Antidiabetic Activity of Self Nanoemulsifying Drug Delivery System from Bay Leaves (*Eugenia polyantha* Wight) Ethyl Acetate Fraction

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Abstract. Insulin resistance is caused by inability of target tissues to insulin response. Bay leaves (*Eugenia polyantha* Wight) fraction or extract have been used for the treatment of antidibetic mellitus type-2 resistance insulin (ADMRI) but it has low solubility and bioavailability. To overcome these problems, ethyl acetate fraction of bay leaves was formulated into self nanoemulsifying drug delivery system (SNEDDS) using Virgin Coconut Oil (VCO) as a carrier oil. This study aims to produce nanoherbal medicine, determine effect of nanoherbal preparation derived from bay leaves as an anti-ADMRI. The results showed that the optimum SNEDDS formula was tween 80 : PEG 400 : Virgin Coconut Oil (30% : 60% : 10%) in 5 mL. It has emulsification time 13.00 seconds with the average of droplet size value 84.5 nanometer and zeta potential value ± 0.2 mV. Morphological observation showed the nanoemulsion particles has spherical shaped and stable in different pH media. Hypoglycaemic effect of single dose metformin, SNEDDS, combination a-half dose of SNEEDS with metformin value is 28.3 % ; 15.6% ; 34.6% respectively.

1. Introduction

Bay leaves as one of herb plants has some therapeutic effects which are decreasing blood glucose degree so it can be used to be anti-diabetes agent [1]. Formula in the form of nanoemulsion is a choice that is expected to increase solubility and oral bioavailability of Bay leaves ethyl acetate fraction. Nanoemulsion can be formulated through Self-Nanoemulsifying Drug Delivery System (SNEDDS) which is a nanoemulsion pre-concentrate formed from the mixture of isotropic drug, oil, and surfactant. When they are combined with water phase in agitation condition, slowly, it will form nanoemulsion of oil phase in water (M/A) spontaneously [2].

The suitable oil in SNEDDS formulation as drug carrier is oil containing high medium-chain fat acid so it will be easier to be emulsified than long-chain fat acid. It is appropriate with Virgin Coconut Oil (VCO) that contains high lauric acid while lauric acid is medium-chain saturated fat acid [3].

Tween 20 and Tween 80 are surfactant having alcil-chain structure that has oil penetration effect to surfactant layer that enables nanoemulsion formation. Thus, the both are appropriate surfactant to be used in SNEDDS formulation [4]. Besides, Tween 20 and Tween 80 have high HLB values which are

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16.7 for tween 20 and 15 for tween 80. The higher HLB value, the easier it will decrease the surface tension between oil and water [5].

Meanwhile, co-surfactants that are choosen are *propylene glycol* and *polyethylene glycol* 400, they are commonly used in formulating SNEDDS. It is because they are short-chain alcohol so it is more effective in decreasing surface tension [6]. They are ampifilic compound having affinity towards water phase and oil phase [7].

Ethyl acetate fraction from bay leaves is used as a sample research because it has low solubility in the body. The successfulness of this research is expected to be an innovation for nanoemulsion availability of ethyl acetate fraction from bay leaves and be a product that can be used as alternative of herb medical antidiabetic treatment with high bioavailability.

Diabetes mellitus (DM) is a disease which can be identified from the over blood glucose level (hyperglycaemic) as the result of the lack of insulin [8]. According to International Diabetes Federation (IDF), DM is the top seven death causes in the world [9]. Diabetes mellitus is divided into two categories. They are DM type 1 and DM type 2. Diabetes mellitus type 1 is caused by autoimmune damage that is mediated by pancreas cell causing insulin deficiency [10]. Meanwhile, hyperinsulinemia occurs in DM 2. However, insulin cannot bring glucose to get into tissue because of the occurrence of insulin resistance which is the reduction of insulin ability to stimulate glucose removal by peripheral tissue [11]. The use of Oral Hypoglycaemic Drug (OHM), for example metformin, still have side effect [12]. Recently, human tend to return to the nature, including the use of antidiabetes herb drugs that is Bay leaves (*Eugenia polyantha Wight*). The use of plant active extract and fraction still has weaknesses because of the lack of oral bioavailability [13]. Therefore, in order to make herb drug bioavailability becomes maximum, the size of the particle must be changed into nano-size (nanoemulsion).

