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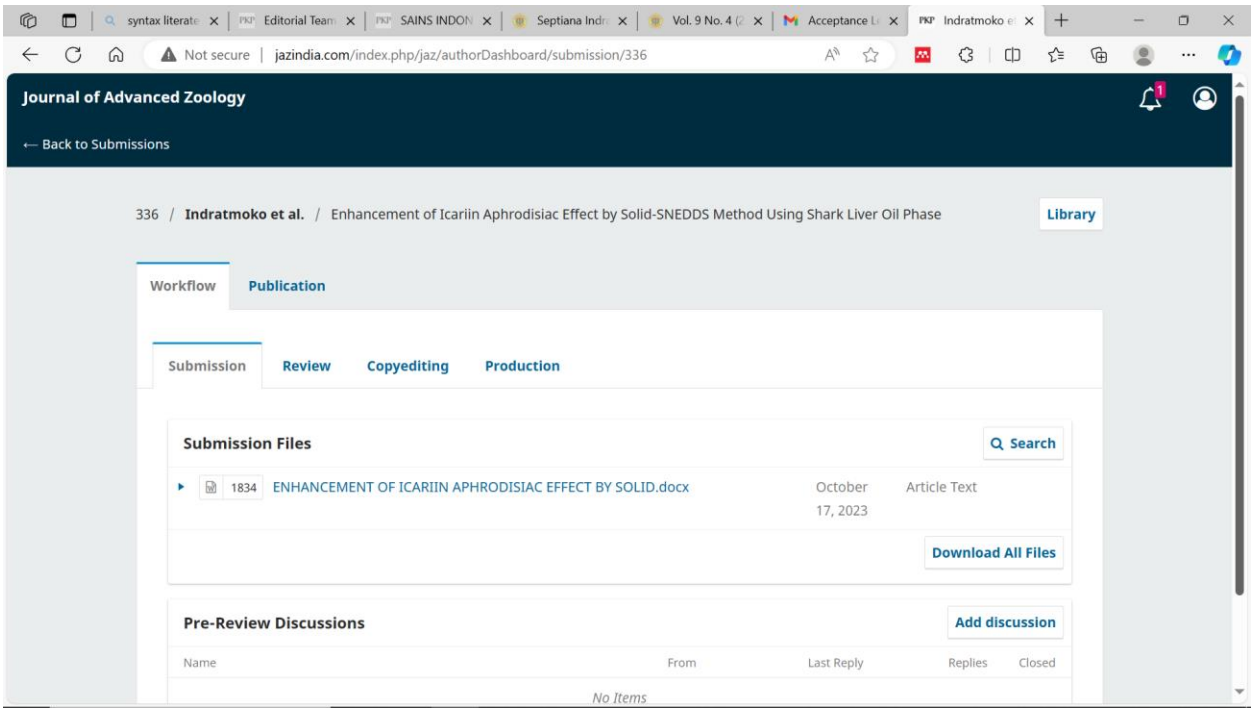

