ATLANTIS PRESS

Advances in Health Sciences Research, volume 26

2nd Bakti Tunas Husada-Health Science International Conference (BTH-HSIC 2019)

Pharmaceutical Cocrystal of Ethyl p-Methoxycinnamate: Formulation and Characterization

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***Abstract*—Ethyl p-methoxycinamate (EPMC) is a compound derived from kencur (Kaemferia galanga) and has a pharmacological effect as an antiinflammatory. EPMC has poorly water solubility and need to be modified to increase its solubility. The aim of this study was to improve the water solubility of EPMC by cocrystallization method. Cocrystal formation between EPMC and tartaric acid as a coformer with various ratio of 1:1, 1:2, and 1:3 were produced by solvent drop grinding technique utilizing methanol solvent. EPMC-tartaric acid cocrystal were characterized by fourier transform Infrared (FTIR) spectrophotometer, X-ray diffraction (XRD), scanning electron microscopy (SEM), and water solubility study. The result showed that the EPMC- tartaric acid cocrystal have new diffraction and changing in peak intensity of crystallinity and aggregation occurs, but the cocrystallization did not damage the particles. The water solubility of EPMC-TA cocrystals ratio of 1:1, 1:2, and 1:3 increased 1.39; 1.50; 1.44 folds respectively. It could be concluded that cocrystal formation succeeded increase solubility of EPMC by utilizing solvent drop grinding as technique, tartaric acid as coformer, and methanol as solvent.**

Keywords: ethyl p-methoxycinnamate, tartaric acid, cocrystals

I. INTRODUCTION

Kencur (*Kaemferia galanga*) is a plant that is often used by Indonesian people to deal with inflammation (1). Ethyl p-methoxycinnamate acid (EPMC) which is responsible for anti-inflammatory was successfully isolated (2, 3, 4). EPMC includes esters containing benzene rings and methoxy groups which are non-polar and carbonyl groups that bind ethyl are slightly which is polar and therefore tend to be insoluble in water (5).

The solubility of active pharmaceutical ingredient (API) in water is very important in the absorption process. The bioavailability of API given orally is very dependent mainly on solubility in the gastrointestinal tract and drug permeability across the gastrointestinal membrane. API molecules must be in dissolved form so they can be transported across biological membranes. Low water solubility can delay or limit drug absorption (6, 4). Therefore, EPMC which has low solubility in water can be problematic in the absorption process so it is necessary to develop a formulation to increase its solubility.

One method that has been proven successful in increasing the solubility of API in water is the cocrystallization method. The cocrystalization method is proven to be able to change the physicochemical properties of a compound including its solubility. Cocrystallized particles are called cocrystal. Cocrystal is a combination of two compounds in the same crystal lattice, namely API and other components known as coformer (7). Cocrystal has advantages compared to hydrate and amorphous forms. Hydrates often turn into anhydrous crystal form so as to produce significant changes in physical properties, appearance, and integrity. Amorphous has a higher surface area and better wetting, but is not thermodynamically stable (8). These problems can be overcome by the formation of cocrystal (9).

Coformer is a compound with a small molecular weight used in cocrystal formation. Coformer that is often used to increase the solubility of API in water is tartaric acid. Tartaric acid is registered by the FDA (Food and Drug Administration) as GRAS (generally recognized as safe) so it is safe to use as a coformer in cocrystal formation. Tartaric acid has 4 hydrogen donors and 2 hydrogen acceptors which allow hydrogen bonds to form cocrystal. The carboxylic acid functional groups in tartaric acid can bind to the ester functional groups on API. The ester function group itself is contained in EPMC making it very possible that tartaric acid is used as an EPMC coformer (10).

In producing cocrystal, the most commonly used technique is solvent drop grinding because it has several advantages which are inexpensive, easy, and environmentally friendly (11). In addition, the solvent drop grinding technique can reduce particle size, fast processing, high yields, isolate polymorphs from the same cocrystal, and can use different stoichiometric ratios (12).

EPMC has not been modified to improve its water solubility previously, while water solubility is very important in the pharmaceutical formulation technology. EPMC as a marker compound for kencur, a typical Indonesian plant, has many pharmacological effects and low side effects. So, EPMC has the potential to become a

new API by improving its water solubility in order to optimize its pharmacological effects.

