The Curcuminoids Extract of *Curcuma xanthorrhiza* RoxB. Loaded Solid Lipid Nanoparticles

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Abstract: Curcuma xanthorrhiza RoxB. is the medicinal plant that widely used in Indonesian traditional herbal medicine (Jamu). The curcuminoids is well known for its pharmacological properties such as antioxidant, anti-inflammatory, and anti-carcinogenic. The curcuminoids is weakly soluble in water that restrict its bioavailability. This problem could be overcome by incorporated curcuminoid into solid lipid nanoparticles (SLN). The purpose of this study was to produce and characterize curcuminoidsextract loaded solid lipid nanoparticles (curcuminoids-SLN) fromC. xanthorrhiza. The curcuminoids-SLN was prepared using different composition of curcuminoid extract, surfactant and lipid. It was produced by using homogenization-ultra sonication methods. The particle was characterized for its size, properties and entrapment efficiency. Result showed that the best composition for producing curcuminoids-SLN was palmitic acid: curcuminoids: surfactants with weight ratio 1:0.5:1.5 with entrapment efficiency 72.98%. The particle size was 285.5 \pm 76.7 nm. The FTIR spectral data of curcuminoids-SLN was reflection of raw materials spectrum and XRD measurement showed that crystallinity of curcuminoids-SLN in the good agreement with palmitic acid.

Keywords: curcuma xanthorrhiza, solid lipid nanoparticle, curcuminoids, bioavailability, ultra sonication

1. Introduction

Curcuma xanthorrhiza RoxB., namely temulawak in Indonesia, is one of several plants that widely used in the Indonesian traditional herbal medicine (Jamu). The rhizome of this plant, beside rich in sesquiterpenes (like xanthorrizol, bisacumol, bisacurol, bisacurone, and zingiberene) also contains curcuminoids (1-2%) [1]. The characteristic yellow color of temulawak rhizome is due to curcuminoids. The curcuminoids is an orange-yellow crystalline powder practically insoluble in water [2]. The pharmacology activities of curcuminoids has been shown to the exhibit of anti-inflammatory. antioxidant. antimicrobial. and anticarcinogenic activities. Additionally, the hepato- and nephro-protective, antirheumatic, and hypogvicemic effects of curcuminoids also well established [3]. The clinical trials indicated that curcuminoids is well tolerated when taken as high as 12 grams per day but have low bioavailability. The major reason for low bioavailability of curcuminoid is almost insoluble in water at acidic or neutral pH, and thus is difficult to absorb, rapid metabolism, and rapid systemic elimination [3, 4].

Numerous approaches have been undertaken to improve the bioavailability of curcuminoids, involve use of adjuvant, liposome, nanoparticle, and phospholipid that can resulting long-therm circulation and better permeability [3]. This problem can be addressed by incorporated curcuminoids into colloidal carrier system. Among modern drug delivery carriers solid lipid nanoparticles (SLN) seemed to be a promising colloidal carrier system [5].

SLN are developed as an alternative system for polymeric nanoparticles, liposome and emulsion. SLN have unique properties like small size, large surface area, and high drug loading. SLN are submicron colloidal carrier composed of physiological lipid, dispersed in water or in aqueous surfactant solution [6]. Compared with other particulate carriers, SLN have a number advantages as a drug delivery system, such as good tolerability and biodegradation, high bioavailability, efficient targeting, and are easy to prepare and sterilize on a large scale [7]. Other advantages of SLN as drug delivery system are possibility of controlled drug release and drug targeting, increased drug stability, incorporation of lipophilic hydrophilic drugs feasible, no biotoxicity of the carrier, and avoidance of organic solvents [8].

Many methods are developed to prepare SLN, such as high pressure homogenization, microemulsion, solvent emulsion diffusion, solvent emulsification evaporation, high speed stirring and ultrasonication. Few novel techniques also used are supercritical fluids, membrane contractor, solvent injection, and multiple emulsion technique [9]. Ultrasonication technique is the widely used technique due to simplicity of the method and effective to production SLN without organic solvent. The problem of this method is broader particle size distribution ranging into micrometer range. Potential metal contamination due to ultrasonication is also a big problem in this method. To overcome this problem, high speed stirring (homogenization) and ultrasonication are used combined and performed at high temperature [10].Study about solid lipid as carrier system of curcuminoids has not been reported. The palmitic acid was done for microparticles development of curcuminoids coating materials [5]. The purpose of this study was to prepare curcuminoids extract loaded solid lipid nanoparticles with homogenization and ultrasonication method, and to characterize curcuminoids loaded solid lipid nanoparticles as drug delivery system.

2. Materials and Methods

Dry-powdered rhizome of *C. xanthorrhiza* were obtained from BALITTRO Bogor-West Java, Indonesia.Chemicals curcuminoids standard, palmitic acid for synthesis,

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poloxamer 188, deionized water, ethanol 96%, and n-hexane were obtained from Merck.

