Original Article

Enhancement of Icariin aphrodisiac effect by self nano emulsifying drug delivery system (SNEDDS) method

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ABSTRACT

The epimedium plant (Epimedium brevicornum Maxim) has activity as an aphrodisiac derived from a flavonoid compound, namely icariin. Icariin has low solubility and bioavailability. The utilization of icariin using the SNEDDS (Self Nanoemulsifying Drug Delivery System) method is expected to increase the solubility and bioavailability of icariin. This study aims to determine the optimum formula, characteristics, physical properties, and aphrodisiac activity of SNEDDS icariin. The optimum formula used is tween 80 (75%): PEG 400 (12.5%) and cucut fish oil (12.5%). The characteristics of SNEDDS icariin obtained were droplet size with an average of 15.63±0.058 nm, PI 0.144±0.062, zeta potential -22.67±2.06 mV, emulsification time 12.30 seconds, and transmittance 97.2%. The physical test of SNEDDS icariin produced in the form of a clear preparation in yellow color, pH 6.52, the viscosity of 120 cps, as well as the SNEDDS icariin dissolution in aqueous media in the 60th minute was 75.40% colluded. SNEDDS icariin dose 50 mg/KgBW has a better aphrodisiac effect than pure icariin 100 mg/kgBW.

Keywords: Aphrodisiac, Epimedium, Icariin, SNEDDS

Introduction

Sexual dysfunction disorders include reduced libido, abnormal ejaculation, and erectile dysfunction [1]. Erectile dysfunction has a considerable impact on the psychology and quality of life of men such as anxiety and depression [2, 3]. The use of drugs with the target of inhibiting phosphodiesterase type 5, such as sildenafil, tadalafil, vardenafil, and avanafil is one of the main options for the treatment of erectile dysfunction [1]. However, the use of synthetic phosphodiesterase type 5 inhibitor compounds (PDE5) causes some side effects such as headaches, priapism, redness, and visual impairment [4].

In recent years, compounds of plant origin have increasingly been studied for their safety in the treatment of sexual dysfunction [5].

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One of them is the epimedium plant. The main content in epimedium plants is icariin [6]. Icariin has various pharmacological effects such as anti-osteoporosis [7], cardiovascular protection [8], anti-tumor [9], anti-inflammatory [10], Immunoprotection [11], and improvement of sexual dysfunction [12]. Based on research icariin can fix erectile dysfunction problems by inhibiting cGMP PDE5 [13].

The use of icariin for treatment is limited due to its low solubility in water, which results in minimal bioavailability. Icariin exhibits poor solubility and low membrane permeability (water solubility of <100 µg/mL, logP = 0.81 and pKa = 7.07), as it is a flavonoid aglicon [14]. In recent years, the method used to increase the solubility and reliability of active substances is by making microemulsion formulas [15], nanoemulsions [16], mucoadhesive [17], nanoparticles [18], liposomes [19], transfersom [20], self-emulsifying [21], Self-Nanoemulsifying [22], and solid-SNEDDS [23].

Self-Nanoemulsifying Drug Delivery System (SNEDDS) is an isotropic mixture of oils, surfactants, cosurfactants, and drugs that form nanoemulsions when meeting the water phase [24, 25]. SNEDDS spread easily within the gastrointestinal tract and with digestive motility of the stomach and intestines provide the agitation necessary for the formation of emulsion systems [26].

In this study, the development of icariin nanoparticles made by the SNEDDS method was carried out. The composition of SNEDDS is surfactants, co-surfactants, and oils. Surfactants as emulsifiers of oil into water through the formation and stability guard of the interface film layer, and co-surfactants help the task of surfactants as emulsifiers [24]. The resulting icariin SNEDDS preparations were subsequently carried out to characterize physical properties and test aphrodisiac activity in rat test animals.

