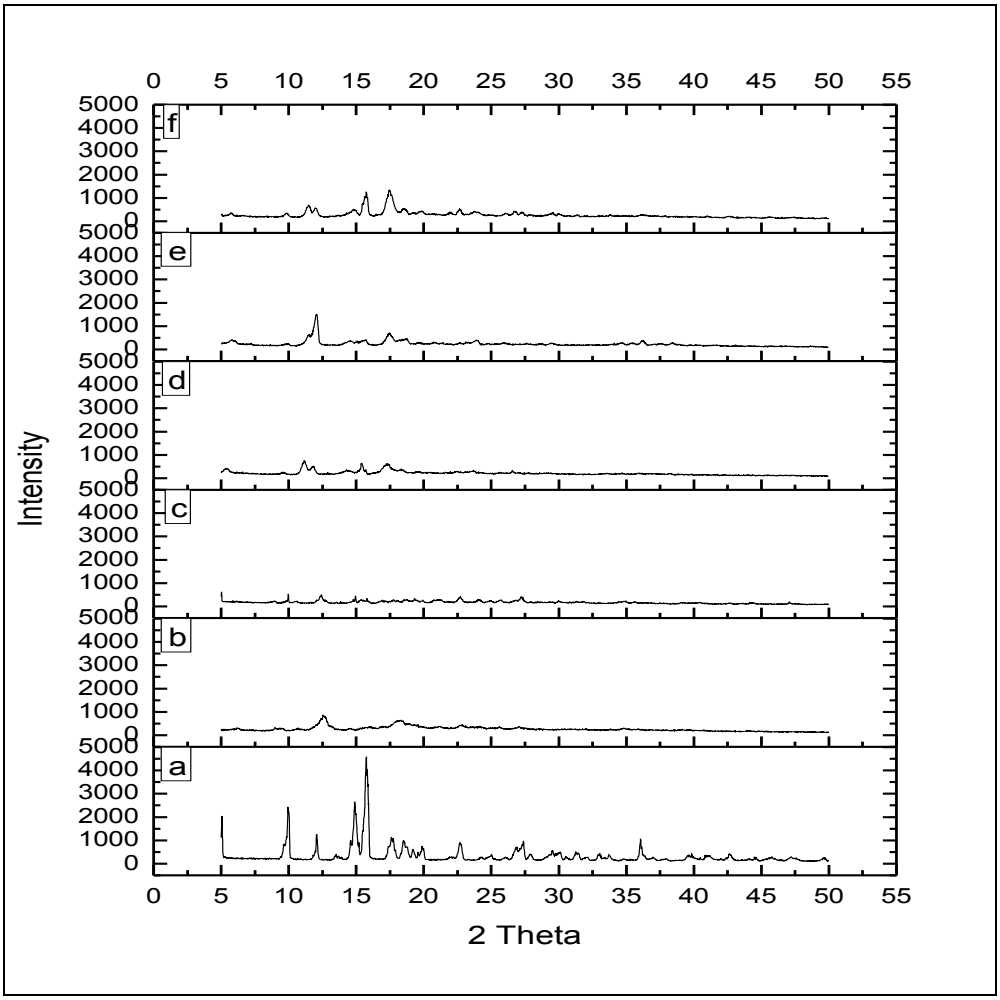

FIG. 1: RESULT OF DIFFRACTOGRAM X-RAY POWDER: [a] AG, [b] BCD, [c] PHYSICAL MIXTURE OF AG - BCD, [d] INCLUSION COMPLEX OF AG - BCD (1:1), [e] INCLUSION COMPLEX OF AG - BCD (1:2), [f] INCLUSION COMPLEX OF AG-BCD (2:1)

Physical Characterization Using DSC: In **Fig. 2a**, the DSC thermogram shows an endothermic peak at 231.5 °C which is a melting events of the components AG. This is consistent with the data obtained from the melting point on the reference. AG is a crystalline solid with a regular internal structure, so that the melting point which is a transition change from solid to liquid phase could be clearly seen.

According to the reference, BCD melting point is 228 - 230 °C. Above the temperature, the BCD will be degraded [7]. In **Fig. 2b**, The BCD thermogram shows that below 300 °C it does not show any sharp peak. This may be caused by the amorphous shape of BCD so the melting points could not be seen by a sharp peak. As known that the amorphous solid form does not provide a thermogram peak because amorphous is a substance with an irregular internal structure so it does not give a clear melting point. Thermogram of physical mixture of AG-BCD can be seen in **Fig. 2c**. The melting point of AG on the thermogram is at a temperature of 228.1°C. The melting point of AG decreased from 231.5 °C into 228.1 °C. The decrease is due to the mixing of a material with another material by fusing the second material into the crystal lattice of the other materials then depress the melting point of a substance. In **Fig. 2e**, thermogram of AG-BCD inclusion complex at the ratio of 1:2, did not show a melting point of AG clearly. This phenomenon may indicate AG have entered into the BCD cavity so that the melting point of AG could not be seen at the temperature of 228 - 231 °C. The thermogram differences of AG with the physical mixtures and inclusion complexes AG-BCD (1:2) suggested the possibility of the formation of inclusion complexes between AG and BCD. When guest molecules are embedded in BCD cavities; their melting, boiling, or sublimating points generally shift to different temperatures or disappear completely [8]. The entry of the molecule AG into the cavity of the molecule BCD causes the melting point of AG is no longer visible at the thermogram inclusion complex (**Fig. 2e**).