2.1 Preparation of curcuminoids extract and analysis

Dry-powdered rhizome of *C. xanthorrhiza* was macerated in ethanol 96% for 48 hours. The residue of maceration was sequentially extracted by soxhlet with ethanol 96%. The extraction by soxhlet was finished after the solvent have not color. The ethanolic extract (resulted by maceration and soxhlet) was liquid-liquid extracted with n-hexane (1:1). The ethanol fraction was evaporated by rotary evaporator and furthermore freeze-dryed.

The content of curcuminoid from the extract was analyzed by using HPLC. The elution was carried out with gradient solvent system with a flow rate 1 mL/min. The mobile phase consisted of methanol (A), 2% acetic acid (B), and acetonitrile (C). Quantitative levels of curcuminoids were determined using the above solvents programmed linearly from 45 to 65% acetonitrile in B for 0-15 min. The gradient then went from 65 to 45% acetonitrile in B for 15-20 min, with a constant of 5% A [11].

The curcuminoid extract was characterized using FTIR spectroscopy (Tensor 37, Bruker) and compared with curcuminoids standard. The FTIR spectra were recorded by KBr disc method and scanning was conducted from 4000 to 400 cm⁻¹.

2.2 The curcuminoids extract loaded SLN production

The oil phase consisting of lipid (palmitic acid) and curcuminoid was heated to 75°C, which exceeds the melting point of lipid. The aqueous phase was prepared by dissolving poloxamer 188 in 100 mL deionized water and heated to the same temperature of oil phase. Hot oil phase was added to aqueous phase and stirred for 5 minutes. Homogenization was carried out using Ultra-Turrax homogenizer at 13,500 rpm for 1 minute. Coarse oil in water emulsion obtained then ultrasonicated using probe sonicator and immediately cooled to room temperature in water bath [5, 12]. The formulas that used in this research was described in Table 1.

 Table 1: The raw materials composition of curcuminoids

 extract loaded SLN production

Formula	Palmitic Acid (g)	Curcuminoids extract (g)	Poloxamer 188 (g)	Deionized Water (mL)
S0.5	1.00	0.10	0.50	100
S1.0	1.00	0.10	1.00	100
S1.5	1.00	0.10	1.50	100

2.3 The curcuminoids extract loaded SLN characterization

Particle size measurement. The mean size of curcuminoids extract loaded SLN was determined by dynamic light scattering (DLS) using a laser particle sizer (Delsa Nano C, Beckman Coulter). The particle size data was evaluated using the number distribution.

FTIR analysis. The functional groups of curcuminoids extract loaded SLN was characterized using FTIR spectroscopy (Tensor 37, Bruker) to ensure that nanoparticle product has no damage during production process. The curcuminoid loaded SLN spectra as compared to raw materials spectra.

X-rays diffraction. The crystallinity of curcuminoid extract loaded SLN was characterized using x-rays diffraction (PW1710, Philips) to ensure that nanoparticles product has good solidity.

Entrapment efficiency. The curcuminoids extract loaded SLN product was centrifuged 14,000 rpm (18,626 x G) at 4 °C for 40 minutes and the supernatant was decanted. The residue was macerated by methanol to extract of the curcuminoid from solid lipid nanoparticles and then recentrifuged. The methanolic supernatant absorption was measured by spectrophotometer UV-Vis (UV-1700, Pharmaspec) at 425 nm. Entrapment efficiency was calculated by equation:

Entrapment Efficiency (%) = $\frac{[entrapped curcuminoids]}{[added curcuminoids]} \times 100\%$

3. Results and Discussion

3.1 Preparation of Curcuminoids Extract and Analysis

Ethanolic extract from the both maceration and soxhlet extaction were liquid-liquid extrcted with n-hexane for removal the volatile oils [11]. The ethanol fraction was evaporated by rotary evaporator and furthermore freezedryed. Total extraction yield from the both extraction methods was 7.62%.

HPLC analysis of curcuminoids standard (curcumin, demethoxycurcumin, and bisdemethoxycurcumin) that isolated from Curcuma longa showed peaks at retention times 7.853 min, 8.460 min, and 9.090 min, respectively. The peaks chromatogram of HPLC has been investigated for bisdemethoxycurcumin, demethoxycurcumin, and curcumin [11]. The HPLC chromatogram (Figure 1) of curcuminoids extract of temulawak showed two major peaks at retention times 8.423 min and 9.050 min, also one lower peak at retention times 7.817 min. Its result indicated that the major components of curcuminoids extract of temulawak was demethoxycurcumin (27.51%) and curcumin (65.42%), also very low component of bisdemethoxycurcumin (3.36%). The result was different that curcuminoids of temulawak rhizome only consist of curcumin and bisdemethoxycurcumin [13]. From peaks area in chomatogram also known that curcumin was the highest component in the ethanolic extract of temulawak.

