



ISSN 0975-7058

International Journal  
of  
Applied Pharmaceutics



## Vol 11, Special Issue 5 (Sep), 2019

4th International Conference on Pharmacy and Pharmaceutical Science (ICPPS) 2019

PUBLISHED: 15-09-2019

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## FORMULATION AND CHARACTERIZATIONS OF SELF-NANOEMULSIFYING DRUG DELIVERY SYSTEM OF EXTRACT *PETIVERIA ALLIACEA* (SINGAWALANG) LEAVES

ARIFA MUSTIKA<sup>1\*</sup>, NURMAWATI FATIMAH<sup>1</sup>, GADIS MEINAR SARI<sup>2</sup>

<sup>1</sup>Department of Pharmacology and Therapy, Faculty of Medicine, Universitas Airlangga, Prof. Moestopo 47, Surabaya, Indonesia. <sup>2</sup>Department of Physiology, Faculty of Medicine, Universitas Airlangga, Prof. Moestopo 47, Surabaya, Indonesia. Email: arifa-m@fk.unair.ac.id

Received: 19 December 2018, Revised and Accepted: 22 July 2019

### ABSTRACT

**Objective:** Formulation of Singawalang leaves extract should be considered because the extract contains a variety of compounds so that there may be a competitor in the absorption process and will cause the absorption of active ingredients in the gastrointestinal decline. One way to increase the absorption and disposition of active ingredients on target organs is to use a nanoparticle formulation. Therefore, this study will conduct research on self-nanoemulsifying drug delivery system (SNEDDS) formulation of Singawalang (*Petiveria alliacea*) leaves extract.

**Methods:** The systems were developed by investigating the solubility Singawalang leaves extract in various carrier oil, the suitable surfactant, and cosurfactant, construction of SNEDDS of Singawalang leaves extract and characterization of droplet size through particle size analyzer and transmission electron microscopy.

**Results:** The results of this study indicate that the optimum carrier oils for Singawalang leave extract are miglitol and virgin coconut oil (VCO), the compatible surfactant component is tween 80 and the compatible cosurfactant is propylene glycol (PG). The average droplet size is 13 nm and polydisperse index 0,004 and 0,006.

**Conclusion:** It can be concluded, the present study demonstrated that the optimum SNEDDS formulations of Singawalang leave extract are the mixture of VCO, tween 80, PG at ratio 1:8:1 and miglitol, tween 80, and PG at ratio 2:5:3.

**Keywords:** *Petiveria alliacea*, Self-nanoemulsifying drug delivery system, Miglitol, Virgin coconut oil.

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### INTRODUCTION

Singawalang (*Petiveria alliacea*) is a plant widely found in Indonesia and empirically used by residents to treat various diseases such as diabetes and bleeding cough and is also used to protect plants from pests. The ethanol extract of Singawalang leaves has the ability to lower blood sugar levels [1,2]. Mustika *et al.*, 2015, also showed that extract of Singawalang leaves decreased blood sugar level in the mouse model of type 2 diabetes mellitus (T2DM) with the optimum dosage of 360 mg/kg BW and also increased 5' adenosine monophosphate-activated protein kinase expression on liver [3]. This phenomenon raises the hope that the extract can be used as a phytopharmaca drug in the management of T2DM.

The extract of Singawalang leaves contains a complex of active compounds so that there may be a competition between several active compounds in the absorption process and will cause the absorption of active compounds in the gastrointestinal decline. Herbal medicine also has some problems such as instability and solubility formulation, low absorption because unable to cross the lipid membranes of the cells, larger molecular size, numerous large of chemical molecules, degradation during gastric emptying, and extensive metabolism [4,5]. Therefore, the formulation of drug dosage forms and active material delivery systems plays an important role in determining their therapeutic effects. Therefore, it is necessary to do good research on the formulation of the dosage form of leaves extract Singawalang.

