Micronization of *Curcuma xanthorrhiza* Extract with Addition of PVP Using Supercritical CO_2 as Anti-solvent

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Abstract *Curcuma xanthorrhiza Roxb*, known as Temulawak or Javanese ginger, is a plant species. Its rhizomes are used as a medicinal herb. It contains curcumin as an active compound and ethereal oils mainly consisted of sesquiterpenes. In this work, *Curcuma xanthorrhiza Roxb* ethanolic extract was micronized with an addition of PVP using supercritical antisolvent (SAS) method. The ethanolic extract was obtained from dried *Curcuma xanthorrhiza Roxb* using soxhletation. For the micronization, the extracted compound solvent was a mixture of acetone and ethanol (90:10 (v/v)), while the supercritical CO_2 was used as an antisolvent. The effect of operating conditions on the particle size and morphology was evaluated. Through this method, spherical *Curcuma xanthorrhiza*/PVP particles with mean diameter ranging from 191 ± 70 nm to 178 ± 57 nm were successfully formed. The particle size not significantly decreased as the pressure increased from 8 - 12 MPa. The addition of PVP is very effective to reduce the particle size, increase the solubility, and enhance the bioavailability of *Curcuma xanthorrhiza* extract. This work has the potential to improve the use of *Curcuma xanthorrhiza* in pharmaceutical and nutraceutical applications.

1 Introduction

For several years an increasing number of herbal medicines has appeared in worldwide marketplace. There are many varieties of plant that can be studied in deliver its function for herbal medicine. Natural products are known as more drug-likely and biological friendly than synthetic molecule product. Many of them have been proven to have better compatibility with biological system and lesser side effect (Oon *et al.*, 2015). Nowadays, the studies for modern pharmaceutical industry has been more focused on medicine from natural product. One of the most known used on plant-based-medication is the *Curcuma* genus, which consist of more than eighty species. *Curcuma xanthorrhiza Roxb*, known as Temulawak or Javanese ginger, is an original plant from Indonesia that belongs to *Zingiberaceae* family. Today, the cultivation of this plant is grown wild in Thailand, Indochina, The Malay Archipelago, India, Japan, Korea, and Northern Australia (Jantan *et al.*, 2012).

Curcuma xanthorrhiza Roxb has been traditionally used as herbal medicine in Indonesia. There are more than fifty recipes of herbal medicine using *C. xanthorrhiza*. Its rhizome is a major material in the herbal medicine production. *C. xanthorrhiza* known as remedy for many diseases such as stomach diseases, liver disorders, rheumatism, and high cholesterol (Yasni *et al.*, 1994). From previous study showed that extract from *C. xanthorrhiza* proven to have antioxidant, anti-microbial, anti-inflammatory, analgesic, immunostimulant, anti-fungal, antitumor, and anti-diuretic activity. Beside for medical needs, its rhizome is also used as spice on foods, giving a yellow colour on some cuisine, and for cosmetics (Jae *et al.*, 2008; Kim *et al.*, 2007a; Mary *et al.*, 2012; Ozaki, 1990). All the benefits were support by the active compounds in *C. xanthorrhiza* extract such as curcumin, xanthorrhizol, and few volatile compounds (Devaraj *et al.*, 2010; Hwang *et al.*, 2000). The phytochemical studies showed that *Curcuma* rhizomes contain two important bioactive compounds, namely diarylheptanoid (curcuminoid) and terpenoid (mainly sesquiterpenes) (Diastuti *et al.*, 2019). The micronization of *C. xanthorrhiza* extract into microparticles is needed to prevent the rapid oxidation of bioactive compounds.