FTIR analysis was performed to characterize functional groups of a compound. FTIR spectra of curcuminoids standards and curcuminoids extract of temulawak (Figure 2) was shown characteristic functional group frequencies at wave number $3600-3100 \text{ cm}^{-1}$ (O-H stretching), 3000 cm^{-1} (C-H aromatic stretching), $2980-2840 \text{ cm}^{-1}$ (C-H methyl stretching), $2000-1667 \text{ cm}^{-1}$ (overtone of aromatic bands), 1650 cm^{-1} (C=C-C=C conjugated diene stretching), $1640-1580 \text{ cm}^{-1}$ (C=O stretching in tautomeric keto and enol

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forms), 1500–1420 cm⁻¹ (C=C aromatic stretching), 1275 cm⁻¹ (C–O–C asymmetric stretching), 1010 cm⁻¹ (C–O of prime alcohol stretching), 980 cm⁻¹ (C–H olefin out-of-plane band), 805–745 cm⁻¹ (C–H aromatic out-of-plane band), dan 438 cm⁻¹ (C=C aromatic out-of-plane band) [14]. The FTIR spectral data of ethanolic extract generally as well as curcuminoids standard, but have C-O primary alcohol stretching band and higher intensity on O-H stretching region.

3.2 The curcuminoids extract loaded SLN production

In this study, an homogenization-ultrasonication method was employed to produce curcuminoid loaded SLN and palmitic acid was chosen as the solid lipid. The composition and several characteristics of the produced SLN was presented in Table 2. The drugs delivery systems must have high drug loading capacity that expression by percentage of entrapment drug in the lipid phase (entrapment efficiency, EE) [9]. The concentration of curcuminoids measured by spectrometric method at maximum wavelength 424.8 nm. The three Formulas were had same composition of lipid and curcuminoid [5] with different content of surfactant. The Formula with 1.5 g surfactant had lowest particles size distribution and highest entrapment efficiency. This result indicated that surfactant composition was enhanced the stability of solid lipid nanoparticles.



Figure 1: HPLC chromatograms of (a) curcuminoids standard and (b) curcuminoids extract of temulawak



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Table 2: The raw materials composition of curcuminoids extract loaded SLN characteristics								
Formula	Palmitic Acid (g)	Curcuminoids (g)	Poloxamer 188 (g)	Size distribution (nm)	Entrapment efficiency (%			
S0.5	1.0	0.1	0.5	357.6 ± 90.9	44.53			
S1.0	10	0 1	1.0	306.3 + 81.2	52.71			

1.5

3.2 The curcuminoids extract loaded SLN characterization

0.1

1.0

S1.5

The particle size distribution of yellow-bright emulsion obtained was determined was measured using particle size analyzer (Delsa Nano C, Beckman Coulter). The average diameter of curcuminoidsextract loaded SLN was 285.5 \pm 76.7 nm (Figure 3). This result indicated that the particles of curcuminoids loaded had sub-micron and uniform size. This research was produced the smaller particles size than solid lipid nanoparticles was produced (131 \pm 2.22 µm) with the same methods [5].

FTIR spectra of curcuminoids extract-SLN (Figure 4d) was shown broad band frequency at wave number 3550–3200 cm⁻¹ that indicated the intermolecular hydrogen bonding from curcuminoids and poloxamer 188 (Figure 4a and 4c). The characteristic spectra of curcuminoids extract-SLN at wave number 3300–2500 cm⁻¹, 3000 cm⁻¹, 1700 cm⁻¹ resulted from palmitic acid, curcuminoids, and carboxyl group of palmitic acid, respectively. FTIR spectra of curcuminoids, palmitic acid, and poloxamer 188). This results indicated that occured physical interaction only between raw materials.

Crystallinity is one of important parameters in of curcuminoids extract-SLN production. X-rays diffraction was used to investigate the crystallinity of curcuminoids-SLN. The result of X-rays diffraction analysis was shown characteristic peaks of curcuminoids extract-SLN (Figure 5b) had same pattern with palmitic acid (Figure 5a) but had weaker intensity because of curcuminoids that loaded in the solid lipid particles. The characteristic peaks of curcuminoids-SLN that had same pattern with palmitic acid was located at 20: 12, 17, 19, 20, 21, 24, 30, and 40.

 285.5 ± 76.7

72.98



Figure 3: Particle size distribution of curcuminoids extract loaded SLN



Figure 4: FTIR spectra of (a) curcuminoids, (b) palmitic acid, (c) poloxamer 188, and (d) curcuminoids extract-SLN



Figure 5:X-rays diffractograms of (a) palmitic acid and (b) curcuminoids

4. Conclusion

The curcuminoids of temulawak extract consist of curcumin and demethoxycurcumin, also a few component of bisdemethoxycurcumin. The characteristic of curcuminoid extract from temulawak was similar with curcuminoids standard. The best formula for SLN production was with surfactant composition 1.5 g having high entrapment efficiency 72.98%. The particle size distributions of curcuminoids loaded solid lipid nanoparticles have average diameter 285.5 \pm 76.7 nm. The characteristic FTIR spectrum of curcuminoids extract-SLN was indicated that occurred physical interaction between raw materials. The X-rays diffractogram of curcuminoids extract-SLN was shown that recrystallization of solid lipid was occurred in a good process. However, further research needs to be done for preclinical and clinical of curcuminoids extract-SLN.

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