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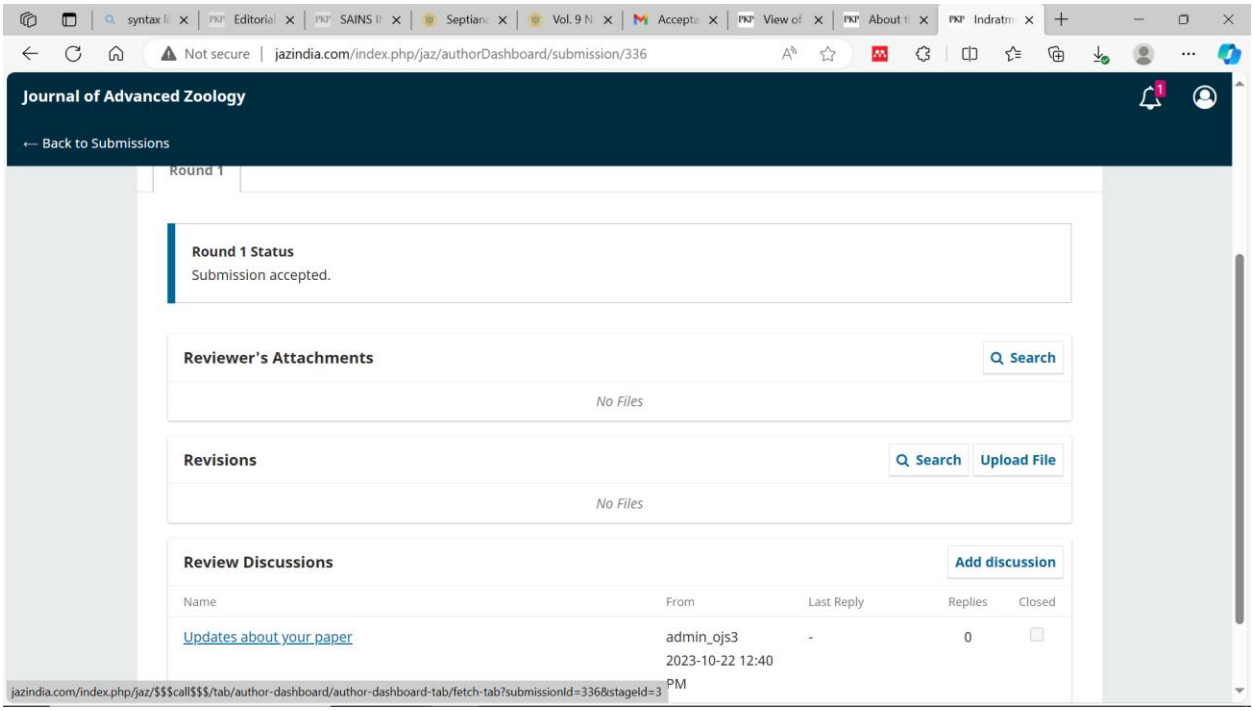

Line	Koreksi dari reviewer untuk diperbaiki
5	Another drug
14	How about bioavailability?
27	What is its chemical structure?
155	In english
204	In english
210	What is the importance of the dissolution test?

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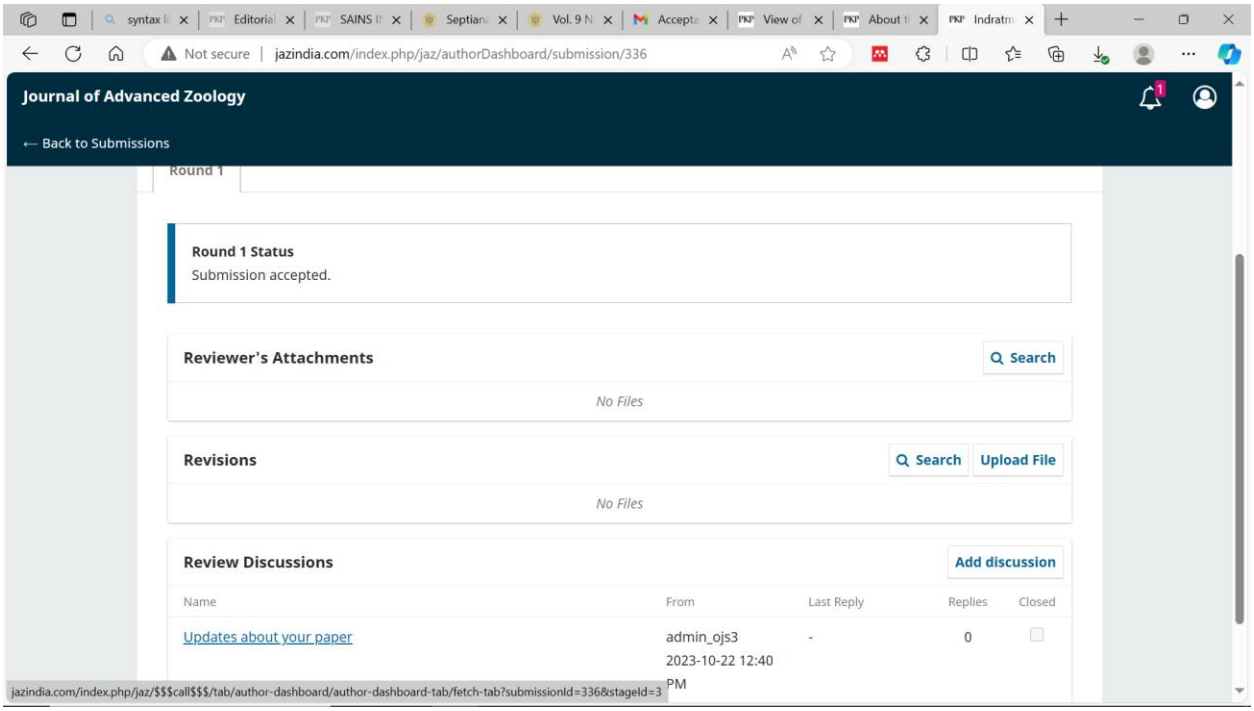