Based on the background above, a test was conducted to examine the availability of ethyl acetate fraction from bay leaves in SNEEDS formula as antidiabetes 2 which is resistance to insulin.

2. Experimental

2.1 Tool and Materials

Materials which were used in this research are Bay leaves were obtained from Sukoharjo, Indonesia; component of SNEDDS: aquades, chloroform, ethyl acetate, Virgin Coconut Oil (VCO), PEG 400, propylene glycol, tween 20, tween 80 (Brataco); materials of artificial gastric fluid (AGF) and artificial intestinal fluid (AIF) consist of aquades (general), NaCl, sulfuric acid, MgCl2, CaCl2, NaHCO3, and KCl (E. Merck); male white mice (*Rattus norvegicus*) on Wistar strain around 3 months old with weight about 150-200 gram. It was diet administration in composition of woof (BR comfeed) (80%), lard (15%), duck yolk (5%), fructose; Na-CMC liquid 0,5%; reagent Glucose GOD (FS dyasis), metformin and glibenclamide (Generic).

Equipment which were used in this research are oven, blender, flannel, stopwatch, rotary evaporator ((RVO 400 SD Boeco Germany), waterbath (Grant), vortex (Maxi Mix II Thermolyne), sonicator (Branson 1510), magnetic stirrer (Cimarec), yellow tip (Kan Jian), flacon, spectrophotometer UV/Vis (Perkin Elmer Lambda 25), micro pipettes 50-200µL, particle size analyzer (Horiba SZ-100), Transmission Electron Microscope, analytical scale (Metler Teledo), and glass made tools (Pyrex).

2.2. Procedure

2.2.1. Ethyl acetate fraction from bay leaves

Bay leaves simplisia powder was extracted by using maceration method with chloroform solvent for 5 days. Macerate was filtered through glass funnel to separate macerate from simplisia powder. The simplisia powder then remaceration treatment with ethyl acetate. Thus, evaporate with rotary evaporator in 55^0C temperature and 6 rotary speeds until it became concentrated.

2.2.2 *Optimization of surfactant, consurfactant, and oil composition*
Ethyl acetate fraction from bay leaves was added to 5 ml carrier component (carrier oil: VCO, surfactant: tween 20 and/or tween 80, and consurfactant: PEG 400 or propyleneglycol) with optimal composition of surfactant and cosurfactant which was appropriate with optimization results that had been done. Then, the comparison of surfactant and cosurfactant with oil is 9:1. The mixture was made to be homogeneous, then sonicated for 15 minutes, and was placed in 45^0C temperature for 10 minutes. The result of the mixture was keep for 24 hours in the room temperature to find out the homogeneity. The homogeneous formula is the chosen formula for SNEDDS formula [14].

2.2.3. *SNEDDS formula selection*
100 µL of SNEDDS formula candidate was added with aquades until the last volume becomes 50 ml . Mixture homogenization was done by using vortex for 30 minutes. The mixture result which is in the form of homogeneous emulsion and give clear visual look are the beginning marks of the successfulness of nanoemulsion making. SNEDDS which had been emulsified was measured on its transmittance by using spectrophotometer on the length wave 650nm with aquades blank to know the clarity level.
The calculation of emulsification time was done towards herb extract nanoemulsion in three medium which were aquades, artificial gastric fluid without pepsin, and artificial intestinal fluid without pancreatic. 500 ml of each medium were placed in 37^0c temperature on the magnetic stirrer with speed about 120rpm. SNEDDS containing 1 ml of herb extract was dropped into medium quickly. The observation was done towards the time needed from the first drop until it forms emulsion. Nanoemulsion that was formed was marked by herb extract SNEDDS which was perfectly dissolved in the medium [15].
Nanoparticle size, size distribution, and zeta potential was measured using Particle Size Analyzer (PSA). Two drops of SNEDDS sample were emulsified into 5 ml of aquades, taken 3 ml and poured into covet to be analyzed. Then, to know the morphology of nanoemulsion particle visually by doing observation using Transmission Electron Microscope (TEM).