The nanomaterials can expressively enrich the pharmacokinetics, pharmacodynamic, and therapeutic index of phytomedicines. In accordance with micrometer-sized, nanocarriers are responsible for more surface area and increase solubility, bioavailability, and enable appropriate nanomedical targeting [4,6,7]. The advantages for herbal

medicines nanoparticles formulation are including increased solubility and bioavailability, protection from toxicity, increased pharmacological activity, increased stability, improved tissue distribution, continuous shipping, and physical protection and chemical degradation [8,9]. Various research has been developed to improve the formulation and delivery system that aims to increase the levels of active ingredients to target organs, one of which is by the delivery of active ingredients in the nanoparticle system [10,11].

The system of lipid-based drugs has achieved increased interest in the oral route of administration of poorly bioavailable drugs. This lipid system is absorbed by lymphatic system through Peyer patch along the gastrointestinal tract. The self-nanoemulsifying drug delivery system (SNEDDS) is one of the systems for the delivery of lipid-based drugs that have now been investigated for its benefit, providing a large interfacial area to partition the drug between oil and gastrointestinal fluids. This technique increases the oral bioavailability of drugs that are difficult to dissolve by increasing solubility and keeping the drug dissolved, in small droplets of oil, throughout its transit through the digestive tract. The SNEDDS has recently emerged as an approach to improve oral solubility, dissolution, and absorption for poorly water-insoluble drugs [12,13].

SNEDDS is a type of self-emulsifying drug delivery system that contains an isotropic mixture of oil, surfactants, cosurfactants, and medicinal substances, which can form nanoemulsion in the digestive tract after oral administration. Emulsions produced with particle sizes <100 nm increase the solubility of hydrophobic drugs and increase their absorption. SNEDDS is an isotropic mixture of oil, surfactants, and cosurfactants that form oil-in nanoemulsions in fine water, in mild agitation, followed by administration into aqueous media, such as gastrointestinal fluids [14].

Thus, considering its potential benefits, we have developed a novel SNEDDS of Singawalang leaves extract and evaluated its characterization. The aim of this study was to make a formulation of Singawalang leaves extract in a SNEDDS and their characterization. The SNEDDS were developed by investigating the solubility of Singawalang leaves extract in various carrier oil, the suitable surfactant, and cosurfactant, construction of SNEDDS of Singawalang leaves extract, and self-emulsification time and percentage transmittance in artificial gastric fluid (AGF). Furthermore, the droplet size characterization of Singawalang leaves extract was evaluated through particle size analyzer and transmission electron microscopy (TEM).

## MATERIALS AND METHODS

### Materials

*P. alliacea* leaves were collected from Balai Materia Medika (BMM) Batu, East Java, Indonesia and were determined by BMM. Ethanol 96%, virgin coconut oil (VCO), sunflower, olive oil, sesame oil, fish oil, soya oil, canola oil, rice oil, corn oil, span 20, span 80, tween 20, and tween 80 were used. PEG 400, propylene glycol (PG) was purchased from Brataco, miglitol from the Department of Biopharmaceutics, Faculty of Pharmacy of Universitas Gadjah Mada, Yogyakarta.

### Methods

#### Formulation of SNEDDS of Singawalang leaves extract

Extraction of Singawalang leaves  
1000 g of Singawalang leaves powder has been extracted with 70% ethanol solvent. The extraction was done by maceration for 3 days.

#### Selection of SNEDDS components

Solubility test of carrier oil  
Testing solubility of Singawalang leaves extracts in various oil. The oil used is VCO, sesame oil, olive oil, corn oil, sunflower oil, fish oil, soya oil, and miglitol. 50 mg of ethanol extract of Singawalang leaves is put into 5 mL each oil gradually until saturated. The mixtures were vortexed as long as 5 min and sonicated during 3 times–15 min. Solubility will be visually observed.