Line	Koreksi dari reviewer untuk diperbaiki	Perbaikan:
5	Another drug	*..., such as sildenafil, tadalafil, vardenafil, and avanafil,*
14	How about bioavailability?	*Icariin exhibits poor solubility and low membrane permeability (water solubility of <100 µg/mL, logP = 0.81 and pKa = 7.07), as it is a flavonoid aglycon (14).*
27	What is its chemical structure?	*Shark liver oil can dissolve icariin better than other oils because bottled fish oil 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.*

155	In english	*Table 4. Turbidity S-SNEDDS Icariin* *Replication* | *Transmittance (%)* *1* | *97,03* *2* | *98,34* *3* | *98,87* *Avarage ± SD* | *98,08 ± 0,94*
204	In english	*Table 7. Rest angle of S-SNEDDS Icariin* *Repication* | *Rest angle (°)* *1* | *33,663* *2* | *34,837* *3* | *36,979* *Avarage ± SD* | *35,159 ± 1,681*
210	What is the importance of the dissolution test?	*The dissolution test describes the speed of release and dissolution of the active substance in the preparation. The importance of dissolution tests because a drug's availability depends on the substance's ability to dissolve into the solvent medium before being absorbed into the body.*

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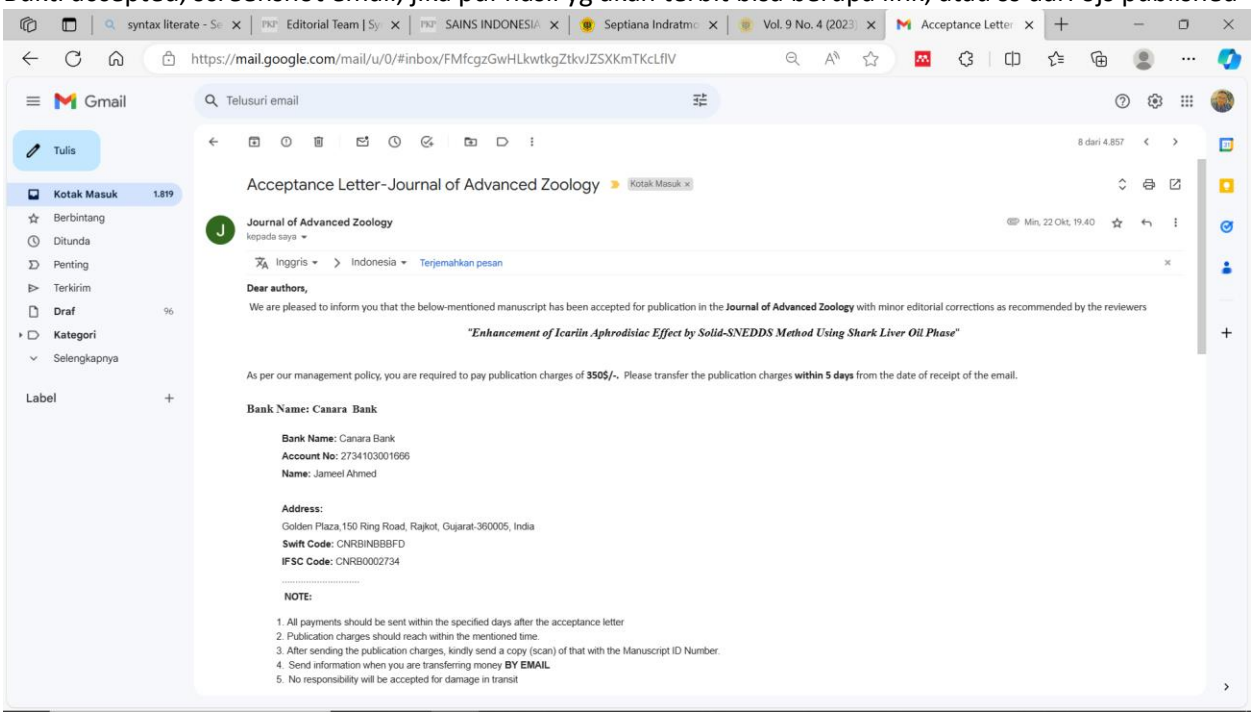

