RESEARCH ARTICLE

Formulation and characterization of self-nano emulsifying drug delivery systems of lemongrass (*cymbopogon citratus*) essential oil

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Abstract

The present research was aimed to characterize the formula of self-nano emulsifying drug delivery systems (SNEDDS) for lemongrass essential oil. The observation variables included: particle size, zeta potential, and morphology. Meanwhile, the materials consisted of the mixtures of oils (lemongrass essential oil and carrier oil), surfactants, and cosurfactants. Carrier oils were screened as candidates for SNEDDS, and the formula was evaluated for transmittance and emulsification time. The value of the formulation component was lemongrass essential oils, carrier oil (Virgin Coconut Oil), surfactant (Tween 80), and co-surfactant (PEG 400) = 8.34, 8.34, 71.43, and 16.67% respectively. The formulation had a mean of the nanoemulsion droplet diameters of 20.7 nm with the polydispersity index (PI) 0.378 and potential zeta -73 mV. The transmission electron microscopy demonstrated spherical droplet morphology. This research produced SNEDDS of lemongrass essential oil with nanoparticle size that can be used as feed additive for poultry.

Keywords: *Cymbopogon citratus*; essential oil; nanoemulsion

INTRODUCTION

The increasing concern of antibiotic residues in poultry products has made many researchers try to find alternatives for the Antibiotic Growth Promoters (AGPs). One of the alternatives is the herbal feed additive such as essential oils (Eos) which known to have antibacterial, antiviral, antioxidant, antifungal, immunomodulator, hypolipidemic properties, and without residues (Gopi *et al.*, 2014), anti-inflammatory, safe food product for human being (Kumar et al., 2014), stimulate feed intake, coccidiostats and antihelmintic activity (Suganya *et al.*, 2016).

LITERATURE REVIEW

The lemongrass (*Cymbopogon citratus*) is a perennial, grows over the years, mainly in the tropical and savannah area. The main constituents of the lemongrass essential oil are citral (aldehydes geranial (40-62%) + neral (25-38%)), the other main components are terpenes (myrcene – monoterpene and geranial – terpenic alcohol) (Negrelle & Gomes, 2007; Ranitha *et al.*, 2014). The citral shows antimicrobial activity against Gram-positive and Gram-negative bacteria as well as the fungi. However, myrcene has no individual antibacterial activity but do enhance activity when combined with others (Onawunmi, 1989; Onawunmi *et al.,* 1984). *In-vitro* studies showed that essential oils of lemongrass have antimicrobial properties against *Campylobacter jejuni*, *Clostridium perfringens* (Wannissorn *et al.*, 2005), *Bacillus cereus*, *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus* (Zulfa *et al.*, 2016), and *Salmonella typhi* (Shin, 2005). A study by Mukhtar *et al.* (2012) showed that

supplementing diets with lemongrass essential oils reduced the blood cholesterol without affecting overall growth performance of broiler chickens. This might be attributed to the ability of active substances to inhibit the hepatic 3-hydroxyl-3-3methylglutary co-enzyme A (HMG-COA).

Essential oils are mixtures of volatile components, which are composed of lipophilic substances in plants (Jeong *et al.*, 2009). Nanoencapsulation of these oils in drug delivery systems have been proposed for improving the solubility, bioavailability, stability, and efficacy of essential oil-based formulation (Eid, *et al*., 2013; São Pedro *et al*., 2013). Low water solubility drug can be developed with self-nano emulsifying drug delivery systems (SNEDDS) (Savale, 2015; Wang, *et al*., 2009; Zhang *et al.*, 2011). The SNEDDS is used for controlling the release rate of poorly water soluble drugs in both intestine and liver (Elnaggar, *et al.*, 2009; Wang *et al.*, 2009). The SNEDDS are the mixtures of oils, surfactants, and cosurfactants, sometimes including co-solvents, which emulsify under gentle agitation conditions, similar to those which would be encountered in the gastro-intestinal tract. It can lead to improved bioavailability of drug from gastro-intestinal tract (Pouton, 1997; Savale, 2015). Tween 80 is the nonionic surfactant that is emulsifying agents in the preparation of the stable oil in water pharmaceutical emulsions. PEG 400 can be used either to suspend agents or to adjust the viscosity and consistency of other suspending vehicles, which can act as emulsion stabilizers (Rowe *et al*., 2006).