#### Emulsification studies of surfactant

A surfactant was selected on the ease of emulsification and solubility by visual. Various surfactants (span 20, span 80, tween 20, and tween 80) were selected to determine the emulsification ability of the selected oil phase. Mixture the amount of selected oil and the amount of each surfactant in the tube with ratio 1:1, mix it by gently shaking the tube to determine the ease of emulsion. The amount of shake is used to determine the ease of emulsion. Allow the emulsion for 2 h, and the optical clarity of aqueous dispersion was visually observed.

#### Emulsification studies of cosurfactant

Cosurfactant was selected on the ease of emulsification and solubility by visual. Various cosurfactants such as PEG 200, PEG 400, and PG were tested to find an appropriate formulation. Mixture the amount of surfactant and the amount of cosurfactant in the tube with ratio 2:1, mix it by gently shaking the tube to determine the ease of emulsion. The amount of shake is used to determine the ease of emulsion. Allow the emulsion for 2 h, and the optical clarity of aqueous dispersion was visually observed.

#### Construction of SNEDDS

The series of SNEDDS was formulated with varying concentrations of oil (25–70% w/w), surfactant (30–75% w/w), and cosurfactant (0–25% w/w) (Table 1) at room temperature (25°C) for 72 h. For any mixture, the total of surfactant, cosurfactant, and oil concentration added was always 100%. Each formulation was homogenated with a stirrer as long as 30 min. The suitable systems were selected based on visually observed and percentage transmittance by ultraviolet (UV)-VIS spectrophotometer at  $\lambda$  650 nm.

**Table 1: Solubility test of the Singawalang leaves extract in various carrier oils**

Oil	Solubility
VCO	Clear
Sesame	Turbid
Olive	Turbid
Corn	Clear
Sun Flower	Turbid
Fish	Turbid
Soya	Turbid
Miglitol	Clear

VCO: Virgin coconut oil

Formulation of SNEDDS of Singawalang leaves extract was prepared based on the optimum suitable composition of oil, surfactant, and cosurfactant.

#### Loading drug

The series of the number of ethanol extracts of Singawalang leaves (50 mg, 75 mg, 125 mg, and 200 mg) was dissolved in 5 mL each Sneed system. The formulation was homogenated by vortex 4 min, sonicator 5 min, and heating in the water bath at 37°C as long as 15 min. The cycle was repeated 3 times. The evaluation of formulations was performed using self-emulsification time and percentage transmittance by UV-VIS spectrophotometer at  $\lambda$  650 nm.

#### Self-emulsification time and percentage transmittance of Sneed formulation

In aquadestilata and AGF  
The emulsification time was evaluated by 1000  $\mu$ L of formulations incorporated into the 100 mL aquadestilata and into the 100 mL AGF with temperature 37°C and at centrifuge at a rate of 148 RPM. Time from starting formula was inserted until dissolved all called emulsification time. A sample was evaluated percentage transmittance of UV-VIS spectrophotometer at  $\lambda$  650 nm.

AGF solution was prepared by mixing 1 g NaCl, 35 mL HCl 37%, and add aqueous until 500 mL. Adjust the pH until 1, 2 by adding NaOH. Adjust AGF solution temperature at 37°C.

#### Characterizations of SNEDDS of Singawalang leaves extract

##### Particle size analysis

Particle size and polydispersity index were obtained using particle size analyzer (HORIBA SZ-100). The polydispersity index reflects the uniformity of particle diameter and it can be used to depict the size distribution of nanoemulsion [15,16]. The sensitivity range was 3–8000 nm. The measurements were performed at 25°C at a fixed angle of 90°.

##### TEM analysis

The extract Singawalang leaves SNEDDS droplet was observed by a transmission electron microscope. A sample was visualized by drying it on a carbon-coated grid and stained negatively with an aqueous solution of phosphotungstic acid 2%. After drying the phosphotungstic acid, the sample was observed under TEM [17]. Samples were analyzed at a lower accelerating voltage of 100 kV with a smaller objective aperture to achieve the contrast. Images were captured at  $\times$ 40,000 magnification.