Acceptance Letter-Journal of Advanced Zoology

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Dear authors,

We are pleased to inform you that the below-mentioned manuscript has been accepted for publication in the **Journal of Advanced Zoology** with minor editorial corrections as recommended by the reviewers

"Enhancement of Icariin Aphrodisiac Effect by Solid-SNEDDS Method Using Shark Liver Oil Phase"

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ENHANCEMENT OF ICARIIN APHRODISIAC EFFECT BY SOLID-SNEDDS METHOD USING SHARK LIVER OIL PHASE

Septiana Indratmoko[1]*, Laela H. Nurani[2], Iis Wahyuningsih[2]

[1] Department of Pharmacy, University of Al-Irsyad Cilacap, Cilacap 53225, Indonesia,
[2] Department of Pharmacy, University of Ahmad Dahlan, Yogyakarta 55185, Indonesia
**Corresponding author: indratmoko86@gmail.com*

Abstract

The Epimedium brevicornu Maxim plant contains icariin, a flavonoid compound known for its aphrodisiac effects. However, icariin has low solubility and bioavailability. This study aims to find the best formula for S-SNEDDS icariin, its physical properties, characteristics, and aphrodisiac activity. Using the S-SNEDDS (Solid-Self Nanoemulsifying Drug Delivery System) method with shark liver oil phase is expected to increase the solubility and bioavailability of icariin. The optimum formula used is tween 80 (72.5%): PEG 400 (13.75%) and shark liver oil (13.75%). The optimal formula of S-SNEDDS icariin with the adsorption method to solid carriers requires aerosol 200 as much as 783±28.86mg per 1mL. S-SNEDDS icariin has characteristics with an average emulsification time of 12.88±0.26 seconds, transmittance value of 98.08±0.94%, droplet size 171.8±8.9 nm, zeta potential -35.2±1.1 mV, flow speed less than 10 seconds, resting angle 35.159°. The dissolution test of S-SNEDDS icariin is better than icariin instead of S-SNEDDS. S-SNEDDS icariin dose 50 mg/KgBW has a better aphrodisiac effect than pure icariin 100 mg/kgBW.

Keywords: aphrodisiac, icariin, shark liver oil, S-SNEDDS

1. Introduction

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

Recently, compounds of plant origin are increasingly being studied for their safety in treating sexual dysfunction (6). One of them is the epimedium plant. The main content in epimedium plants is icariin (7). Icariin has various pharmacological effects such as anti-osteoporosis (8), cardiovascular protection (9), anti-tumor (10), anti-inflammatory (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, as it is a flavonoid aglycon (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), liposome (19), transfersome (20), self-emulsifying (21), Self-Nanoemulsifying (22), and solid-SNEDDS (23).