METHODOLOGY

Materials

The main material used in this study was lemongrass leaf, which obtained from a local suppliers in Bantul Regency, Yogyakarta, Indonesia. Chemicals and other materials used were: Tween 80 (Brataco, Yogyakarta, Indonesia), PEG 400 (Brataco, Yogyakarta, Indonesia), VCO (Healthy Co, Yogyakarta, Indonesia), soybean oil, canola oil, sunflower oil, and corn oil. Artificial gastric fluid (AGF) consisted of distilled water (Brataco, Yogyakarta, Indonesia), NaCl (Merck, Germany), and hydrochloric acid 37% (Merck, Germany).

The essential oil extraction

The extraction technique of lemongrass essential oil was combined with steam distillation method. Leaves of *C. citratus* were chopped into small pieces (about 10-15 cm), the weight was about 4.5 kilogram, and it was loaded into the still distillation tank. Eight liters of water was added into the tank and the rid secured tightly. The plant materials were subjected there to steam distillation with the collection of the oils starting after a heating time of 30-50 minutes and continued until no more essential oil was obtained (4-8 hours).

The formula of SNEDDS

The solubility studies for carrier oil selection: five commonly oils used are; virgin coconut oil (VCO), canola oil, soybean oil, sunflower oil and corn oil, these five kinds of oils were screened.

SNEDDS preparation: SNEDDS was prepared using lemongrass essential oil, carrier oil VCO, non-ionic surfactant Tween 80, and co-surfactant PEG 400 stirred with a magnetic bar. The next, the formulations were subjected to sonication using ultrasonicator (J.P Selecta, Barcelona, Spain) for 10 minutes which reduces droplet size of the emulsion and water bath (Memmert GmbH & Co. KG, Schwabach, Germany) at 37°C for 15 minutes. After that, the formulation of SNEDDS was characterized (Sh, Abdelrazeik, & Rakha, 2015).

Transmittance: 100 µl of SNEDDS was accurately weighed and placed into 5 ml aquadests. The mixture was homogenized with the aid of a vortex for 30 seconds. The homogenous mixing resulted and provided a clear visual display of the beginning of the SNEDDS creation. The obtained emulsion has been measured the absorbance at a wavelength of 650 nm (Pratiwi *et al.*, 2016).

Determination of the emulsification time: In order to determine the emulsification time (the time needed to reach the emulsified and homogeneous mixture, upon dilution). 1µl of SNEDDS was added to 500 ml of artificial gastric fluid at 37°C with gentle agitation using magnetic stirrer. The formula was assessed visually according to the rate of emulsification and the final appearance of the emulsion (Basalious *et al.*, 2010) with the modification.

The character of SNEDDS

Particle size analysis: The emulsion droplet size and size distribution were determined using particle size analyzer (HORIBA SZ-100, Kyoto, Japan). Droplet size was analyzed using dynamic light scattering (DLS) technique. The samples were diluted with a ratio of 1:1000 (v/v) with distilled water and mixed for 1 min using a magnetic stirrer. The droplet size and polydispersity index (PDI) of the formulated nanoemulsion were measured (Sh *et al.*, 2015).

Zeta potential analysis: The nanoemulsion stability is directed to the magnitude of the surface charge. The zeta potential of the selected formulations was determined using particle size analyzer (HORIBA SZ-100, Kyoto, Japan) (Balakumar *et al.*, 2013).

Transmission electron microscopy (TEM): The morphology of SNEDDS was observed by TEM (JEOL JEM 1400, USA). After the sample dilution with water (1:1000), a sample drop was placed on a copper grid. The excess was drawn off with a filter paper. The samples were subsequently stained with 1% phosphotungstic acid solution for 30 s (Basalious *et al.*, 2010).

