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α-Glucosidase Inhibitory Activity and Formulation of Solid Lipid Nanoparticle Containing Ethanol 70% Standardized Extract of Kumis Kucing Leaves (*Orthosiphon stamineus* Benth.)

(Aktivitas Penghambatan α-Glukosidase dan Formulasi Solid Lipid Nanoparticle mengandung Ekstrak Etanol 70% Daun Kumis Kucing Terstandar (*Orthosiphon stamineus* Benth.))

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Abstract: Kumis kucing leaves (Orthosiphon stamineus Benth.) are one type of plants that is able to inhibit the activity of α-glucosidase enzyme. This study aims to formulate Solid Lipid Nanoparticle from ethanol 70% standardized extract of kumis kucing leaves and which give α-glucosidase enzyme inhibitory activity. The result of SLN characteristic for particle size of formula I, II and III shows the result of 51,60 nm; 12.81 nm; 11.87 nm and zeta potential of formula I, II and III show the results -27.67; -10,7; -15.0, respectively. The results of the α-glucosidase enzyme inhibitory activity of the standardized extract, SLN formula I, II and III respectively showed IC50 of 132.9 ppm; 130.3 ppm; 131.4 ppm; 132.2 ppm and Acarbose as comparison showed IC50 of 50.0 ppm. Data processing using t-test statistically at α = 0,05 showed that extract and SLN of formula I had significant difference, thus the best SLN formula was formula I with concentration of tween 80 and propylenglycol of 6% and 10%.

Keywords: kumis kucing leaves, standardized extract, nanoparticle, solid lipid nanoparticle.

Abstrak: Daun kumis kucing (*Orthosiphon stamineus* Benth.) adalah salah satu jenis tumbuhan yang mampu menghambat aktivitas enzim *α-glucosidase*. Penelitian ini bertujuan untuk memformulasi *Solid Lipid Nanoparticle* dari ekstrak etanol 70% daun kumis kucing terstandar dan yang memberikan aktivitas penghambatan enzim *α-glucosidase*. Hasil karakteristik SLN untuk ukuran partikel formula I, II dan III menunjukkan hasil 51,60 nm; 12,81 nm; 11,87 nm dan zeta potensial formula I, II dan III menunjukkan hasil -27,67; -10,7; -15,0. Hasil aktivitas penghambatan enzim *α-glucosidase* dari ekstrak terstandar, SLN formula I, II dan III masing-masing menunjukkan IC_{50} adalah 132,9 ppm;130,3 ppm; 131,4 ppm; 132,2 ppm dan Akarbosa sebagai pembanding menunjukkan IC_{50} yaitu 50,0 ppm. Pengolahan data menggunakan *t-test* secara statistik pada α=0,05 menunjukkan bahwa ekstrak dan SLN formula I memiliki perbedaan yang signifikan, sehingga formula SLN terbaik adalah formula I dengan konsentrasi tween 80 dan propilen glikol adalah 6% dan 10%.

Kata kunci: daun kumis kucing, ekstrak terstandar, nanopartikel, *solid lipid nanoparticle*.

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INTRODUCTION

INTERNATIONAL Diabetes Federation (IDF) states that the prevalence of Diabetes Mellitus (DM) in the world is 1.9% and has made DM as the cause of death of the seventh sequence in the world while in 2012 the incidence of DM in the world is as many as 371 million where the proportion of DM incidence type 2 is 95% of the world population who suffer from DM. The high prevalence of type 2 DM is caused by unchanging risk factors such as gender, age, and genetic factors, the second is the modifiable risk factors such as smoking and alcohol consumption. Symptoms of diabetes include drinking lots, eating lots, rapid weight loss ±5-10 kg in 2-4 weeks, and frequent urination. A person is diagnosed to have diabetes if the above symptoms occur for a long time with normal blood glucose levels <140 mg/dL and fasting blood glucose <100 mg/dL[1].

Diabetes mellitus can be treated using oral antidiabetics with sulfonylureas, biguanides and inhibition of glucosidase or insulin-injectable enzymes. This therapy has side effects on the patient and requires a high cost. Therefore, an alternative way is needed with the use of medicines from natural materials. One plant that is efficacious as an antidiabetes is a kumis kucing leaves (*Orthosiphon stamineus* Benth.). Kumis kucing leaves contain essential oils, saponins, polyphenols, flavonoids, and potassium salts[2]. Based on research conducted by Ameria[3], phytochemical screening of ethanol 70% extract of kumis kucing leaves has done and showed that kumis kucing leaves contain flavonoid, saponin, steroid, triterpenoid, tannin, quinone, coumarine and essential oil and on test of inhibition of enzyme α-glucosidase with a dose of 225 µg/mL showed percent (%) inhibition of 87.11% and IC50 131.2 ppm[3].