Solid-self nanoemulsifying drug delivery system (S-SNEDDS) is an isotropic mixture of oils, surfactants, cosurfactants, drugs and carrier matrix that form nanoemulsions when meeting the water phase (24). S-SNEDDS spread easily within the gastrointestinal tract, and the digestive motility of the stomach and intestines provides the agitation necessary for emulsion systems (25).

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In this study, the development of icariin nanoparticles made by the S-SNEDDS method was carried out. 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 (26). Shark liver oil can dissolve icariin better than other oils because bottled fish oil contains squalene with a similiar chemical structure. The resulting icariin S-SNEDDS preparations were subsequently carried out to characterize physical properties and test aphrodisiac activity in rat test animals.

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2. Material and Methods

Biomaterials and Chemicals

Icariin (Liftmode, USA), standard icariin (Sigma-Aldrich 96%), aquades (Bratachem, Indonesia), PEG 400 (Bratachem, Indonesia), Tween 80 (Bratachem, Indonesia), Shark liver oil (Bumi Wijaya), aerosil 200 (Aloin Labora), 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 change in weight 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. From the stock solution, concentrations of 6 ppm, 8 ppm, 10 ppm, 12 ppm, and 14 ppm, were made. Precision was determined by applying 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.

S-SNEDDS Formula Optimization

Making S-SNEDDS begins with the selection of the most optimal Liquid-SNEDDS formula. The most optimal liquid-SNEDDS are then solidified into S-SNEDDS. Making solid SNEDSS based on the publication of Buya et al (2020), using the adsorption onto solid carriers method using aerosil 200. SNEDDS liquid icariin is inserted into a solid carrier namely aerosil 200 in a mortar until a non-sticky powder is formed.

Characterization of SNEDDS Icariin

1. *Emulsification Time Test*
   A total of 1 part of S-SNEDDS was dissolved in 500 mL aquadest using a magnetic stirrer at a speed of 500 rpm at room temperature while calculating the time to achieve emulsification using a stopwatch (27).
2. *Turbidity Test*
   The determination of turbidity is carried out using the results of emulsification time. The emulsion that has been obtained is measured absorption using a UV-VIS spectrophotometer at a wavelength of 650 nm with an aquadest blank (28).
3. *Particle size characteristics of S-SNEDDS icariin*

There are two parameters to determine the characterization of droplet size of icariin nanoemulsions, namely droplet size and droplet size distribution with a Particle Size Analyzer tool and zeta potential measurement. The test was carried out by taking a preparation of S-SNEDDS icariin as much as 2 g dissolved in 100 mL water, the mixture was homogenized using vortex. Then measured droplet size and droplet size distribution.

4. *Flow Speed Test*
   A total of 100 g of icariin powder was flowed on the flowability tester test kit. It is noted the time it takes for the powder to flow. The powder flow speed is said to be good when it has a flow time of ≤ 10 seconds.
5. *Break Angle*
   A total of 100 g of powder is input in the flowability tester test equipment, then cultured for flow and recorded radius (r) and height (h). The angle of rest is calculated using the formula:

$$tg\alpha = \frac{h}{r} \qquad (1)$$

A stationary angle value of <40° indicates that the granule flows easily (29).

Icariin Release Rate in S-SNEDDS System

Drug release or dissolution tests are carried out to determine the rate of release of the active substance using a disolution 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 S-SNEDDS Icariin

The S-SNEDDS icariin aphrodisiac test was performed using 25 male white rats and 25 female white rats. The rats were divided into five treatment groups, namely the negative control group (aquadest), negative control (S-SNEDDS base), positive control (Sildenafil 50 mg), S-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 observations made were coitus activities between male and female rats (30).

Data Analysis

Quantitative data on the activity of coitus obtained were analyzed using the oneway anova method then continued with the LSD (Lease Significant Different) test.