The particle mironization of bioactive compound with biopolymer addition has been found to be particular interest because it can improve drug delivery system by preventing the rapid degradation of bioactive compounds, increasing the bioavailability in the body, and enhancing the solubility in the water (Chhouk *et al.*, 2018; Nuchuchua *et al.*, 2017). Polyvinylpyrrolidones (PVP), water-soluble carriers with high molecular weight, have been the most used biopolymer for particle micronization. According to previous study, the dissolution of drug in physical mixtures were higher with the present of PVP. The ability to form good solubility in water and various

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solvent had become a suitable reason to use PVP as a carrier. The *C. xanthorrhiza* extract has low solubility in water. Therefore, in this work, the addition of PVP was expected to give a great influence in increasing the solubility of *C. xanthorrhiza* extract. The formation of hydrogen bonds between PVP and the phenolic compound in the extract plays an important role in particle formation (De Almeida *et al.*, 2018).

The size of particle holds an important effect in the drug delivery system and its bioavailability. Decreasing the particle size will increase the surface area and enhance the solubility itself. There are many conventional techniques used to produce coprecipitates, such as solvent evaporation, coacervation, and spray drying. However, these techniques have some shortage such as the particle size obtained relatively large, the thermal-degradation of bioactive compounds, and the large amount of solvent needed that can affect residue in final product (Kwon *et al.*, 2011). Supercritical Fluids (SCFs) based techniques has been used to overcome the shortage in conventional methods. Supercritical fluid with the antisolvent (SAS), one of supercritical assisted micronization method, is widely used for microparticles formation from natural products (Boonnoun *et al.*, 2013; Chhouk *et al.*, 2018; Kim *et al.*, 2007b; Lestari *et al.*, 2019; Xia *et al.*, 2012; Yoon *et al.*, 2016). This method suitable for bioactive compounds that difficult to dissolve in supercritical fluid. In SAS method, an organic solution of solute is streamed through a nozzle and is conjugated with a supercritical fluid in a chamber simultaneously. The phenomenon of mass transfer occurs between solution and supercritical fluid, which plays role as an anti-solvent, leading to supersaturation state and then resulting in the formation of solute particles. SAS method generates smaller particle size, better morphology and even more equitable particle distribution. Carbon dioxide (CO_2) is the most commonly used as supercritical fluid due to it is non-inflammable, non-toxic, inexpensive, available in abundant amount, eco-friendly, and its properties are controlled easily.

In this study, the SAS method using carbon dioxide as the anti-solvent was done for particle micronization of *C. xanthorrhiza* extract with the addition of PVP as a biopolymer. The effect of operating conditions on the particle size and morphology was examined.

2 Materials and Methods

2.1 Chemicals reagents

Carbon dioxide (CO_2, purity ≥ 99.9%) was obtained from Samator (Surabaya, Indonesia). Ethanol (≥ 99.5%) and Acetone (≥ 99.7%) was provided by Merck (Germany) and PT. Smart Lab Indonesia (Tangerang, Indonesia). Polyvinylpyrrolidone (PVP; molecular weight of 29000) was provided by Sigma Aldrich.

2.2 Plant materials

Curcuma xanthorrhiza Roxb were purchased from local suppliers in Surabaya, East Java, Indonesia. The sample preparation from *C. xanthorrhiza* were dried at room temperature to decrease its moisture content. Then the dried sample were grounded into fine powder.

2.3 Equipment, methods, and procedure

2.3.1 Extraction of C. xanthorrizha

Fine powder of *C. xanthorrhiza* were extracted using soxhletation with ethanol as the solvent for 15 hr. Then the ethanol was removed using rotary vacuum evaporator (RE-1000VN, B-ONE, Indonesia). The crude from *C. xanthorrhiza* extract were obtained. This method was done to achieve *C. xanthorrhiza* extract in higher yield than using another extraction method (Leblebici *et al.*, 2012; Tambun *et al.*, 2017).