In this study, the novel method on particle preparation was proposed. The objective of the present investigation is to enhance the water solubility of EPMC by preparing particle using the cocrystalization method utilizing tartaric acid as coformer. Functional groups, crystallinity, surface morphology, and water solubility studies of as prepared EPMC-tartaric acid cocrystal were investigated.

II. EXPERIMENTAL

A. Preparation of EPMC-Tartaric Acid Cocrystal

Each batch of cocrystal was prepared by accurately weighed equimolar proportions of EPMC and tartaric acid 1:1; 1:2; 1:3 as we can see in Table 1.

TABLE 1. THE RATIO OF EPMC AND CITRIC ACID

Ratio	Concentration		Volume of methanol (µL)
	EPMC	Tartaric acid	
EPMC-tartaric acid cocrystal 1:1	0.800 g (1mmol)	0.582 g (1mmol)	345.5µL
EPMC-tartaric acid cocrystal 1:2	0.800 g (1mmol)	1.164 g (2mmol)	491.0 µL
EPMC-tartaric acid cocrystal 1:3	0.800 g (1mmol)	1.746 g (3mmol)	636.5µL

EPMC and tartaric acid are crushed separately and mixed in mortars. EPMC-tartaric acid is crushed again for 4 minutes then methanol is added then evaporated at room temperature. Untreated EPMS as a comparison was physically crushed using in a mortar for 4 minutes.

B. Characteristic of Particle

Untreated EPMC and EPMC-tartaric acid cocrystal were characterized by means of fourier transform infrared spectroscopy (FT-IR) Thermo Scientific Nicolet iS5 spectrophotometer to characterize functional groups, by means of x-ray diffractometry (XRD) Shimadzu 4.5 to characterize crystallinity, and scanning electron microscopy (SEM) Phenom FEI to characterize surface morphology.

C. Solubility Studies

Untreated EPMC and EPMC-tartaric acid particles (equivalent to 20 mg of EPMC) were added to 50 ml of water, shaken at speed of 240 rpm for 24 hours at temperature of 25±1 °C. The sample of 5 mL was filtered through a membrane filter 0.45 µm then determined by UV spectrophotometer Shimadzu at a wavelength of 310 nm.

III. RESULTS AND DISCUSSION

Functional group analysis using the Fourier Transform Infra Red (FTIR) spectrophotometer was performed on EPMS, tartaric acid, EPMS-tartaric acid cocrystal of 1:1, EPMS-tartaric acid cocrystal of 1:2, and EPMS-tartaric acid cocrystal of 1: 3. The FTIR spectrum can be seen in figure 1.