3. Results and Discussion

Standard curve, Accuracy, and Precision of Icariin

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 value of the correlation coefficient 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 ranging from 98.13% to 103.00%, this is in line with the provisions of the percentage of recovery analytes in the sample in the range of 95-105%. 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%.

S-SNEDDS Formula Optimization

The optimum composition of the formula can be seen in Table 1 with a ratio of the composition of tween 80: PEG 400: Shark liver oil which is 72.5%: 13.75%: 13.75% which produces the highest percentage of Transmittance of 97.3±0.77%, the fast emulsification time is 12.48±0.82 seconds and the smallest particle size is 17.82±0.62 nm.

Table 1. *Material Composition, Transmittance Results and Emulsification Time Base L-SNEDDS*

Run	Comparison in %			Transmittance (%)	Emulsification time (second)	Particle Size (nm)
	Tween 80	PEG 400	Shark liver oil			
1	70	15	15	82.7±0.32	18.5±0.53	28.43±0.44
2	72.5	13.75	13.75	97.3±0.77	12.48±0.82	17.82±0.62
3	75	12.5	12.5	87.2±0.21	16.3±0.56	22.57±0.48

L-SNEDDS icariin is converted into S-SNEDDS icariin using the adsorption method to solid carriers by mixing aerosil 200 into SNEDDS icariin then stirring until homogeneous. This method is the simplest method also has advantages including better uniformity of granule size and the drug can be adsorbed up to 70% w/w with suitable carriers. The manufacture of S-SNEDDS icariin is carried out using adsorption techniques to solid carriers where aerosil 200 used as an absorbent has the property of being able to absorb L-SNEDDS formulations. The addition of aerosil is done little by little until the desired powder period is obtained. After the addition of aerosil 200 obtained a powder of pale yellow color. In 1 mL L-SNEDDS icariin requires 783.33 mg of aerosil 200 so that L-SNEDDS icariin can be absorbed until a powder period is formed. Data on the results of the addition of aerosil 200 can be seen in Table 2.

Table 2. *Addition of Aerosil 200 per 1 mL L-SNEDDS Icariin*

Adsorben	R1	R2	R3	Average ± SD
Aerosil (mg)	800	750	800	783.33± 28.86

The addition of aerosil 200 as an adsorbent can increase the ability to bind high moisture, the ability to overcome the stickiness of particles to each other so as to minimize friction that occurs between particles and maintain good flowability (31).

Characteristics of S-SNEDDS Icariin

1. Emulsification Time S-SNEDDS Icariin

Emulsification time is the time it takes for S-SNEDDS to form nanoemulsions starting when the initial 1 part of S-SNEDDS is dripped until the consistency of the nanoemulsion is formed when it encounters a liquid medium in the presence of mild agitation (32). An emulsification time test is performed to determine how fast the S-SNEDDS formula forms an emulsion. An S-SNEDDS formula is good when emulsification occurs rapidly in less than 1 minute with visually clear or transparent observations (33). Emulsification time testing performed 3 replications on the dissolution tester tool on aquuadest media and artificial gastric fluid (AGF) media shown in Table 3.

Table 3. *Emulsification Time S-SNEDDS Icariin (15 mg)*

Replication	***Aquadest (second)***	***AGF (second)***
1	*13,05*	*17,53*
2	*12,57*	*18,12*
3	*13,01*	*18,87*
Avarage ± SD	*12,88 ± 0,26*	*18,17 ± 0,67*

From the results of the emulsification time test, it was found that the emulsion formation time on aquadest media was 12.88± 0,26 seconds and on AGF media nanoemulsions were formed at 18.17± 0,67 seconds so that it can be said that S-SNEDDS icariin meets good criteria because the time needed is less than 1 minute (34).