2.2.4. Hypoglicemic Effect Test
To make mice resistance to insulin, that it can be done by feeding the mice with high fat woof which consists of woof (80%), lard (15%) and duck yolk (5%). Meanwhile, diet on fructose was also given 3,3 gram/kg BB of perioral mice for 80 days. To know whether the mice are resistance to insulin or not, a measurement was conducted on blood glucose level which comes from glibenclamide hypoglycaemic activity.
The test of pharmacology compound activity was conducted for 15 days. the sample were divided into 5 groups, which are, group 1 (normal), group II (negative), group III (metformin 91.75 mg/kg of weight), group IV (SNEDDS of Bay leaves ethyl acetate fraction 183,5 mg/kg of weight), and group V (the combination of both metformin 22.5 gram/kg of weight and SNEDDS of Bay leaves ethyl acetate fraction 91.75mg/kg of weight). Blood measurement was done by using reagent Glucose GOD FS. Blood measurement data was obtained from the 0 day, 80^{th} day, and 95^{th} day. The results of blood glucose level measurement were analysed by using paired t-test, independent t-test, and Anova test which is continued by LSD test.

3. Result and Discussions

3.1. Optimization or surfactant and cosurfactant, and VCO

This optimization aims to determine the composition ratio of surfactant and co-surfactant with oil (Virgin Coconut Oil) which is capable of producing a homogeneous phase after mixing. Composition optimization results surfactant and co-surfactant with Virgin Coconut Oil (VCO) (Table 1) showed that combination of tween 80 : propylene glycol : VCO was not able to produce a homogeneous mixture in various proportions, while combination of tween 80 : PEG 400 : VCO capable of producing a homogeneous mixture in the ratio of surfactant: co surfactant is 1:2 and 1:3. Combination

of tween 20 : PEG 400 : VCO was not able to produce a homogeneous mixture in various proportions, while combination of tween 20 : propylene glycol : VCO capable of producing a homogeneous mixture in the ratio of surfactant: co-surfactant is 1:1 and 2:3. Combination of surfactant (Tween 80 and Tween 20), co-surfactant (propylene glycol or PEG 400) and VCO was not able to produce a homogeneous mixture in various proportions.

Table 1. Optimization of surfactant (Tween 80, Tween 20 and combination Tween 80 : Tween 20), co-surfactant (Propylene Glycol and PEG 400), and oil phase (Virgin Coconut Oil)

Surfactant	**Composition of surfactant : co-surfactant**	**VCO : surfactant-cosurfactant (1 : 9)**	
		PG	**PEG**
T80	1 : 1	x	X
	2 : 1	x	X
	3 : 1	x	X
	3 : 2	x	X
	2 : 3	x	X
	1 : 3	x	v*(F1)
	1 : 2	x	v*(F2)
T20	1 : 1	v*(F3)	X
	2 : 1	x	X
	3 : 1	x	X
	3 : 2	x	X
	2 : 3	v*(F4)	X
	1 : 3	x	X
	1 : 2	x	X
T80 : T20	**Composition of surfactant**	**T80/T20 : PG (3 : 1)**	**T80/T20 : PEG (3 : 1)**
	1 : 1	x	X
	2 : 1	x	X
	3 : 1	x	X
	3 : 2	x	X
	2 : 3	x	X
	1 : 3	x	X
	1 : 2	x	X

x : not homogeneous (split); v: homogeneous (not split)

3.2. SNEDDS Formula Selection

This selection was done on SNEDDS formula which is chosen from optimization phase of surfactant, cosurfactant, and oil composition which are formula F1, F2, F3, and F4. Transmittance observation result (%) at 650 nm wave length shows that formula with the highest transmittance is formula F2. The average transmittance value F2 is 90,95% while emulsification time average in aquades is 13.00 seconds, in AGF is 13.09 seconds, and in AIF is 16.05 seconds. Nanoemulsion particle size was measured by using Dynamic light scattering method using PSA. From the measurement, the result of nanoemulsion size is 84,5 Nm. Nanoemulsion has size about 1-100nm [16].

An emulsion can be said as nano-sized emulsion if it is passed by light having wave length about 650 nm [17[. The light can be continued until the solution looks transparent. The high transmittance value which is closer to aquades is 100%. Emulsification tima is time that is calculated from the beginning of sprinkling until the formation of nanoemulsion in three medium, those are aquades, Artificial Gastric Fluid (AGF) without pepsin, and Artificial Intestinal Fluid (AIF) without pancreatin. The faster process will fasten the nanoemulsion formation.