In this experiment, ethanol 70% standardized extract of kumis kucing leaves was prepared, then was tested for α-glucosidase inhibitory activity of the extract and Solid Lipid Nanoparticle (SLN). In this study the extract was formulated into a nanoparticle form, which is expected to have uniform size in the nano level so that contact with the enzyme becomes more effective. The nanoparticle technology performed by formulating Solid Lipid Nanoparticles (SLN) with cetyl alcohol as a matrix and varying the concentrations of tween 80 and propylene glycol in each of the formulas[5].

MATERIALS AND METHOD

MATERIALS. *Orthosiphon stamineus* Folii Extractum Spissum, pure water, α-glucosidase enzyme (G5003100 UN, Sigma), p-nitrofenyl-α-D-glucopyranoside substrate (N1377-IG, Sigma), bovine serum albumin (A2153-10G, Sigma), acarbose (Bayer), sodium carbonate, sodium hydroxide, monobasic potassium phosphate, cremophor RH-40, cetyl alcohol, tween 80, and propylenglycol.

METHOD. Preliminary Test or Optimization of Enzyme Conditions. **Inhibitory Activity Test Against α-glucosidase Enzyme from Ethanol 70% Standardized Extract of Kumis Kucing Leaves**. The enzyme solution was prepared by dissolving 1.0 mg of α-glucosidase enzyme in phosphor buffer solution (pH 7) containing 200 mg of bovine albumin serum, previously used, enzyme diluted 10 times with phosphate buffer (pH 7). The reactant mixture comprises 250 µL p-nitrophenyl α-D-glucopyranoside (p-NPG) 2 mM as a substrate, 400 µL phosphate buffer solution (pH 7) and 100 µL sample solution in DMSO. Then the mixture was incubated at 37°C for 5 minutes, after which the enzyme solution was added 250 µL and incubated for 15 minutes. The enzyme reaction was stopped by adding Na_2CO_3 200 mM as much as 1000 µL. Then the solution is measured at 405 nm wavelength. Acarbose is used as a reference standard with the same treatment as the sample. The experiment was done 3 times repetition.

Identification of raw materials. **Manufacture of SLN (Solid Lipid Nanoparticles) Containing Ethanol 70% Standardized Extract of Kumis Kucing Leaves**. Preparation of SLN (Solid Lipid Nanoparticles) from ethanol 70% standardized extract of kumis kucing leaves begins with melting cetyl alcohol at a temperature of 8±5 °C until melting. Then cremophor RH-40 and standardized extract of kumis kucing leaves were added, then homogenized at 200 rpm for 2 minutes (mixture A). Preheat the propylenglycol and water sufficiently at 80 °C then add tween 80 and mix A then homogenize at 200 rpm for 5 minutes. Hot water was added to the mixture to 100 mL and homogenize with ultra turrax at 25000 rpm for 8 minutes. The temperature was set to 75±5 °C, then cooled. Homogenized back at 600 rpm for 7 minutes then 800 rpm for 8 minutes. Allow the SLN mixture to cool for 30 minutes and then stir the manual for 5 minutes. The formula of SLN (Solid Lipid Nanoparticles) from ethanol 70% standardized extract of kumis kucing leaves with variation concentration of propylenglycol and tween 80 can be seen on Table 1.

Characterization of SLN (Solid Lipid Nanoparticles) containing ethanol 70% standardized extract of kumis kucing leaves:

a. Determination of distribution and particle size
b. Determination of potential zeta
c. Morphology

Table 1. Composition of SLN formula of standardized extract of kumis kucing leaves.

F	Standardized extract of kumis kucing leaves	Cremophor RH-40	Propylene glycol	Tween 80	Cetyl alcohol	Water
I	1%	2,5%	10 %	6 %	2 %	
II	1%	2,5 %	15 %	9 %	2 %	Ad 100 mL
III	1%	2,5 %	20 %	12 %	2 %	

Evaluation of SLN (Solid Lipid Nanoparticles) containing ethanol 70% standardized extract of kumis kucing leaves:

a. Organoleptic examination

b. Stability test

c. In vitro inhibitory activity against α-glucosidase enzyme.