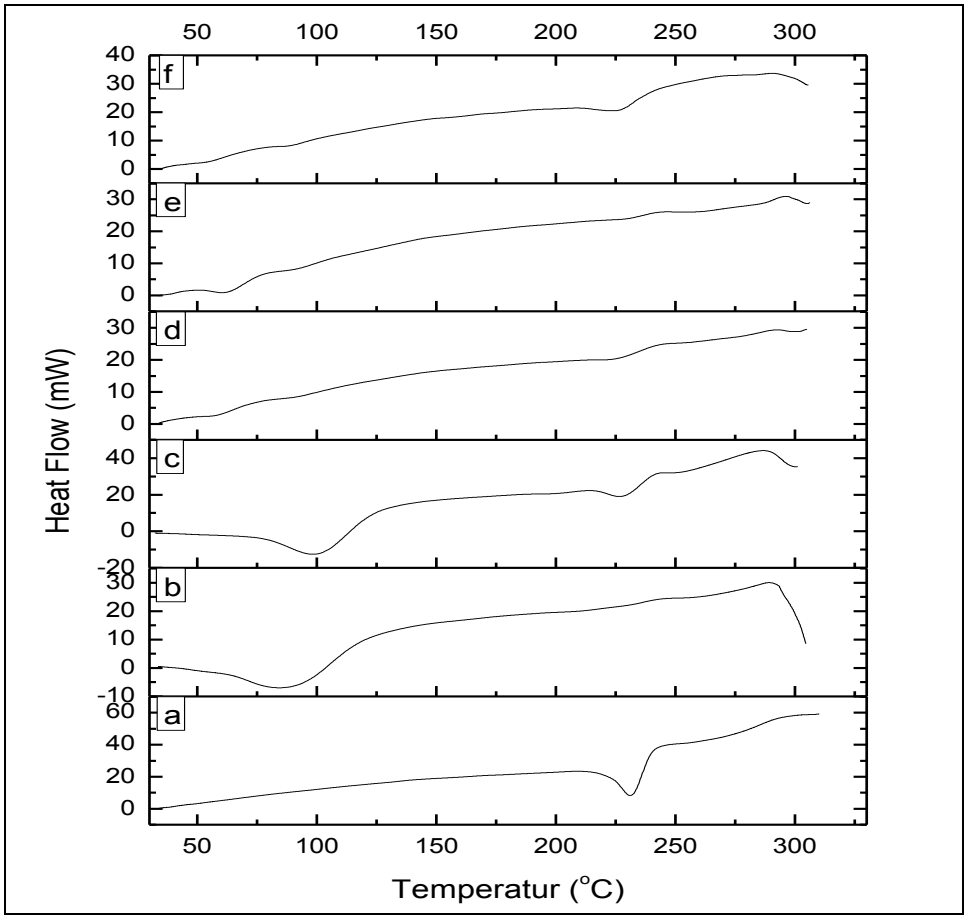

FIG. 2: RESULT OF POWDER DSC MICROGRAPH: [a] AG, [b] BCD, [c] PHYSICAL MIXTURE OF AG - BCD, [d] INCLUSION COMPLEX OF AG - BCD (1:1), [e] INCLUSIONS COMPLEX OF AG - BCD (1:2), [e] INCLUSION COMPLEX OF AG - BCD (2:1)

Scanning Electron Microscope: In the SEM micrograph, pure AG (**Fig. 3a**) shows the habit flattened rod shape or needle. This is different from pure BCD (**Fig. 3b**) which is a tubular cylinder, although it is not seen clear. However, the solid from the results of AG-BCD inclusion complex show the different habits compared to each single component of the AG and BCD habit which forming the irregular or amorphous. This indicated the possibility of interaction between the AG to BCD to form an inclusion complex compound. If compared to the third comparison AG-BCD inclusion complex, the ratio of 1:2 (**Fig. 3e**) showed the smallest particles that are less than 10 μm and with amorphous habit. This indicated that the interaction between the AG and BCD formed an inclusion complex compound.