## RESULTS AND DISCUSSION

### Formulation of SNEDDS of Singawalang leaves extract

#### Selection of SNEDDS components

The result showed that Singawalang leaves extract dissolved on VCO, miglitol, and corn oil (Table 1). Hence, these three oils can be used as oil components to make the nanoemulsion formulations. The result of

the test can be concluded that the compatible surfactant used to form the nanoemulsion formulation was tween 80, and cosurfactant was PG (Table 2).

**SNEDDS formulation**

The compatibility of oils, surfactants, and cosurfactants to acquire a higher transmittance was an essential foundation in the formulation of the nanoemulsion.

We choose ten compositions of SNEDDS formulation based on visual examination of oil, surfactant, and co-surfactant. The result of a visual examination, time of emulsification, and percentage transmittance are shown in Table 3.

In accordance with the results of visual observations, the emulsification time and percentage transmission, the formulas A, B, C, D, E, and F are chosen as the SNEDDS formulation.

**Loading drug**

The six SNEDDS formulations have been added the extract Singawalang leaves at dose 1 is 50 mg, dose 2 is 75 mg, dose 3 is 125 mg, and dose 4 is 200 mg. The result of visual observation, emulsification time, and percentage transmittance in the AGF are shown in Table 4.

**Characterizations of SNEDDS of extract Singawalang leaves**

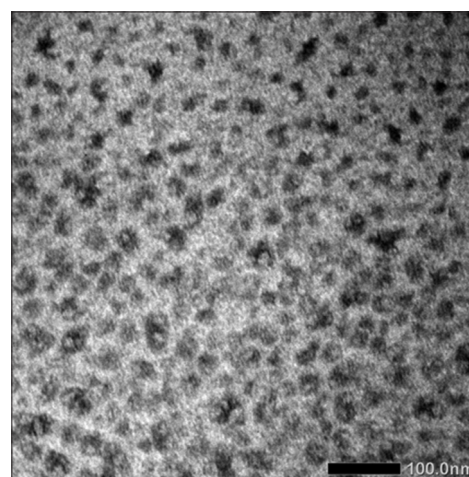
The mean of droplet size and the polydispersity index of SNEDDS extract Singawalang leaves are shown in Table 5.

The morphology of optimum SNEDDS and formulation of Singawalang leaves extract by TEM are shown in Fig. 1.

In this study, VCO, corn oil, and miglitol have been chosen as the carrier oil because the extract can dissolve well into the oil. VCO has a shorter chain of C atoms when compared with corn oil, thereby increasing the solubility of extract. The results of this study are also in accordance with the research conducted by Tri, 2018, that the solubility of *Amomum compactum* in VCO is better than in corn oil [18].

The solubility and emulsification studies were carried out to identify the suitable carrier oil, surfactant, and cosurfactant for the formulation of SNEDDS of Singawalang leaves extract. The result inferred that miglitol and VCO were found to solubilize of Singawalang leaves extract, tween 80, and span 80 as surfactant, and PG as cosurfactant. Tween 80 as a non-ionic surfactant was found to have good solubility and better emulsification ability that allowed to rapid dispersion when in contact with a biological fluid. PG is a compatible cosurfactant that it can be used to enhanced solubility and bioavailability [18].

The data in the study show that all the test compositions of carrier oil, surfactants, can be used as SNEDDS formulations. Based on the



**Fig. 1: The transmission electron microscopy image of droplet size of the Singawalang leaves extract self-nano emulsifying drug delivery system (SNEDDS) formula D4. The dark spherical shapes are indicated nanoparticle of SNEDDS contains Singawalang leaves extract. Magnification ×40,000**

**Table 2: The surfactant and cosurfactant emulsification study**

Surfactant	Oil			Cosurfactant		
	Corn	Miglitol	VCO	PEG200	PEG400	PG
Span 20	Turbid	Turbid		Clear homogeneous	Clear homogeneous	Turbid
Span 80	Turbid	Clear homogenous	Clear homogenous	Turbid	Turbid	Turbid
Tween 20	Turbid	Turbid		Turbid	Turbid	Turbid
Tween 80	Clear homogeneous	Clear homogeneous	Clear homogeneous	Turbid	Clear homogenous	Clear homogenous