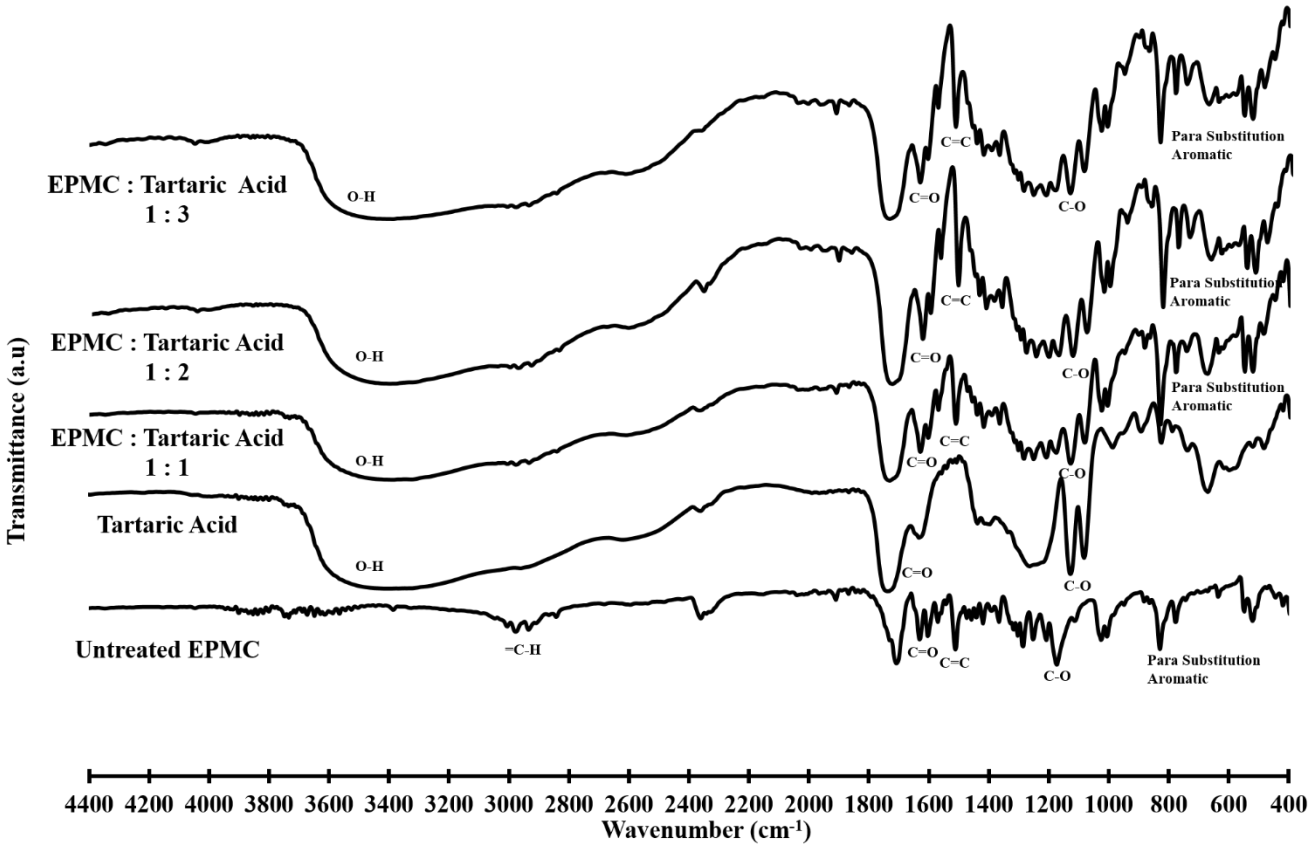

Fig 1. FTIR spectrum of epmc-tartaric acid 1:3, epmc-tartaric acid 1:2, epmc-tartaric acid 1:1, tartaric acid, and epmc

Based on Figure 1, the cocrystallization method does not damage EPMC or tartaric acid. This is indicated by the presence of EPMS and tartaric acid functional groups in the cocrystal of EPMC-tartaric acid.

The FTIR spectrum shows that EPMS has a typical peak at wave number 2978-2935 cm^{-1} indicating aliphatic C-H bonds, at wave number 1631 cm^{-1} indicating C = O carbonyl bonds, at wave number 1512 cm^{-1} indicating C = C bonds aromatic, at wave number 1172 cm^{-1} indicating C-O bond binding to aromatic, at wave number 1022 cm^{-1} indicating C-O bond of ester groups, and at wave number 829 cm^{-1} indicating aromatic substituted para. The FTIR spectrum shows tartaric acid has a typical peak at wave number 3402 cm^{-1} indicating the presence of a hydroxy function group, at wave number 1732 cm^{-1} indicating the C = O carboxylic acid bond, and at wave number 1126 cm^{-1} indicating C-O.

The FTIR spectrum shows a 1:1, 1:2, and 1:3 ratio of EPMC-tartaric acid cocrystal have some typical peaks that correspond to peaks that appear on EPMS and tartaric acid. The FTIR spectrum of EPMC-tartaric acid 1: 1 cocrystal showed a shift in the peak of the C = O carbonyl group from wave number 1631 cm^{-1} to 1627 cm^{-1}, CO ester group from wave number 1022 cm^{-1} to 1026 cm^{-1}, and the group CO is bound to aromatics from wave number 1172 cm^{-1} to 1130 cm^{-1}. The FTIR spectrum of EPMC-tartaric acid 1:2 cocrystal showed a shift in the peak C = O carbonyl ester group from wave number 1631 cm^{-1} to 1627 cm^{-1}, CO ester group from wave number 1022 cm^{-1} to 1026 cm^{-1}, and CO groups that bind to aromatics from wave number 1172 cm^{-1} to 1130 cm^{-1}. The FTIR spectrum of EPMC-tartaric acid 1:3 cocrystal shows the peak of the C = O carbonyl group of fixed esters, the CO ester group of wave numbers 1022 cm^{-1} becomes 1026 cm^{-1}, and the CO groups that bind to aromatics of wave numbers 1172 cm^{-1} to 1130 cm^{-1}.