Materials and Methods

Biomaterials and chemicals

Icariin (Liftmode, USA), standard icariin (Sigma-Aldrich 96%), aquades (Bratachem, Indonesia), PEG 400 (Bratachem, Indonesia), Tween 80 (Bratachem, Indonesia), sunflower oil (Sukanda Djaya), corn oil (Sukanda Djaya), coconut oil (Barco), VCO (Tyara), soybean oil (Sukanda Djaya), olive oil (Bratachem, Indonesia), cucut fish oil (Bumi Wijaya), sildenafil 50 mg (Novell Pharmaceutical Laboratories).

Experimental animals

Male and female white rats of the Wistar strain (Lab. Pharmacology and Toxicology, Pharmacy UMP). Ethical clearance for experimental animals has been registered with the ethics committee of the Faculty of Medicine Jenderal Soedirman University with no. 024/KEPK/PE/V/2022. The rats need to be acclimatized for at least 1 week before use and the weight was observed every day to ensure the weight change did not exceed 10%.

Standard curve of icariin

Standard curves are created using the maximum wavelength. The maximum wavelength is obtained by scanning wavelengths from 200-400 nm. Accuracy was determined using a standard solution of Icariin 1 mg in 10 mL of methanol. The stock solution made concentrations of 6 ppm, 8 ppm, 10 ppm, 12 ppm, and 14 ppm. Precision was determined by the repetition method using a standard solution of Icariin with a concentration of 5 mg/mL. Precision is performed by measuring absorbance using a 6-repeat UV-Vis spectrophotometer.

Solubility test of icariin in oil

The composition of the SNEDDS system is determined with surfactants (tween 80), co-surfactants (PEG 400), and oils (sunflower oil, corn oil, coconut oil, VCO, soybean oil, olive oil, and cucut fish oil) that can dissolve icariin well. The solubility test was carried out by weighing as much as 5 mg of icariin. Then it is dissolved separately into a 10 mL carrier. The mixture is conditioned in a magnetic stirrer at 40°C for 10 min [27]. Then the mixture is dissociated for 15 minutes to maximize the dissolving process and left for 24 hours at room temperature to know its homogeneity [28].

SNEDDS formula optimization

Design the SNEDDS system formula using Design Expert 10.0 (Simplex Lattice Design). From this design, 14 formulas were formed, and then the physical characterization of SNEDDS icariin was carried out to determine the optimal formula with parameters of percent transmittance and emulsification time of the nanoemulsion system formed.

Optimization of icariin drug loading in the SNEDDS system

Optimization was carried out on the icariin weight series of 5, 10, 15, 20, and 25 mg into 1 mL of the optimal SNEDDS formula. The highest concentration that produces a clear mixture without free icariin particles is the maximum concentration.

Characterization of SNEDDS icariin

Icariin nanoemulsions were prepared from 100 µL of icariin SNEDDS plus aquadest up to a volume of 5 mL. Characterize droplet sizes with the Particle Size Analyzer tool and potential zeta using the zeta sizer. Calculation of emulsification time was carried out on icariin nanoemulsions in aquadest. Organoleptic tests are carried out by observing the visual appearance of the preparation's shape, taste, color, smell, and stability. SNEDDS icariin pH testing is carried out using a calibrated pH meter.

Stability observations of SNEDDS icariin

SNEDDS containing 100 µL of icariin plus aqueous and artificial gastric fluid of 10 mL. The mixing results are observed every hour for 4 hours to determine its stability [29].

Observation of icariin release rate in SNEDDS system

Drug release or dissolution tests are carried out to determine the rate of release of the active substance using a dissolution tester. One capsule is inserted into the dissolution tube, air bubbles are removed from the surface of the tested preparation immediately the tool is executed at a rate of 100 rotations per minute for 90 minutes.