2. Turbidity S-SNEDDS Icariin

The turbidity test aims to determine the level of clarity of the preparation, where a good transmittance value is close to 100%. The measurements were made using UV-Vis spectrophotometry at a wavelength of 650 nm. In testing, aquades are used as a blank solution or comparison because aquades are neutral and minimize interference with S-SNEDDS components when taking data.
The test was carried out using a solution that had passed the emulsification time test stage, taken 5 mL. Vortex 30 seconds and measured absorption at a wavelength of 650 nm with an aqueous blank to determine the level of clarity. Turbidity test results can be seen in Table 4.

Table 4. *Turbidity S-SNEDDS Icariin*

Replication	***Transmittance (%)***
1	*97,03*
2	*98,34*
3	*98,87*
Rata-rata ± SD	*98,08 ± 0,94*

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From the data obtained, the transmittance value of S-SNEDDS icariin meets the requirements, which is close to 100%, with the average replication value obtained at 98.08± 0,94 %, which is the value of water transmittance.

3. Droplet Size and Zeta Potential of Icariin S-SNEDDS Nanoemulsion

In the particle size characteristics test, there are 2 parameters: droplet size measurement and zeta potential test. Droplet size or droplet size is important in making S-SNEDDS because it determines the size of droplets produced to determine the rate of drug release and absorption (35). Meanwhile, the Polydispersity Index (PI) value measures the molecular mass distribution in a particular sample, which is used as a parameter of uniformity and reliability of nanoemulsion manufacturing methods. There is also a zeta potential test to determine the characteristics of nanoemulsion preparations. The zeta potential value is a stability parameter of a system in which globules are dispersed through the presence of opposite forces between particles with the same charge when close together. Data on droplet size, PI, and zeta potential can be seen in Table 5.

Table 5. *Droplet Size, Polydispersity Index, and Zeta Potential of Icariin S-SNEDDS Nanoemulsion Zeta*

Replication	***Droplet Size (nm)***	***Polydispersity Index (PI)***	***Zeta Potential (mV)***
1	*162,2*	*0,475*	*-34,7*
2	*173,6*	*0,491*	*-34,5*
3	*179,8*	*0,483*	*-36,5*
Avarage ± SD	*171,8±8,9*	*0,484±0,008*	*-35,2±1,1*

The results of the droplet size test on S-SNEDDS icariin using the Particle Size Analyzer (PSA) tool obtained size of 171.8±8.9 nm, the value of the nanoparticle droplet size is in the range of 20-200 nm so that the droplet size value of S-SNEDDS icariin is said to meet the range of requirements, where the smaller the droplet size, the faster the absorption, the solubility increases, and the pharmacological effect is faster. The Polydispersity Index (PI) value obtained an average value of 0.484±0.008 PI value below 1 or close to zero, which means that the distribution is good and said to be homogeneous.

The zeta potential measurement of S-SNEDDS icariin is an average of -35.2±1.1 mV, and no flocculation occurs. Zeta potential values of more than ± 30 mV provide nanoemulsion droplet stability in the system so that flocculation does not occur. The negative charge from the droplet surface occurs due to free fatty acids derived from the S-SNEDDS component, namely surfactants, co-surfactants and shark liver oil containing fatty acids (36).

4. Icariin S-SNEDDS Flow Speed

The flow velocity test of S-SNEDDS icariin is performed to determine the characteristics of granules that are qualified to be produced to become oral solid preparations. Granules from S-SNEDDS icariin are expected to have a good flow speed so that the capsule preparation size can be uniform when filling into capsules. The granule is said to have a good flow speed. If the flow speed is not less than 10g/second or 100 g of granule the flow time is not more than 10 seconds. The flow velocity test results can be seen in Table 6.

Table 6. *Icariin S-SNEDDS Flow Speed*

Replication	***Flow speed (seconds)***
1	*10*
2	*9,25*
3	*9,27*
Avarage ± SD	*9,50 ±0,42*

A good flow speed is less than 10 seconds; the data obtained is the average flow speed carried out in 3 replications, which is 9.50±0.42 seconds.

5. S-SNEDDS Icariin Break Angle

The rest angle test is a fixed angle between the cone-shaped particle pile and the horizontal plane when the powder flows into the tool. The higher the granule cone, the higher the resting angle. The values of the break angle can be seen in Table 7.

