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Snedds (Self-nanoemulsifying Drug Delivery System) Formulation of *Sarang Semut* Extract on Cervical Cancer Cells (HeLa) with MTT Assay Method

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Abstract. *Sarang semut (Myrcomedia pendans*) is traditional medicine plant that has been widely used as alternative treatment for diabetics and cancer. Flavonoids in sarang semut ectract known as active copounds for pharmacology activity, and it has low solubility in water. The methods can be used to improve the availability of water-insoluble compounds for better to deliver the active compounds into the body is SNEDDS (Self –Nanoemulsifying Drug Delivery System). The purpose of this study is to get the formulation and characterized of SNEDDS of sarang semut. The parameters used were particle size, polydisperse index (PI), zeta potential. The results showed capryol 90 as oil vehicle, tween 80 as the surfactant, and propylene glycol as co-surfactant, and particle size is 12.53 nm, the polydisperse index is 0.27 Đ and value of zeta potential is -51,43 mV. Physical stability of the three formula has shown good results. MTT Assay test results obtained with an amount of 258.755 ppm and 389.955 ppm are included in the range of toxic enough for IC50 is in the range ≤ 1000 ppm. The results obtained show that the sarang semut SNEDDS have good physical and chemical properties, has good organoleptic and have an inhibitory effect on the activity of Hela cells.

1. Introduction

Cancer is a chronic disease characterized by uncontrolled growth of cells/tissues, continues to grow, immortal and suppress surrounding organs or nerves. Based on the data of World Health Organization (WHO) states that deaths caused by cancer can reach 45% 2007-2030, which is about 7.900.000-11.500.000 deaths. In Indonesia, based on Riskesdas's report, the prevalence of cancer is about 4.3 out of every 1,000 residents, and the seventh disease that causes of death (5.7%)(1). Sarang semut (Myrmecodia pendans) is a plant comes from Indonesia which has been widely experimented and has good activity of compounds in human's body(2). Some studies reported that sarang semut contains important compounds, these are terpenoids, flavonoids, and tannins(3). Medicinal plants are rich in antioxidants such as polyphenols, and vitamins A, C, and E, which are essentia for good health and useful for therapeutic purposes against various diseases(4). It is traditionally used as a remedy against ulcer, hemorrhoid, nosebleed, backache, allergy, uric acid disorder, stroke, coronary heart problem, total blood count, tumor, cancer, and lactagogue. In our previous study, sarang semut was found to be

a rich source of phenolic compounds(5). Some research has also proven that pharmacological activities owned by sarang semut as an antibiotic, antidiabetic, Antidiarrhea, and antineoplastic(6). Besides, sarang semut also contain tocopherol and alpha-tocopherol, a highly active substance with free radicals inhibition capability(7). Several studies have determine the efficacy of sarang semut for the cancer therapy(8,9). Nanotechnology has been utilized to create a variety of delivery systems for the encapsulation, protection and controlled release of bioactive and nutraceuticals(10). Nanoparticle herbal medicine takes a lot of attention because it has an active system as the formulation of drugs that can easily reach the target(11,12). In the drug discovery, a significant proportion of new chemical entities and many existing drug molecules exhibit poor water solubility and hence poor oral absorption(13). An innovation strategy which would overcome this barrier is self-nanoemulsifying drug delivery system (SNEDDS) that so results in improving the oral bioavailability of poorly water-soluble and lipophilic drugs(14). Self-Nanoemulsifying Drug Delivery System (SNEDDS) appeared as an effective drug delivery system because of its ability which has proven of increasing the bioavailability of lipophilic drug(15,16). Therefore, SNEDDS have the advantages in possessing higher solubilization capacity, leading to the incorporation of poor water-soluble pharmaceutical inside the oil phase(17). Self-emulsifying drug delivery system is one of the useful methods in addressing solubility issues(18,19). The purpose of the present study was to development a preparation of sarang semut SNEDDS for the effect of anticancer activity using MTT Assay into cervical cells.

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2. Material and Methods

2.1. Material

The tools used in this study oven (Memmerth, Germany), vaccum evaporator (IKA, Germany), analytical balance (Mettler Toledo, Germany), magnetic stirrer (IKA, Germany), ultrasonic homogenizer (Biologics, USA), particle size analyzer (Horiba, Japan), and waterbath (Memmerth, Germany). The materials used in the study sarang semut was collected from Papua Indonesia. Oils (capryol 90; Gattefose, olive oil; Bratachem, and VCO; Bratachem), Surfactant (tween 20 and tween 80; Kao), Co-surfactant (PEG 400, PEG 600, and Propylene glycol; Brathachem) which had been provided in Pharmaceutical Technology Laboratory of Pharmacy Program Study of Universitas Islam Indonesia.