2.3.2 Micronization with SAS method

The particles micronization process started by heating the oven drying chamber into the desired temperature. Then using a coil, the supercritical CO_2 was streamed through a chiller to maintain the liquefaction of CO_2 and prevent cavitation in pump. With a high-pressure pump (PU-1586 Intelligent prep. pump, JASCO, Japan), the liquid CO_2 was pumped into the system at a constant flow rate of 15 mL/min. The operating pressure in the system was maintained by using a back-pressure regulator (BPR, Tescom, U.S.). It was equipped with a heater to prevent the CO_2 that passing through the BPR from freezing and clog the precipitator tube. After the operating pressure and temperature were achieved, another pump (PU-980 Intelligent HPLC pump, JASCO, Japan) was used to inject the crude extract and PVP solution with ratio varied from 0 to 1:10 (w/w) into the system at a constant flow rate of 0.25 mL/min. The concentration of feed solution about 2 mg/mL with a mixture from acetone and ethanol ((90:10, (v/v)) as organic solvent to dissolve the extract and PVP. The supercritical CO_2 with a constant flowrate of 15 mL/min and the solution were contacted through a precipitator tube inside the heating chamber for 90 min. This process leads SC-CO_2 dissolved into organic solvent and causing supersaturation condition, thus resulting in the formation of fine particles. After the micronization process was completed, the SC-CO_2 was continuously pumped for 90 min to wash out the remaining solvent in precipitator. The obtaining particles were collected inside a collector with 0.5 μm filter (Swagelok, USA). Then the micronized particles were kept in desiccator to prevent degradation of the particle due to light until further analysis.

2.3.3 Product analysis

The Scanning Electron Microscopy (SEM, S-4300, Hitachi) was used to determine the surface morphology of the particles. Before conducting SEM analysis, the sample of particles was dispersed on a carbon tab in the aluminium holder and coated with thin layer of gold at high pressure evaporator. The mean particle size and distribution were calculated using Image J analysis

software from approximately ± 250 particles observed in SEM image.

Fourier Transform Infrared (FTIR) spectrophotometry was used to determine chemical structure and the existence of remaining solvent after micronization process in *C. xanthorrhiza* extract, PVP, and *C. xanthorrhiza*/PVP particles. Data were obtained using wavelength range of 4000–400 cm^{-1}.

2.4 Dissolution studies

The dissolution rate of *C. xanthorrhiza* extract and *C. xanthorrhiza*/PVP particles were analysed using UV spectrophotometer (Genesys 10-S, Thermo Fisher Scientific, US) at wavelength of 425 nm as the highest absorbance peak. Sample from *C. xanthorrhiza* particles and *C. xanthorrhiza*/PVP particles were tested in 10 mL of ultra-pure water with equal concentration as dissolution medium. Through a disposable filter, aliquots of sample were filtered and then measured the absorbance with interval time up to 24 hr. The solubility of particles was compared with particles solubility in ethanol as a complete dissolution (Lestari *et al.*, 2019).

3 Result and Discussion

3.1 SAS micronization of *C. xanthorrhiza*/PVP

In supercritical fluid with antisolvent method, knowing the thermal properties of CO_2 and its organic solvents is important to assure the micronization process conducted in the right condition. There are many studies done for determine the phase diagram of CO_2 in a mixture with acetone/ethanol. Acetone and ethanol as organic solvent have a higher solubility in CO_2 at high-pressure than low-pressure system. Their binary mixture achieves the critical state of CO_2 at an enough high-pressure system (Day *et al.*, 2002; Hsieh and Vrabec, 2015). Hence, based on the P-T diagram reported by Ziegler *et al.* (1995), it can conclude that the operating condition in this study were conducted in the mixture critical point (MCP). When the operating condition above the MCP, interfacial tension between *C. xanthorrhiza*/PVP solution and supercritical CO_2 disappear then the precipitate particles were formed as the result (Yoon *et al.*, 2016).