The shift of the wave number in the cocrystal spectrum pattern occurs due to the hydrogen bonding between the hydroxy function group found in tartaric acid and the carbonyl functional group found in the EPMC. Hydrogen bonds at C = O, C-O esters and C-O aryl will extend the bonds C = O, C-O and C-O aryl so that the strength of the bonds is reduced and the vibration band appears at lower numbers.

Crystallinity analysis using an X-ray diffractometry (XRD) spectrophotometer was performed on EPMC, tartaric acid, EPMC-tartric acid cocrystal 1:1, EPMS-tartaric acid cocrystal 1: 2, and EPMS-tartaric acid cocrystal 1: 3. Difractogram can be seen in Figure 2.

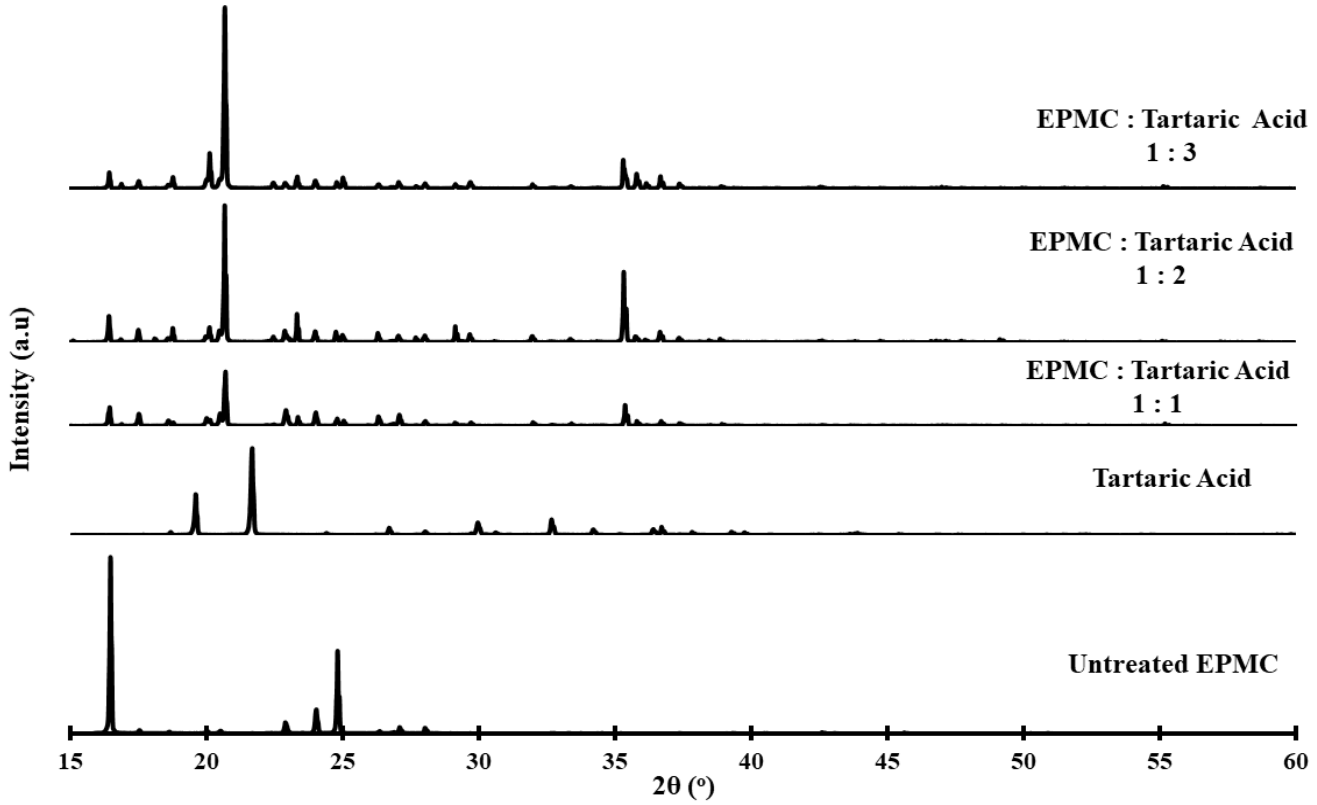

Fig 2. Difractogram of epmc-tartaric acid 1:3, epmc-tartaric acid 1:2, epmc-tartaric acid 1:1, tartaric acid, and epmc

Each material has a specific reflection angle. In XRD angle 2θ is used as a variable to measure the reflection angle of each atom. Angle 2θ is the angle between incident ray and reflected ray. The angle can be determined based on the similarity of the angle of reflection with the angle of incidence. At an angle of 2θ reflected very much light due to differences in atomic and crystal structure of the sample. FWHM (Full Width at Half Maximum) shows the crystallinity of a substance. Narrow FWHM shows that a particle has a crystalline or high crystallinity. A wide FWHM indicates that a particle has an amorphous or low crystallinity.