2.2. Methods

2.2.1. Preparation of plant extract

Sarang semut was washed by water before drying at 60°C for 24 hours. And Then extracted by maceration method with ethanol 70% ratio of 1: 7 (w/v). Then concentrated by rotary evaporator.

2.2.2. Solubility study in oils

Preliminary screening of surfactants, co-surfactans, and oils. This approximate solubility tests with observations visually. The solubility of sarang semut in various oils (virgin coconut oil, olive oil, and capryol) and surfactant (tween 20 and Tween 80) then co-surfactan (PEG 400, PEG 600, and Propylene Glycol). Each 1 mg sarang semut extract added to 1 ml oils, surfactants and cosurfactans will be used.

2.2.3. Preparations and characterizations of SNEDDS

Extract of sarang semut mixed with capryol 90 then added a surfactant and co-surfactant mixed until homogeneous in stirrer 450 rpm . Determination of the particle size, polydisperse index, and zeta potential sarang semut SNEDDS using the PSA (Particle Size Analyzer). Each formula of 1 mL dispersed in 100 mL of aqua pro injection (100X dilution) is stirred slowly to form nanoemulsion.

2.2.4. Physical stability studies

The objective of these tests was to figure out the stability and phase integrity of Sarang semut SNEDDS under different conditions of temperature variation and centrifugal force. Centrifugation Study, Formulations were centrifuged at 5000 rpm for 30 minutes, and were then checked visually for instability such as phase separation, creaming, cracking or drug precipitation. Heating and cooling cycle performed involved three cycles between 40C and 450C with storage at each temperature for not less than 48h. The formulation that passed at these temperatures, without undergoing any creaming, cracking, coalescence, phase separation or phase inversion, was chosen for the freeze-thaw test. Freeze-thaw cycle involved three freeze-thaw cycles at temperatures between -200C and + 250C with storage at each temperature for not less than 48h. The formulation was then visually observed for phase separation. The only formulation that was stable to phase separation were selected for cytotoxicity assay study.

2.2.5. Cytotoxicity assay

This research used MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), a tetrazolium salt which is commonly used in the quantitative purpos of living mammalian cells or cell proliferation using in vitro calorimetric method. These methods can only be used on live cells and not in dead cells because the test was based on degrees of cell activation. Cells with a density of 10,000-50,000 cells / ml were taken as many as 100 mL, then put on microplate (96 wells). Microplate incubated for 24 hours in the CO2 incubator with a temperature of 37ºC. Incubated microplate was added 100 µl test samples with each specified level and incubated for 24 hours at incubator C02. After that a new complete media and added 10 mL of 0.5% MTT assay. Then, incubated for 3 hours in the CO2 incubator with a temperature of 37ºC. The incubated microplate was added SDS 10% and incubated for 24 hours at room temperature and dark. The reading of the results was done using Elisa reader at 550 nm wavelength.

3. Results and discussion

3.1. Solubility test

Selection of a oils phase as the vehicle in the preparation of SNEDDS is crucial, a used in the development of SNEDDS should be able to dissolve the material thoroughly. In this study used several oils, surfactants, and co-surfactants. Capryol 90 was reportedly very well used for liquid emulsifying on oral dosage; Tween is a nonionic surfactant that provides benefits to pharmaceutical formulations, propylene glycol is safe co-surfactant given for oral, The results of solubility test of sarang semut can be seen in the following table 1..

3.2. Preparations and characterizations of SNEDDS

Preparations of Snedds were made by gentle mixing capryol 90 as oil phase, tween 80 as surfactant and propylene glycol as co-surfactant in various concentrations. The test results indicate that particle size on formula 3 has the smallest particle, it is 12,53 nm. The smaller particle size has, the larger surface area for drug absorption and increasing bioavailability. The polydisperse index values obtained in formula 3 are homogeneous and have a good uniformity of particle size is 0.27 Đ. Then for zeta potential result shows a value is -51,43 mV so its the preparation is electrically stable (table 2.).

3.3. Physical stability studies.

The main difference between emulsions and nanoemulsions is kinetic stability, reflecting the thermodynamic stability of the two systems. Therefore to check the stability, the formulation was exposed to centrifugation study, heating and cooling cycle and freeze-thawing cycle to eliminate the metastable ones. The results of the centrifugation for 30 minutes at 5000 rpm indicates the physical changes that look visually, The centrifugation results in formula 3 is not physical changes. However,

the formulas 1 and 2 show the physical changes were seen visually, such as creaming, cracking, or precipitation. The results of the heating-cooling cycle test and freeze-thaw cycle test showed no signs of physical damage to the preparation (figure 1.)