In order to observe the morphology, particle of PVP, *C. xanthorrhiza* extract, and *C. xanthorrhiza*/PVP that obtained from SAS method were analysed using SEM. As shown in Figure 1, the particle form of *C. xanthorrhiza* extract were irregular with agglomeration and PVP particle form also irregular but almost spherical. Whereas the morphology for particle of *C. xanthorrhiza* extract with the addition of PVP were spherical more evenly. The range particle size of *C. xanthorrhiza* extract from 307 ± 87 nm to 349 ± 68 nm while *C. xanthorrhiza*/PVP was ranged from 178 ± 57 nm to 191 ± 70 nm. From the result, the existence of PVP in *C. xanthorrhiza* extract solution exhibit a completely different morphologies at the same operating condition. The *C. xanthorrhiza* extract were completely micronized or encapsulated in biopolymer microspheres. PVP was simply attached on the surface of the *C. xanthorrhiza* extract and resulting an improvement in the particle morphology. Smaller and better spherical particle were formed.

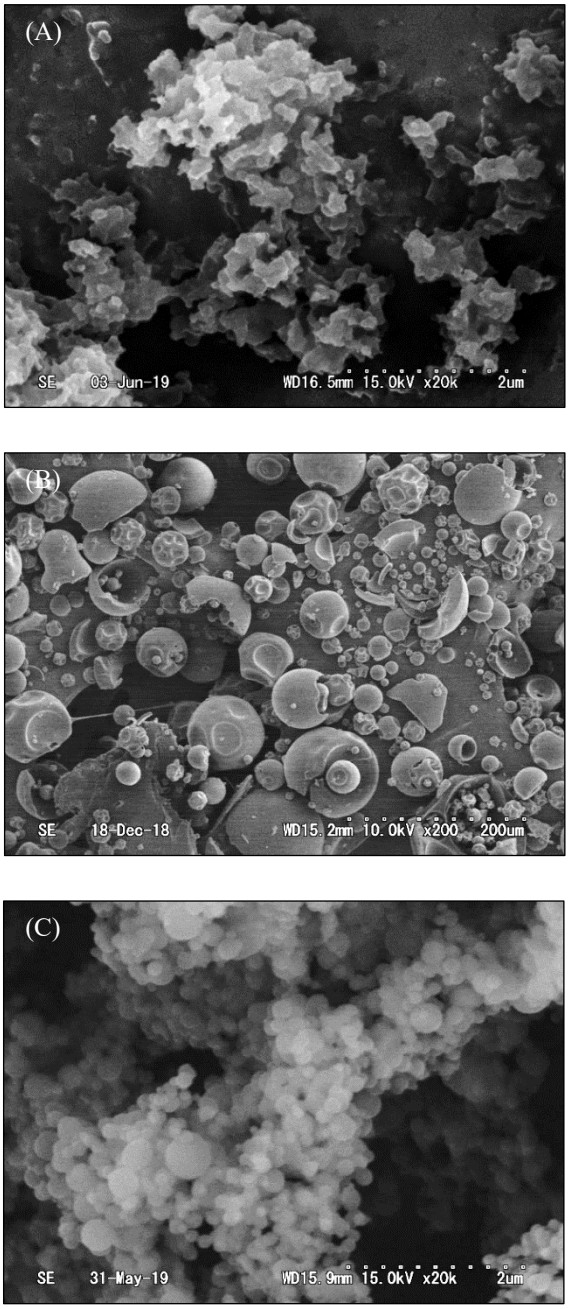

Figure 1. SEM Image of (A) *C. xanthorrhiza* extract, (B) PVP, and (C) *C. xanthorrhiza*/PVP particles.

3.2 Effect of pressure

The micronization of *C. xanthorrhiza*/PVP using SAS method was carried out at various pressure in the range 8 MPa to 12 MPa. At a temperature of 40ºC, CO_2 constant flow rate of 15 mL/min, *C. xanthorrhiza*/PVP ratio of 1:10, the feed concentration of 2 mg/mL, the morphology and particle distribution were examined. When the pressure raised from 8 MPa to 12 MPa, the morphology of particle was less agglomeration as shown in Figure 2. The formation of spherical particle was triggered by

raising the operating pressure. When the pressure was increased, the density difference in CO_2 and organic solvent (acetone/ethanol) were decreased and generate a higher mass transfer between them, faster supersaturation, then resulting smaller mean particle size with better spherical (Kim *et al.*, 2007b).