Aphrodisiac test SNEDDS icariin

The SNEDDS icariin aphrodisiac test was performed using 25 male and 25 female white rats. The rats were divided into five treatment groups, namely the negative control group (aquadest), negative control (SNEDDS base), positive control (Sildenafil 50 mg), SNEDDS icariin dose 50 mg / KgBW, pure icariin dose 100 mg / KgBW. Observation of sexual activity was carried out using tools in the form of closed-circuit television (CCTV) devices. The activity of coitus is taken into consideration in this observation [30].

Data analysis

Quantitative data on the activity of introducing, climbing), and coitus obtained were analyzed using the one-way ANOVA method then continued with the LSD (Lease Significant Different) test.

Results and Discussion

Based on the study, a standard curve was obtained with the equation y = 0.0369x + 0.005 with the value of the relation coefficient (r) = 0.9978. The correlation coefficient value greater than 0.99 indicates that the analysis method used has good linearity and can provide a response comparable to the concentration of analytes in the sample. Based on the value of % recoveries obtained from 98.13% to 103.00%, this is in line with the provisions of the percentage of recovery analytes in the sample in the 95-105% range. The RSD obtained in precision measurement is equal to 1.84%. The value obtained is quite good because the RSD value is less than 3.7%.

The solubility test aims to determine oils, surfactants, and cosurfactants that can dissolve icariin to produce a homogeneous SNEDDS formula. The results of Icariin Solubility can be seen in **(Table 1)**.

Cucut fish oil (CFO) can dissolve icariin better than other oils because CFO contains squalene with a chemical structure of $C_{30}H_{50}$ which is similar to the structure of icariin $C_{33}H_{40}O_{15}$ so that it is almost the same level of polarity.

Table 1. Solubility of Icariin in Various Phases of Oil, Surfactant, Cosurfactant at 40°C.

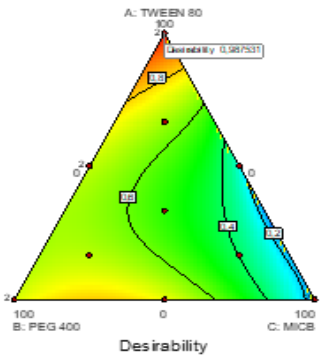
Carrier	Solubility of Icariin (mg/mL)
Sunflower oil	3.04
Corn oil	1.52
Coconut oil	2.54
VCO	3.97
Soybean oil	1.02
Olive oil	2.23
Cucut fish oil*	5.38
Tween 80*	>10
PEG 400*	>10

The results of Design Expert 10.0 (Simplex Lattice Design) a total of 14 formulas were then transmitted to determine the system density and emulsification time to obtain the results as in **(Table 2)**.

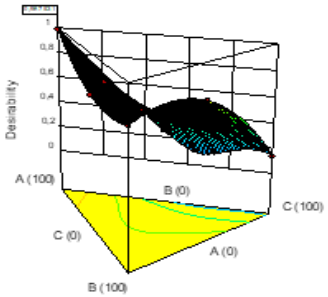
Table 2. Material Composition, Transmittance Results, and Emulsification Time Base SNEDDS

Run	Comparison in %			Transmittance (%)	Emulsification time (second)
	Tween 80	PEG 400	CFO		
1	66.625	16.625	16.625	87.3	16.48
2	75	12.5	12.5	97.2	12.3
3	62.5	12.5	25	82.7	18.5
4	62.5	25	12.5	94.2	16.45
5	62.5	15.62	15.62	92.7	14.98
6	75	12.5	12.5	96.8	12.24
7	62.5	18.75	12.5	95.3	14.24
8	62.5	12.5	25	84.5	19.22
9	68.75	18.75	12.5	93.4	16.4
10	68.75	18.75	12.5	94.8	14.3
11	64.58	14.58	20.83	91.4	15.35
12	70.83	14.58	14.58	93.2	14.9
13	64.58	20.83	14.58	95.6	16.2
14	68.75	12.5	18.75	93.4	20.46