RESULTS AND FINDINGS

The preparation of SNEDDS

Solubility studies for oil selection (Table 1) visual observation for solubility study of lemongrass essential oil in various oils show that VCO and canola oil have good solubility; on the other hand, soybean oil, sunflower oil, and corn oil have inadequate solubility. The further research combination of carrier oil, surfactant (Tween 80) and co-surfactant (PEG 400) (Table 2), VCO has the highest transmittance rate compared with canola and soybean oil which indicating the good dissolving ability. The VCO was selected for carrier oil, combined with Tween 80 and PEG 400 (Table 3) and obtained good transmittance value with low Tween 80 is the formula 1: 4: 1 (ml), respectively. The result of VCO combination, lemongrass essential oil, Tween 80 and PEG 400 (Table 4) with ratio 0.5, 0.5, 4, 1 ml respectively was the best formula with the highest lemongrass essential oil composition (8.33%) for transmittance, visual observation, and emulsification time. Emulsification time means of self-emulsification assessment is visual estimation; the SNEDDS should disperse completely and quickly when subjected to aqueous dilution under agitation (Patel *et al.*,2011).

Table 1 Solubility study of lemongrass essential oil in various oils.

Oils	Visual observation
VCO	Good
Soybean oil	Bad
Canola oil	Good
Sunflower oil	Bad
Corn oil	Bad

Table 2 Combination of carrier oil, Tween 80 and PEG 400.

Carrier oil : Tween 80 : PEG 400 (ml)	% Transmittance		
	VCO	Canola oil	Soybean oil
1 : 1 : 1	86.5	23.2	26.3
1 : 2 : 1	85.0	15.3	59.9
1 : 3 : 1	96.1	67.0	49.7
1 : 4 : 1	99.5	32.1	85.7
1 : 5 : 1	99.8	72.4	85.9
1 : 6 : 1	99.5	52.6	93.9
1 : 7 : 1	99.4	99.5	99.1

Table 3 The combinations without lemongrass essential oil.

VCO : Tween 80 : PEG 400 (ml)	% Transmittance ± SD
1 : 1 : 1	9.37 ± 5.27
1 : 2 : 1	55.00 ± 2.05
1 : 3 : 1	70.40 ± 13.36
1 : 4 : 1	98.43 ± 0.85
1 : 5 : 1	98.73 ± 0.92
1 : 6 : 1	99.13 ± 0.67
1 : 7 : 1	98.60 ± 0.36

Table 4 The combination of lemongrass essential oil.

Formulation (VCO : lemongrass essential oil : Tween 80 : PEG 400) (ml)	% Transmittance ± SD	Visual observation	Emulsification time ± SD
0.75 : 0.25 : 4 : 1	01:04.0 ± 0.01	Good	99.5 ± 0.12
0.5 : 0.5 : 4 : 1	01:06.7 ± 0.02	Good	99.2 ± 0.21
0.25 : 0.75 : 4 : 1	01:10.4 ± 0.02	Bad	94.2 ± 1.39

The characteristics of SNEDDS

Particle size analysis

Size (d.nm)
Z-average (d.nm): 20.7 PDI: 0.378
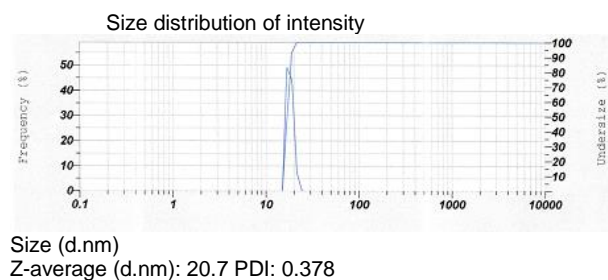
Fig. 1 Particle size analysis of nanoemulsion.

In this current research, the particle size and polydispersity index (PI) (Fig 1.) values were obtained from testing the size and the particle size distribution of distilled water media which were 20.7 nm and 0.378, respectively. The test was performed on distilled water media. Particle size distribution is used as uniformity (Pratiwi *et al.*, 2016). Droplet size was the crucial factor in the SNEDDS performance (Jyothi & Sreelakshmi, 2011), it determines the rate and the extent of drug release as well as drug absorption. The smaller particle size was the larger interfacial surface area which may lead to more rapid absorption and improve the bioavailability. The criteria of SNEDDS were the system with a mean droplet size below 200 nm, and less than 50 nm indicating their efficiency as SNEDDS (Nasr *et al.*, 2016). The particle size distribution was depending on the concentration of surfactant and co-surfactant (Taha *et al.*, 2004). The polydispersity index (PI) less than 0.5 signaled a homogenous distribution, while a greater than 0.5 signaled a higher heterogeneous dispersion (Chavda *et al.*, 2013).