Figure 2 shows EPMC, tartaric acid, and cocrystal having a sharp diffraction pattern or a narrow FWHM. This shows that EPMC-tartaric acid cocrystal was successfully formed in crystal form. However, the crystallinity of EPMC-tartaric acid is different from the crystallinity of its constituent components, namely EPMC and tartaric acid. The crystallinity of EPMC-tartaric acid cocrystal increases with the appearance of new peaks.

EPMC-tartaric acid cocrystal 1:1 diffractogram showed significant new peaks at 2θ = 20.19°, 20.52°, and 35.36°. EPMC-tartaric acid cocrystal 1:2 diffractogram showed significant new peaks at 2θ = 20.17°, 20.52° and 35.37°. The EPMS-tartaric acid cocrystal 1:3 diffractogram showed significant new peaks at 2θ = 20.15°, 20.71°, and 35.31°. The existence of a new peak in the cocrystal indicates the formation of a new crystal formation. This shows that the position of the atom occupying the lattice is different.

Surface morphology analysis using scanning electron microscopy (SEM) was carried out on EPMS, tartaric acid, EPMS-tartric acid cocrystal 1:1, EPMS-tartric acid cocrystal 1:2, and EPMS-tartaric acid cocrystal 1:3. Surface morphology can be seen in Figure 3.

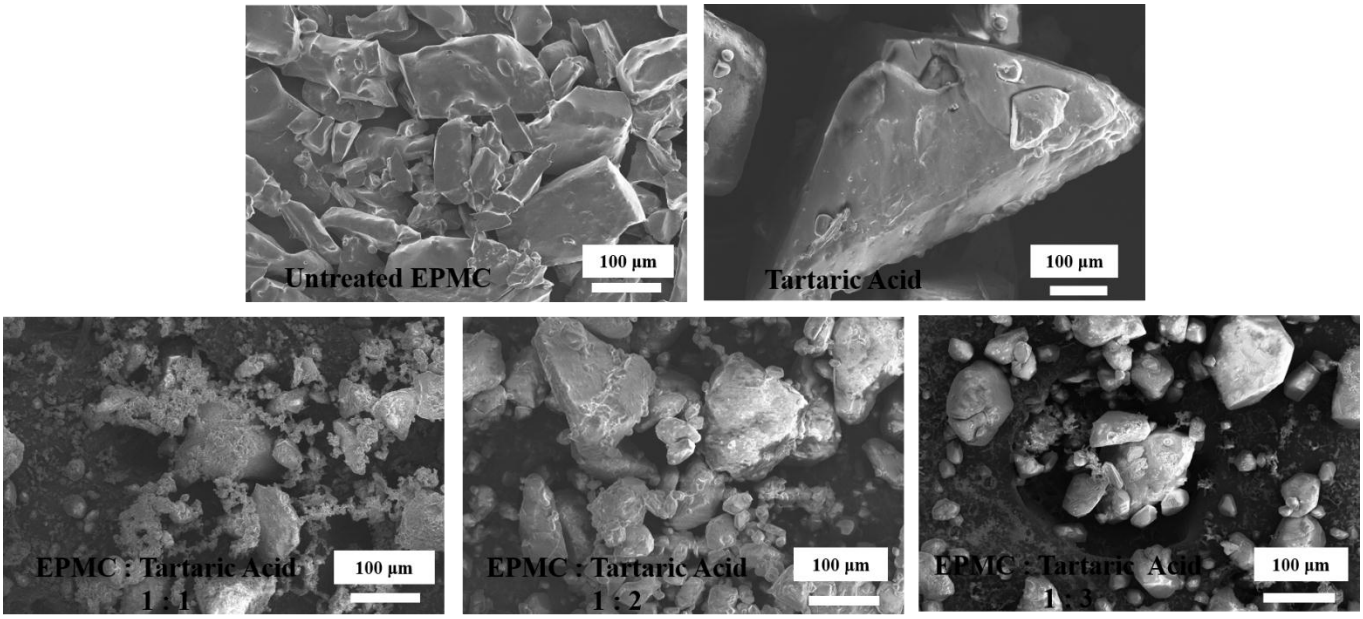

Fig 3. Surface morphology of epmc, tartaric acid, epmc-tartaric acid 1:1, epmc-tartaric acid 1:2, epmc-tartaric acid 1:3