The optimum composition of the formula can be seen in **(Figure 1)** with a ratio of the composition of tween 80: PEG 400: bottled fish oil which is 75%: 12.5%: 12.5% which produces the highest percentage of Transmittance of 97.2%.



a)



b)

Figure 1. a) Countor plot desirability (value 0.987), b) Countor plot optimal formulation of software with predicted transmittance value of 96.84% and emulsification time value of 12.20 seconds; X1- Tween 80, 75% parts; X2- Co-Surfactant, 12.5% part; X3- Oil, 12.5% parts

To determine whether there is a significant difference in the character of the prediction formula with the trial formula, an analysis was carried out using SPSS with one simple t-test method. A comparison of transmittance and emulsification time prediction and trial formula results can be seen in **(Table 3)**.

The transmittance significance value between the prediction and the experiment was 0.649, and the emulsification time significance value of the design expert and experiment's prediction was 0.38. These results showed that the design expert's prediction with experimental data did not have a significant difference because the resulting significance value was more than 0.05.

Table 3. Test Results of One Sample T-Test Transmittance Value and Emulsification Time Prediction Formula Compared to Trial Optimum Formula

Response	Predictions	Experiment	Sig.	Conclusion
Transmitan	96.84	97.2	0.649	Not significantly different
Emulsification time	12.20	12.30	0.38	Not significantly different

Based on the results of drug loading, the SNEDDS formula can dissolve a maximum icariin weight of 15 mg / mL. Compared to aquades, it can only dissolve icariin by 29.2 µg/mL at room temperature. From the results of drug loading obtained, it can be said that the formation of L-SNEDDS can increase the solubility of icariin by 30 times.

The test results of particle size and droplet distribution of nanoemulsions show that the size of SNEDDS icariin particles is 15.63 ± 0.058 nm. The results of the Polipersity Index (PI) measurement obtained were 0.144 ± 0.062. The results showed that the nanoparticle size in the icariin SNEDDS preparation has a uniform particle size and a narrow size distribution. The measurement results of the obtained potential Zeta SNEDDS icariin were -22.67±2.06 mV. The value indicates that the production of the Icariin-SNEDDS is stable, and there is no precipitation during the storage period. The negative charge on the surface layer of the particles occurs due to the presence of free fatty acids derived from the components of the oil and surfactants, which causes a sufficiently strong repulsive force that can prevent flocculation.

Organoleptic tests are performed to determine and identify the physical appearance of SNEDDS. SNEDDS icariin formed from the composition of tween 80 as a surfactant, PEG 400 as a cosurfactant, and cucut fish oil in a ratio of 75%: 12.5%: 12.5% resulted in a physical appearance of the preparation as can be seen in **(Table 4)**.

Table 4. Organoleptic SNEDDS Icariin

Parameters	Result
Shape	Viscous liquid
Color	Yellow
Smell	Typical Fishy
Taste	Bitter
Clarity	Clear
Physical stability	No separation occurs
pH	6.52

The results showed that the droplets of icariin nanoemulsions remained stable until the 4th hour. Icariin nanoemulsions in aquades and AGF media were not subjected to phase separation, clots, or precipitate for 4 hours. So it can be said that icariin nanoemulsions can hold strong gastric juices and protect drugs.

The SNEDDS icariin dissolution test was conducted using a type I apparatus dissolution tester in aqueous media at 37 ± 0.5°C. It aims to determine the influence of the SNEDDS method and whether it can quickly increase the icariin dissolution rate. The results of determining icariin levels based on the dissolution test of SNEDDS icariin 50 mg and icariin powder 50 mg in the media can be seen in **(Figure 2)**.