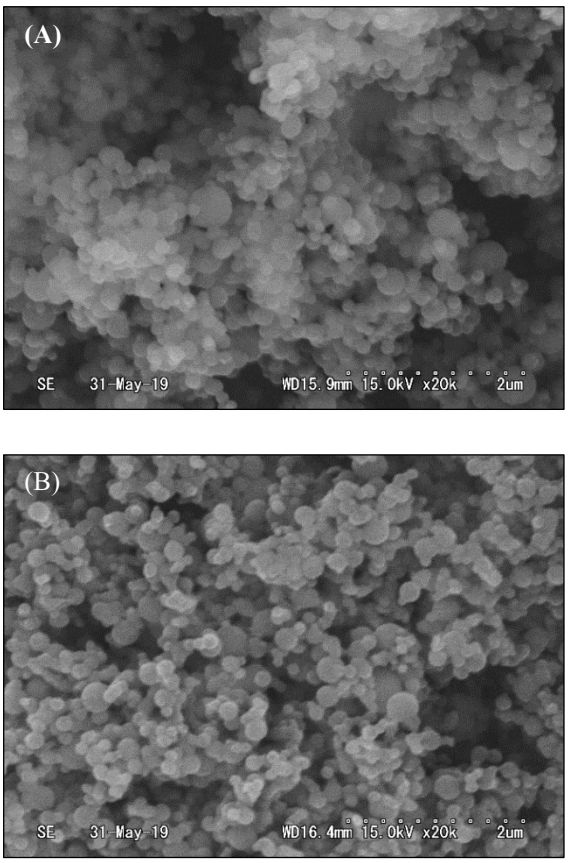

Figure 2. SEM Image of *C. xanthorrhiza*/PVP particles at pressure (A) 8 MPa and (B) 12 MPa.

From SEM image, the mean particle size and the distribution of particles can be determine using Image J analysis software. As pressure increased from 8 MPa to 12 MPa, the mean particle size was slightly decrease from 191 nm to 178 nm. Figure 3 shown a change in pressure has an insignificant effect on particle size distribution.

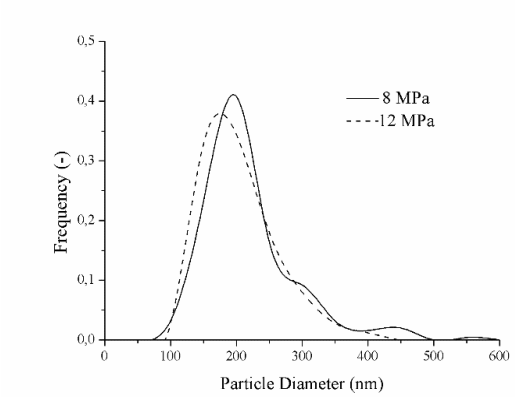

Figure 3. Particle size distribution (PSD) at various pressure.

3.3 FTIR analysis

FTIR analysis were conducted to determine the modification of the particle chemical structures. From Figure 4, the particle produces from *C. xanthorrhiza* extract represent a characteristic absorption band at 3328 cm^{-1} that related to hydroxyl O-H group. Another peak was appeared at 2922, 1514, and 1031 cm^{-1} which attribute to the stretching of C-H aliphatic, C=O stretching, and C-O-C stretching, respectively. The FTIR spectra of PVP represent a characteristic absorption band around 1649 cm^{-1} corresponded to C=O stretching band. Absorption band at 2952 and 3419 cm^{-1} were corresponded to C-H and O-H stretching. Meanwhile for particles of *C. xanthorrhiza*/PVP shows a similar peak with PVP alone at 1649 cm^{-1} with slightly difference in their intensity. This FTIR result indicates the presence of PVP gave an interaction in the *C. xanthorrhiza*/PVP particles.