Figure 3 shows EPMC and tartaric acid are solid particles with a rough surface texture. At the same magnification, which is 200x, the EPMC shows a smaller particle size compared to tartaric acid. Figure 3 shows the EPMC-tartaric acid cocrystal undergoing more rough surface morphology and smaller particle size compared to EPMC although not significant. The smaller particle size causes a larger surface area so that the EPMC-tartaric acid cocrystal has a wider contact surface with solvent. The third form of EPMC-tartaric acid cocrystal is irregular and forms aggregates which cannot be easily separated from each other.

The water solubility study was carried out on EPMS, EPMS-tartaric acid cocrystal 1: 1, EPMS-tartaric acid cocrystal 1:2, and EPMS-tartric acid cocrystal 1:3. The results of the water solubility study can be seen in Figure 2.

TABLE 2. WATER SOLUBILITY OF EPMC-TARTARIC ACID COCRYSTAL

Sample	Solubility (µg/ml)
Untreated EPMS	3.01±0.008
EPMC-tartaric acid cocrystal 1:1	4.20±0.024
EPMC-tartaric acid cocrystal 1:2	4.52±0.020
EPMC-tartaric acid cocrystal 1:3	4.34±0.004

Table 2 shows the water solubility of EPMC increased after cocrystal was formed using tartaric acid as a coformer. EPMC-tartaric acid cocrystal 1:1 has water solubility 1.39 folds compared to untrated EPMC. EPMC-tartaric acid cocrystal 1: 2 has water solubility 1.5 folds compared to untrated EPMC. EPMC-tartaric acid 1:3 has water solubility 1.44 folds compared to untrated EPMC.

The increase in water solubility of EPMC after cocrystal formed is due to several things, namely hydrogen bonds formed, smaller particle size, and tartaric acid which is water soluble. This hydrogen bond was seen in the FTIR spectrum formed in Figure 1. The hydrogen bond formed makes it easier for water to bind to EPMC and break it. The smaller particle size of EPMC-tartaric acid compared to untreated EPMC causes the surface area of EPMC-tartaric acid cocrystal to be greater compared to untreated EPMC. The smaller particle size of EPMC-tartaric acid cocrystal can be seen in Figure 3. The larger surface area of EPMC-tartaric acid cocrystal causes more water to contact EPMC so that the solubility of EPMC-tartaric acid cocrystal is higher than untreated EPMC. Tartaric acid as a water soluble coformer helps EPMC dissolve in water because of its ease of bonding with water and breaking EPMC.

However, the solubility of EPMC-tartaric acid cocrystal does not increase significantly because of its higher crystallinity compared to untreated EPMC as shown in Figure 2. High crystallinity shows strong intermolecular bonds. High molecular bonds need a lot of energy to break them. In addition, the aggregate formed in EPMC-tartaric acid cocrystal causes inhibition of EPMC-tartaric acid cocrystal solubility in water. The aggregate can be seen in Figure 3. Therefore, the cocrystal of EPMC-tartaric acid increases its solubility in water, but it is not significant.

The bond position between EPMC and tartaric acid also determines the bond strength and its solubility in water. The increase in water solubility between EPMC-tartaric acid 1:1, 1:2, and 1:3 does not appear to be significantly different because the likelihood of binding between EPMC and tartaric acid is considerable. The bond between EPMC and tartaric acid can occur in the same or different positions. Possiblility of bond positions that occur between EPMC and tartaric acid were not examined in this study.

IV. CONCLUSION

It can be concluded that the cocrystallization method utilizing tartaric acid with a variety of concentrations succeeded in increasing the water solubility of EPMC.

EPMS-tartaric acid cocrystal in the ratio of 1:1, 1:2, and 1:3 increased water solubility by 1.39, 1.50, and 1.44 folds respectively. The cocrystallization process does not damage the EPMC and tartaric acid components in the cocrystal system, increasing the crystallinity and the surface area of EPMC.

ACKNOWLEDGMENT

This research was supported and fund by Hazanah Foundation for Fundamental Research Grant of Indonesian School of Pharmacy

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