Table 1. *Sarang semut* extract solubility in various oil as vehicle (10 mg/mL)

Solubility	Oil phase			Surfactant		Co-surfactant		
	Capryol 90	VCO	Olive Oil	Tween 20	Tween 80	PEG 400	PEG 600	Propylene Glycol
Soluble	√				√			√
Not soluble		√	√	√		√	√	

Table 2. Particle size, polydisperse index, and zeta potential (n=3)

Formula	**Particle Size (nm) mean±SD**	**PI (Đ) mean±SD**	**Zeta Potential (mV) mean±SD**
1	12.56 ± 1.22	0.49 ± 0.08	-45.50 ± 1.03
2	13.03 ± 2.05	0.47 ± 0.1	-53.86 ± 0.83
3	12.53 ± 0.92	0.27 ± 0.1	-51,43 ± 1.77

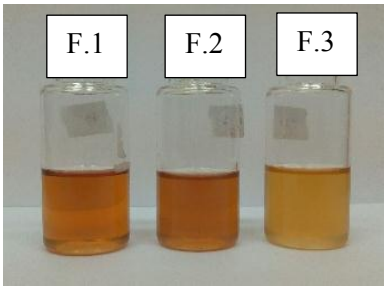

Figure 1. The results of the heating cooling cycle test and freeze thaw cycle test showed no signs of physical damage to the preparation (no phase separation after testing).

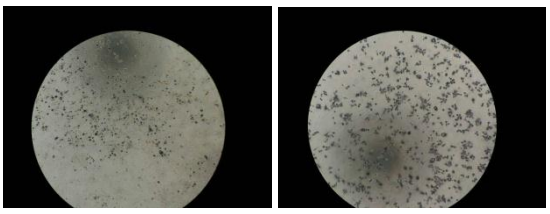

Figure 2. Cytotoxicity assay based cystal formation 10mg extract of sarang semut (a) and snedds formulation contains 10 mg sarang semut (b).

3.4. Cytotoxic assay

The cytotoxic test provides an overview of the potential of the test compound in inhibiting the growth of the test cell. The parameters used are 50% inhibition concentration (IC50). The higher the concentration of the dose, the higher the percentage of cervical cell death so that SNEDDS sarang semut can be used as anti-cervical cancer. IC50 values show concentration values that produce 50% cell death and indicate the toxic potential of a compound against the hela cell (table 3.). The greater the value of IC50 the compound is increasingly not toxic. An extract is considered toxic to cancer cells if it has an IC50 value of less than 1000 ppm[20]. Thus, from extracts or SNEDDS with a value of 258.755 ppm and 389.955 ppm are included in the range of toxic enough for IC50 is in the range ≤

1000 ppm. The results obtained show that the ant nest SNEDDS formulations have good an inhibitory effect on the activity of Hela cells than an extract its self (figure 2.)

Table 3. The cytotoxic test provides an inhibition concentration (IC50) that provides inhibit 50% growth of cells. Snedds have an inhibitory effect on the activity of Hela cells

Sample	Concentrations (ppm)	Abs (ave)	Death Cell (%)	IC_{50} (ppm)
Extract	500	02.10	70.741	258.755
Sarang	250	0.223	68.063	
Semut	125	0.345	42.994	
	62.5	0.473	16.689	
	31.25	0.474	16.414	
	15.625	0.483	14.629	
	7.8125	0.490	13.186	
SNEDDS	500	0.256	61.263	389.955
Sarang	250	0.369	37.980	
Semut	125	0.469	17.376	
	62.5	0.437	24.107	
	31.25	0.470	17.307	
	15.625	0.461	19.162	
	7.8125	0.473	16.552	
Average cell abs.				0.554
Average control media				0.068

4. Conclusion

In this study, Sarang semut snedds formulations composed of capryol 90 as the oily phase, tween 80 as the surfactant, propylene glycol as co-surfactants, were selected. Sarang semut SNEDDS has good characteristic based on the standard parameter evaluation test with the result obtained that particle size is 12,53 nm, the PI value is 0,27 Đ, zeta potential is -53,43 mV, good solubilization, and good organoleptic properties. MTT Assay test results obtained the value of 258.755 ppm and 389.955 ppm are included in the range of toxic enough for IC50 is in the range ≤ 1000 ppm. Thus the sarang semut SNEDDS has anticancer activity by inhibition of Hela cell activity.

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Reference

1. IARC. Cervical Cancer Estimated Incidence, Mortality and Prevalence Worldwide in 2012 [Internet]. 2012 [cited 2017 May 30]. Available from: http://globocan.iarc.fr/old/FactSheets/cancers/cervix-new.asp
2. Achmad H, Suryani A. A, Supriatno, F. Singgih M. Anti-Cancer Activity And Anti-Proliferation Ant Nests Flavonoid Fraction Test (Myrmecodya Pendans) Human Tongue Cancer Cells In Sp-C1. IOSR J Dent Med Sci IOSR-JDMS. 2014;13(6):01–5.
3. Engida AM, Kasim NS, Tsigie YA, Ismadji S, Huynh LH, Ju Y-H. Extraction, identification and quantitative HPLC analysis of flavonoids from sarang semut (Myrmecodia pendan). Ind Crops Prod. 2013 Jan;41:392–6.