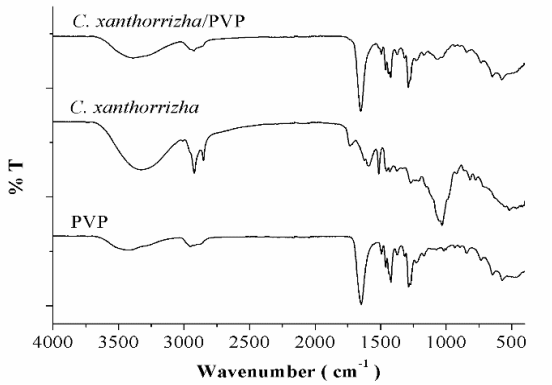

Figure 4. FTIR spectra comparison of PVP, *C. xanthorrhiza* extract particles, and *C. xanthorrhiza*/PVP particles.

3.4 Dissolution studies

Dissolution studies were conducted by dissolving an equal concentration of *C. xanthorrhiza* extract particles and *C. xanthorrhiza*/PVP particles into 10 mL ultra-pure water for 24 hr. The sample mixture was stirred and heated at 100 rpm and 37ºC. The dissolution rate was examined by comparing the absorbance of micronized particle in aqueous solution with the absorbance of *C. xanthorrhiza* extract particles in ethanol. The highly solubilities of *C. xanthorrhiza* extract particles in ethanol was define as complete dissolution of the particles. After 24 hr, the *C. xanthorrhiza*/PVP particles were approximately 71% dissolve in aqueous solution while the *C. xanthorrhiza* extract particles only 40% dissolved. The UV spectra scanning result from *C. xanthorrhiza* extract and *C. xanthorrhiza*/PVP particle in aqueous solution have a different peak absorbance. As shown in Figure 5, the peak absorbance was higher than without the addition of PVP. The significant increase in solubility of *C. xanthorrhiza* by PVP can be explained due to the formation of soluble complexes between water-soluble biopolymer and low-soluble active ingredients (Sethia and Squillante, 2004). The reduction of particle size in *C. xanthorrhiza* extract particles with PVP also contribute in

enhancing the bioavailability and dissolution ability (Perrut *et al.*, 2005).

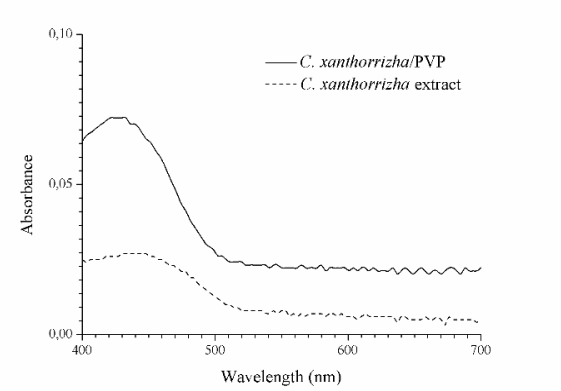

Figure 5. UV Spectra scanning of *C. xanthorrhiza* extract and *C. xanthorrhiza*/PVP particle in aqueous solution.

4 Conclusions

Supercritical antisolvent (SAS) micronization using acetone was successfully produce fine particle of C. *xanthorrhiza* extract with addition of PVP. The variety in operating pressure induced an insignificant effect on the morphology and the particle size distribution. The mean size of particle slightly reduced in line with increased pressure. The FTIR analysis shows that *C. xanthorrhiza* extract exist inside the particle and coated by PVP. The addition of PVP produced a smaller and better spherical particle. From the dissolution study, micronized particle of *C. xanthorrhiza*/PVP has a higher ability to dissolve in aqueous solution and enhance bioavailability. Therefore, this experiment will improve the use of *C. xanthorrhiza* in the pharmaceutical and nutraceutical applications.

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