VCO: Virgin coconut oil, PG: Propylene glycol

**Table 3: The effect of carrier oil, surfactant, and cosurfactant ratio on visual examination, time of emulsification, and percentage transmittance**

Oil	Ratio			Result			Code
	Oil	Surfactant (tween 80)	Cosurfactant (PG)	Visual	Emulsification time (s)	Transmittance (%)	
Corn	1	1	1	Non-Homogeneous	X	X	
	1	7	2	Turbid	X	X	
	2	5	3	Turbid	X	X	
	2	6	2	Turbid	X	X	
VCO	1	8	1	Turbid	X	X	
	1	1	1	Turbid	X	X	
	1	7	2	Clear	14	95,5	A
	2	5	3	Turbid	X	X	
Miglitol	2	6	2	Turbid	X	X	
	1	8	1	Clear	23	95,5	B
	1	1	1	Turbid	X	X	
	1	7	2	Clear	25	95,4	C
	2	5	3	Clear	21	95,5	D
	2	6	2	Clear	15	95,2	E
	1	8	1	Clear	25	95,4	F

VCO: Virgin coconut oil, PG: Propylene glycol

**Table 4: The result of visual observation, emulsification time, and percentage transmittance of self-nanoemulsifying drug delivery system Singawalang leaves extract formulation in artificial gastric fluid**

Formulation	Extract (mg)	Visual	Sediment	Emulsification time (s)	Percentage transmittance $\lambda=650\text{nm}$
A1	50	Clear	None	1:23:6	60,2
A2	75	Clear	None	2:02:3	69,2
A3	125	Clear	None	1:44:6	58,6
A4	200	Clear	None	1:45:1	55,4
B1	50	Clear	None	0:59:1	78,7
B2	75	Clear	None	0:49:4	70,5
B3	125	Clear	None	0:46:4	61,4
B4	200	Clear	None	0:35:8	32,9
C1	50	Clear	None	0:38:1	79,5
C2	75	Clear	None	0:32:2	72,6
C3	125	Clear	None	0:51:3	56,0
C4	200	Clear	None	0:41:2	39,4
D1	50	Clear	None	0:20:5	75,5
D2	75	Clear	None	0:25:1	74,5
D3	125	Clear	None	0:20:2	55,5
D4	200	Clear	None	0:30:2	43,8
E1	50	Clear	None	0:26:5	80,3
E2	75	Clear	None	0:32:9	74,0
E3	125	Clear	None	0:39:1	59,5
E4	200	Clear	None	0:38:1	54,6
F1	50	Clear	None	0:23:8	79,6
F2	75	Clear	None	0:30:9	86,1
F3	125	Clear	None	0:38:8	52,1
F4	200	Clear	None	0:37:2	34,2

emulsification time and the percentage of transmittance parameters, the composition with fast emulsification time is <15 s and percentage transmittance more than 95% is formula A (VCO: Tween 80:PG=1:7:2) and E (Miglitol: Tween 80:PG=1:8:1).

Emulsification time is known as important parameters to describe system stability and prepare emulsification in the gastric fluid characteristic of SNEDDS. The purpose of measuring percent transmittance is to observe the self-emulsification process by measuring the transmittance of the solution during dissolution when the emulsification process takes place.

Singawalang leaves extract up to a dose of 200 mg/5 mL can be entrapped in globules. It can be seen that in the visual observation, the formula remains clear and there is no sediment. The selected SNEDDS formulation was stable for 6 months at room temperature. Another study stated that SNEDDS of *Plantago lanceolata* was stable for 1 month at room temperature [19].