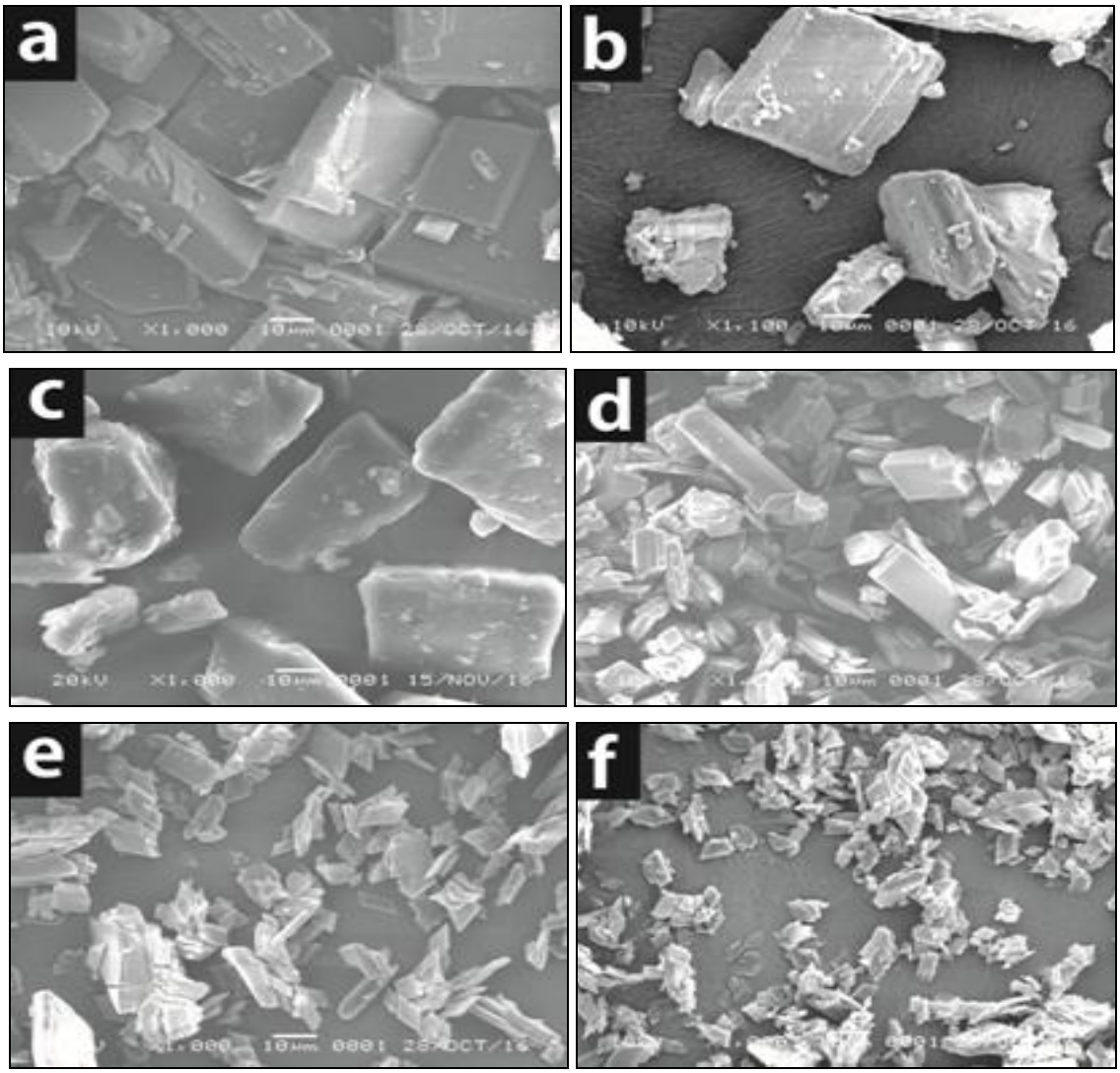

FIG. 3: SEM MICROGRAPH: [a] AG, [b] BCD, [c] PHYSICAL MIXTURE OF AG-BCD, [d] INCLUSION COMPLEX OF AG-BCD (1:1), [e] INCLUSIONS COMPLEX OF AG-BCD (1:2), [f] INCLUSION COMPLEX OF AG-BCD (2:1)

CONCLUSION: Of the three analysis or characterization, it could be concluded that the inclusion complex of AG-BCD was formed at the mole ratio of 1:2.The analysis using PXRD showed the characteristic change from crystalline into amorphous. The DSC analysis showed the invisibility of the AG melting point but shows the thermal profile of BCD. The morphological analysis by SEM indicated that the particle size of inclusion complexes AG - BCD are less than 10 μm with the amorphous habit.

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CONFLICT OF INTEREST: Nil

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