RESULTS AND DISCUSSION

The presence of ethanol 70% standardized extract of kumis kucing leaves and Solid Lipid Nanoparticles may provide inhibitory activity against the α-glucosidase enzyme determined from the uptake of p-nitrophenol formed and measured by Absorbance Microplate Reader ELX 800 at a wavelength of 405 nm. IC_{50} values indicate the concentration of extracts that can inhibit 50% of α-glucosidase enzyme activity. Based on the results of this study IC_{50} from ethanol 70% standardized extract of kumis kucing leaves was 132.9 ppm with 87.0% inhibition at 225.0 ppm concentration. SLN (Solid Lipid Nanoparticles) formula I had IC_{50} 130.3 ppm and 88.07% inhibition at 225.0 ppm, SLN formula II had IC_{50} 131.4 ppm and 87.65% inhibition at a concentration of 225.0 ppm, and SLN formula III has IC_{50} 132.2 ppm and 87.34% inhibition at a concentration of 225.0 ppm. Acarbose has the highest activity of inhibiting α-glukosidase enzyme because acarbose is a synthetic antidiabetic oral drug that has been proven to decrease blood glucose level and this drug belongs to the class of inhibitors of α-glucosidase enzyme. In this study IC_{50} acarbose was 50.0 ppm with an inhibition of 91.38% at a concentration of 225 ppm.

In this study, acarbose has the lowest IC_{50} value than the standardized extract, SLN (Solid Lipid Nanoparticles) of formula I, II and III. This is because acarbose is known to have a high inhibitory activity against α-glucosidase enzyme. The standardized extract of kumis kucing leaves have an IC_{50} greater than SLN of formula I, II and III. This means the required concentration of the standardized extracts to inhibit 50% α-glucosidase enzyme activity than nanoparticle preparations. In this study showed when the the standardized extract was made into nanoparticles, the concentration required to inhibit

Table 2. Characterization of Solid Lipid Nanoparticle (SLN).

Formula	Particle Size (nm)	Zeta Potential (mV)
I	51.60	-27.67
II	12.81	-10.7
III	11.87	-15.0

Table 3. Organoleptic test of SLN extract ethanol 70% of kumis kucing leaves.

Formula	Color	Smell	Taste	Form
I	Brown	Slight	Bitter, slightly sour	Liquid
II	Brown	Slight	Bitter, slightly sour	Liquid
III	Brown	Slight	Bitter, slightly sour	Liquid

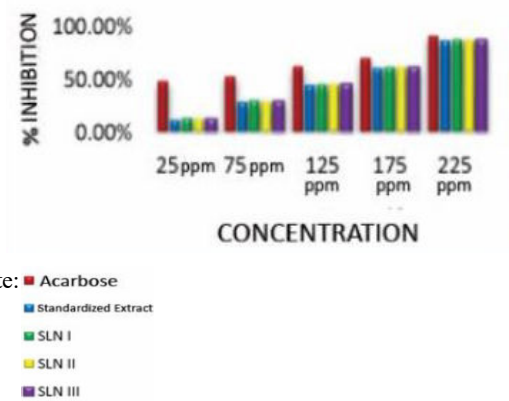
Table 4. Results of inhibitory activity and IC_{50} of SLN extract ethanol 70% of kumis kucing leaves.

No	Inhibitor	Concentration (ppm)	% Inhibition	IC_{50} (ppm)
1	Acarbose	225.0	91.38	50.0
2	Standardized Extract		87.09	132.9
3	SLN I		88.07	130.3
4	SLN II		87.65	131.4
5	SLN III		87.34	132.2

Table 5. Stability of SLN extract ethanol 70% of kumis kucing leaves.

Parameter	Day				
	1	2	3	4	5
Color	Brown	Brown	Brown	Brown	Brown
Turbidity	Stable	Stable	Stable	Stable	Stable
Sediment	-	-	-	-	-

Picture 1. Acarbose inhibitory activity.



50% of α-glucosidase enzyme activity is getting smaller. In this SLN preparation which shows the smallest IC_{50} results are started from formulas I, II and III. Each Solid Lipid Nanoparticle (SLN) formula had a concentration difference of tween 80 and propylenglycol, which gives a difference of IC_{50} results in each of the formulas. The difference in surfactant concentration may affect the homegenity of a Solid Lipid Nanoparticle (SLN) preparation thereby affecting the IC_{50} yield on each of the respective formulas.

The SLN from ethanol 70% standardized extract of kumis kucing leaves had uniform size in the nanometer level so that it can be released slowly and effectively in contact with the enzyme. This can also be due to the size of the nanometer having a larger surface area. Data processing using t test statistically at α=0.05 by processing IC_{50} data from extract and SLN formula I, extract and SLN formula II and extract and SLN formula III, showed that extract and SLN formula I had significant difference, therefore the best SLN formula is in the formula I with each concentration of tween 80 and propylen glycol of 6% and 10%. SLN formula I has IC_{50} 130.3 ppm.

CONCLUSION

The ethanol 70% standardized extract of kumis kucing leaves can be formulated into Solid Lipid Nanoparticles (SLN). Extract and the SLN containing ethanol 70% standardized extract of kumis kucing leaves have the inhibitory activity of α-glucosidase enzyme with IC_{50} successively 132.9 ppm and 130.3 ppm. Based on data processing using t-test statistically at α=0.05 showed that the extract and SLN of formula I had significant difference, the best formula of SLN was formula I.

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