4. Hertiani T, Sasmito E, Sumardi, Ulfah M, editors. Preliminary Study on Immunomodulatory Effect of Sarang-Semut Tubers Myrmecodia tuberosa and Myrmecodia pendens. OnLine J Biol Sci. 2010;10(3).
5. Engida AM, Faika S, Nguyen-Thi BT, Ju Y-H. Analysis of major antioxidants from extracts of Myrmecodia pendans by UV/visible spectrophotometer, liquid chromatography/tandem mass spectrometry, and high-performance liquid chromatography/UV techniques. J Food Drug Anal. 2015;23(2):303–309.
6. Sudiono J, Tri Oka C, Trisfilha P. The Scientific Base of Myrmecodia pendans as Herbal Remedies. Br J Med Med Res. 2015;8(3):230–7.
7. Soeksmanto A, Subroto MA, Wijaya H, Simanjuntak P. Anticancer Activity test for Extract of Sarang Semut Plant(Myrmecodya Pendens) to HeLa and MCM-B2Cells. Pak J Biol Sci. 2010;13(3):148–51.
8. Hasanuddin, Rifayani KS, Supriadi G, Kurnia D, Adhita D. Potential of Terpenoid Bioactive Compound Isolated from Papua Ant Nest as an Alternative Ovarian Cancer Treatment. Open J Obstet Gynecol. 2015;05(07):406–11.
9. Achmad H, Chandra MH, Ramadhany S, Handayani H, Samad R. The Role of Sarang Semut (Myrmecodia pendans) Flavonoid's Fraction in Proliferation and Angiogenesis Inhibition of Human Tongue Squamous Cell Carcinoma. J Biol Agric Healthc. 2014;4(21):65–9.
10. Pal SL, Jana U, Manna PK, Mohanta GP, Manavalan R. Nanoparticle: An overview of preparation and characterization. J Appl Pharm Sci. 2011;01(06):228–34.
11. Bhadoriya SS, Mangal A, Madoriya N, Dixit P. Bioavailability and bioactivity enhancement of herbal drugs by "Nanotechnology": a review. JCPR. 2011;8:1–7.
12. Prabhu V, Uzzaman S, Grace VMB, Guruvayoorappan C. Nanoparticles in Drug Delivery and Cancer Therapy: The Giant Rats Tail. J Cancer Ther. 2011;02(03):325–34.
13. Ranjit K, Baquee AA. Nanoparticle: An Overview Of Preparation, Characterization, And Application. Int Res J Pharm. 2013;4(4):47–57.
14. Wang S-T, Chou C-T, Su N-W. A food-grade self-nanoemulsifying delivery system for enhancing oral bioavailability of ellagic acid. J Funct Foods. 2017 Jul;34:207–15.
15. Dash RN, Mohammed H, Humaira T, Ramesh D. Design, optimization and evaluation of glipizide solid self-nanoemulsifying drug delivery for enhanced solubility and dissolution. Saudi Pharm J. 2015 Oct;23(5):528–40.
16. Zhao D, Li L, Niu W, Chen S. Highly conductive polythiophene films doped with chloroauric acid for dual-mode sensing of volatile organic amines and thiols. Sens Actuators B Chem. 2016;243:380–7.
17. ElKasabgy NA. Ocular supersaturated self-nanoemulsifying drug delivery systems (S-SNEDDS) to enhance econazole nitrate bioavailability. Int J Pharm. 2014;460(1–2):33–44.
18. Sagar K, Kendre P, Pande V, Chaudhari V. Design, Development And Characterization Of Selfnanoemulsifying Drug Delivery System (Snedds) Of Nateglinide. WORLD J Pharm Pharm Sci. 2014;3(8):794–811.
19. Kassem AA, Mohsen AM, Ahmed RS, Essam TM. Self-nanoemulsifying drug delivery system (SNEDDS) with enhanced solubilization of nystatin for treatment of oral candidiasis: Design, optimization, in vitro and in vivo evaluation. J Mol Liq. 2016 Jun;218:219–32.
20. Utami Y, others. Uji Sitotoksisitas Dan Selektivitas Ekstrak Etanol Terpurifikasi Arcangelisia Flava Pada Sel Kanker Payudara Mcf-7. 2016 [cited 2017 Jun 18]; Available from: http://repository.unej.ac.id/handle/123456789/72667