The range of particle size of all selected formulations is between 13 nm and 18,7 nm. The size meets the nano criteria and approaches the DNA size of about 10 nm [20]. The range of particle sizes in the study of SNEDDS formulations containing *P. lanceolata* was between 141,51 and 374,89 [19], while in the study of SNEDDS *A. compactum*, particle size was 13,97 [18]. The average droplet size of tetrandine SNEDDS was 19,75 nm. The composition of SNEDDS formulation in this research consists of 40% (w/w) oleic acid as oil, 15% (w/w) SPC, and 30% (w/w) cremophor RH-40 as surfactant, and 15% (w/w) PEG400 as cosurfactant [21].

Droplet size is an important factor in SNEDDS formulation because it determines the level and rate of drug release and absorption and increases in bioavailability as well there *in vivo* stability smaller droplets have a larger surface area, which then increases the level of drug release. The small droplet size may be due to increased surfactant concentration, which forms a closed layer of surfactant at the interface, so as to stabilize dispersed droplets [22]. Therefore, in this study, the nanosize of SNEDDS Singawalang leaves extract might allow it to be more easily delivered into cells or to pass across some barriers.

The carrier oil positively affects the formation of droplet size, and surfactant causes the interfacial film to stabilize and condense.

**Table 5: The mean of droplet size and the polydispersity index of self-nanoemulsifying drug delivery system extract Singawalang leaves**

Formula	Droplet size mean (nm)	Polydispersity index
A4	13±0,8	0,055
B4	14,4±0,6	0,006
C4	13±1,1	0,126
D4	18,7±0,9	0,004
E4	13,4±0,9	0,195
F4	17,8±1,1	0,249

The polydispersity index of the B formulation (VCO: Tween 80:PG=1:8:1) was 0,006 and in the D formulation (Miglitol: Tween 80:PG=2:5:3) was 0,004. This data show that the system had a narrow size distribution and homogeneity. Polydispersity index is the measure of globule size homogeneity. A value closer to zero, more homogeneous are the particles. The research of Embelin SNEDDS formulation showed that the polydispersity index was found to be 0,15±0,02–0,23±0,04 [23]. The mean droplet size and polydispersity index in myricetin SNEDDS were <200 nm with narrow distribution (<0,4) [15].

The electron microscope images in this study showed a spherical nanoemulsion droplet, measuring <100 nm. A similar morphology of droplet image has been shown in several previous studies such as SNEDDS curcumin and myricetin [15,24]. Spherical, discrete, and nonaggregate clots in the SNEDDS formulation deduce system stability [25].

Future prospects of SNEDDS Singawalang leave extract to have the potential to increase biological activity and overcome the shortcomings of herbal medicines. The latest new challenges in the development of nanotechnology-based drug delivery systems include the feasibility of scale-up process that brings innovative therapeutic techniques to market quickly and the possibility of obtaining a multifunctional system to meet several biological and therapeutic requirements.

At present, health-care costs must also be considered. This reduction in health-care costs is likely to be obtained by increasing nanotherapeutic efficacy, reducing its length hospitalization, reducing personal health-care costs, and effective treatment of major expensive diseases [26].

Subsequent studies must be conducted to determine the factors that lead to increased delivery efficiency and bioavailability.

## CONCLUSION

In the present study, SNEDDS of extract Singawalang leaves was successfully developed. The optimum composition of this system was VCO: Tween 80:PG with a ratio at 1:8:1 and miglitol: Tween 80:PG with a ratio at 2:5:3.

## ACKNOWLEDGMENT

This research was support by the Ministry of Research, Technology and Higher Education of the Republic of Indonesia (Grant no 004/ADD/SP2H/LT/DRPM/VII/2017). The authors are thankful to Ronny Marten from the Department of Biopharmaceutics, Faculty of Pharmacy of Universitas Gadjah Mada, Yogyakarta, for laboratory use guidance and licensing.

## AUTHORS' CONTRIBUTIONS

Arifa Mustika conceived of the presented idea, developed the theory, and performed the research. Nurmawati Fatimah and Gadis Meinar Sari have carried out the research. All authors discussed the results and contributed to the final manuscript. Arifa Mustika wrote the manuscript with support from Nurmawati and Gadis.

## CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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