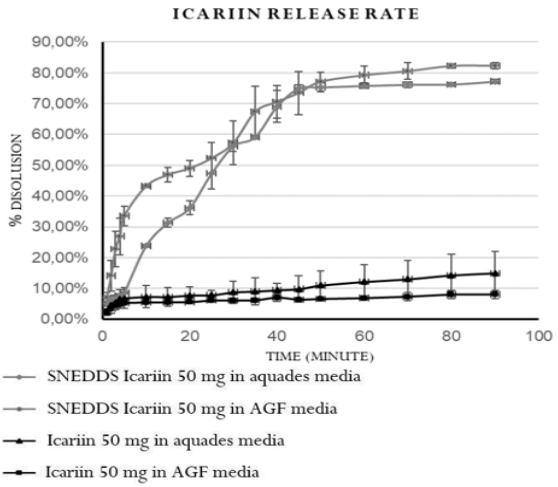


Figure 2. SNEDDS Icariin and Icariin Dissolution Test in Aquades and AGF Medium

Based on the results of the dissolution test shows different dissolution rates. The determination of icariin levels in SNEDDS preparations carried out on aqueous and AGF from the beginning to the end of sampling continued to increase. In the 60th minute SNEDDS icariin in the media aquades and AGF dissolution as much as 75 %. Levels of icariin not formulated in SNEDDS preparations in aquades and AGF media experienced a minimal increase. At the 90th minute, the dissolution of icariin levels reaches only under 20%.

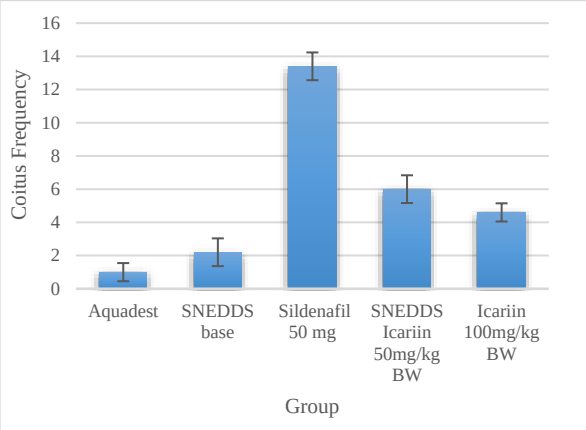


Figure 3. Coitus activity of test animals between treatment groups

SNEDDS icariin at a dose of 50 mg/KgBW provided an aphrodisiac effect in male white rats for better intercourse (coitus) parameters when compared to pure icariin 100 mg/KgBW with a significant differentiation ($p < 0.05$). SNEDDS formulations can increase the solubility of the icariin compound and increase the aphrodisiac effectiveness of animal test rats. The results of the aphrodisiac test between groups can be seen in **(Figure 3)**.

A member of the flavonoid family, icariin is extracted from the epimedium plant. This icariin works by raising levels of cyclic guanosine monophosphate (cGMP), which prevents the synthesis of phosphodiesteration enzyme type 5, to lessen the contraction of the smooth muscles of the corpus cavernous (PDE5). cGMP works to improve erection quality by increasing blood flow to the smooth muscles around the penis. Additionally, flavonoids have a part in raising epiandrogen dehydro levels, which can raise testosterone levels and promote sexual activity.

Conclusion

Icariin, oils, surfactants, and cosurfactants formulated into SNEDDS preparations can produce icariin SNEDDS preparations. The optimum formula obtained is tween 80 (75%), PEG 400 (12.5%), and cucut fish oil (12.5%). SNEDDS icariin has an average particle size of 15.63 ± 0.058 nm, polydispersity index of 0.144 ± 0.062, zeta potential of -22.67±2.06 mV, and maximum drug loading of 15 mg/mL. Dissolution of SNEDDS icariin is better than icariin without SNEDDS. SNEDDS icariin has a higher effectiveness as an aphrodisiac compared to pure icariin.

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Conflict of interest: None

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Ethics statement: Ethical clearance for experimental animals has been registered with the Faculty of Medicine Jenderal Soedirman University ethics committee with no. 024/KEPK/PE/V/2022.

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