Zeta potential
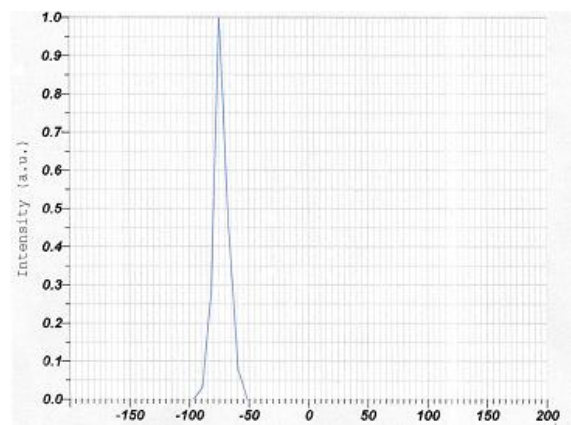
Zeta potential (mV): - 73 mV

Fig. 2 Zeta potential analysis of nanoemulsion

The charge of the oil droplets was another property that was assessed for increased absorption of SNEDDS which was usually found to be negative due to the presence of free fatty acids (Kaur *et al.*, 2013). Tween 80 is a non-ionic surfactant which decreases the zeta potential, because of the amphiphilic property of Tween 80 which deposited at the particle surface and caused the increasing size particle. Furthermore, it could protect surface charge the nanoemulsions led to decrease in zeta potential (Saeedi *et al.*, 2015). The result (Fig 2.) showed that zeta potential was -73 mV, would not exhibit threshold agglomeration as nanoemulsion was stabilized by a greater zeta potential (negative). Nanoparticles with zeta potential values greater than +30 mV or less than –30 mV have high degrees of stability; on the contrary, a low zeta potential value will eventually aggregate due to Van Der Waal inter-particle attractions (Bali *et al.*,

2011; Müller *et al.*, 2001). The zeta potential of more than ± 60 mV was excellent stability (Freitas & Müller, 1998).

Electron microscopic examination
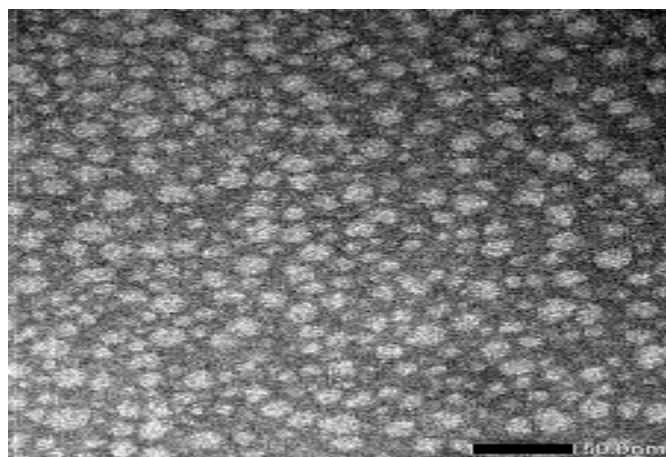
Fig. 3 Transmission electron micrograph of SNEDDS (mag 80 K; 1000-fold with water)

The photograph of the transmission electron microscopy (TEM) was shown in Fig. 3. The nanoemulsion appeared as bright spots on the dark background. The emulsion droplets were spherical and the uniform with the size range between 16 - 25 nm. The figures clearly illustrate that there are no signs of coalescence, indicating the formulation was physically stable (Bandyopadhyay *et al.*, 2012).

CONCLUSION

The study showed that lemongrass essential oil could be employed as the oil phase for the development of SNEDDS. The SNEDDS with relatively good lemongrass essential oil content (8.33%) was prepared with the mean emulsion droplet size of 20.7 nm, the TEM images show nanoemulsion with no agglomeration. Furthermore, the SNEDDS appeared to be effective to improve the solubility.

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