From the visual observation of nanoemulsion morphology of formula 2 SNEDDS availability using Transmission Electron Microscope (TEM), the obtained results are as follows:

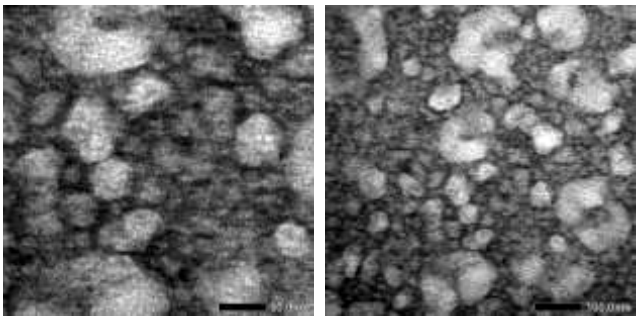

Figure 1. Nanoemulsion morphology of formula 2 SNEDDS using transmission Electron Microscope (TEM) with $2x10^5$ and 10^5 x magnification

The result (figure 1) observation above shows *sferis* particle though there is a minor part of the particle that is not perfectly *sferis*. Particle morphology is important because *un-sferis* particle form will enable inter-particle contact which will lead to aggregation (Couvreur et al., 2002) [18].

3.3. Hypoglycaemic Effect

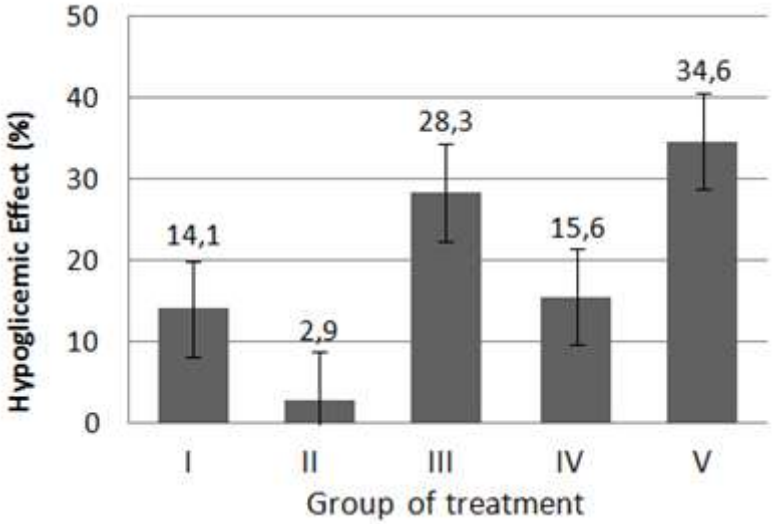

Figure 2. Hypoglycaemic effect of bay leaves treatment. The figure showed CMC Na treatment on normal rat (I), CMC Na treatment on insulin resistance rat (II), single dose of metformin treatment on insulin resistance rat (III), SNEDDS bay leaves fraction on insulin resistance rat (IV) and combination treatment of a half dose SNEDDS bay leaves fraction with metformin on insulin resistance rat (V)

According to figure 2 showed that group III and group V have higher hypoglycaemic effect with value 28.3% and 34.6%% after compared with control group.The result of LSD statistical analysis on the 80th day and 95 day between treatment on group III (single dose of metformin), group IV (SNEDDS), and group V (the combination a half dose of metformin and SNEDDS) showed that there is a significant difference (P>0.05). It means that combination treatment of SNEEDS bay leaves fraction and metformin has potensial to develop as co-therapy of ADMRI. Further molecular target detection to investigate its mechanism need to be conducted.

4. Conclusions

The results showed that the optimum SNEDDS formula was tween 80 : PEG 400 : Virgin Coconut Oil (30% : 60% : 10%) in 5 mL. SNEDDS fraction of bay leaves had emulsification time with value 13.00 seconds, the average of droplet size value 84.5 nanometer and zeta potential value ± 0.2 mV. Morphological observation showed that the nanoemulsion particles had spherical shaped and stable in different pH media. SNEDDS from bay leaves ethyl acetate fraction can decrease blood glucose level with hypoglycaemic effect was 15.6% but combination a- half dose of SNEDDS and metformin from ethyl acetate fraction has higher hypoglycaemic effect (34.6%) compared to a-single dose of metformin (hypoglycaemic effect was 28.3%) on the animal testing with diabetes mellitus type 2 resistance insulin.

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