Tabel 7. *Sudut Istirahat S-SNEDDS Icariin*

Replikasi	***Sudut Istirahat (º)***
1	*33,663*
2	*34,837*
3	*36,979*
Rata-rata ± SD	*35,159 ± 1,681*

From the stationary angle test data in Table 7, it is known that the average test result is 35.16°± 1.681, where this value meets the stationary angle requirement of <40°, and it can be said that the granule flows well (29).

Icariin Release Rate in S-SNEDDS base

Dissolution is releasing drug compounds from preparations and dissolving them in their solvent media. The results of determining icariin levels based on the dissolution test of S-SNEDDS, icariin in capsules and icariin without S-SNEDDS in capsules can be seen in Figure 1.

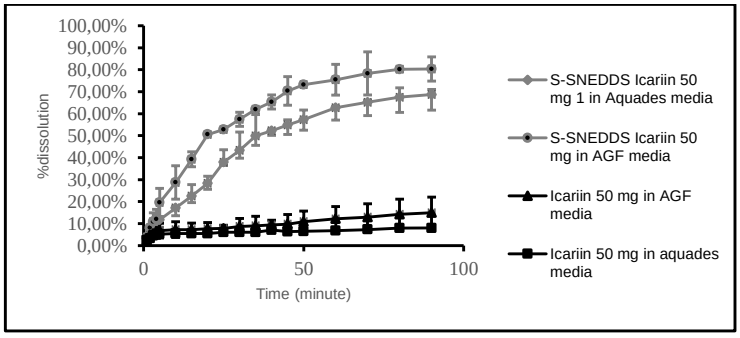

Figure 1. *Dissolution of S-SNEDDS Icariin and Icariin in Aqueous and AGF Media*

From the dissolution test results for determining icariin levels in aqueous media, it can be seen that icariin levels in S-SNEDDS preparations increase with increasing sampling time. Levels of icariin not formulated in the form of S-SNEDDS have increased slowly. S-SNEDDS Icariin has a better solution than Icariin without S-SNEDDS in Aqueous Media and AGF with a significant differentiation ($p < 0.05$).

Aphrodisiac Test S-SNEDDS Icariin

S-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 ($p < 0.05$). S-SNEDDS formulations can increase the solubility of the

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icariin compound and increase the aphrodisiac effectiveness of animal test rats. The results of the aphrodisiac test between groups can be seen in Figure 2.

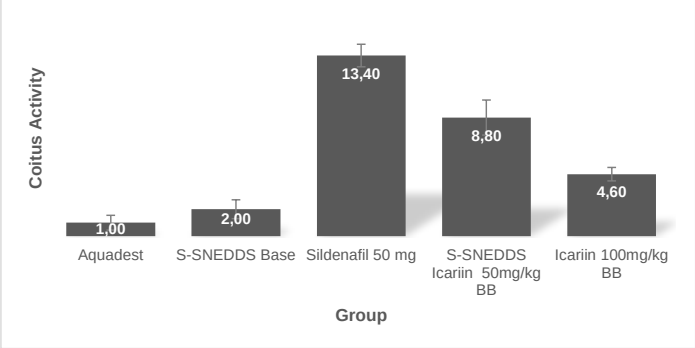

Figure 2. *Coitus activity of test animals between treatment groups*

S-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 ($p < 0.05$). S-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 2.

Icariin is a flavonoid group compound derived from the epimedium plant. This icariin has a role in reducing the contraction of the smooth muscles of the corpus cavernous by increasing levels of cyclic guanosine monophosphate (cGMP) to inhibit the formation of phosphodiesteration enzyme type 5 (PDE5) (37). cGMP serves to increase blood flow to smooth muscles in the penis area so that it can cause an increase in erection. In addition, flavonoids have a role in increasing dihydro levels of epiandrosterone, which can increase testosterone levels and encourage sexual activity (38).

4. Conclusion

The optimum formula used is tween 80 (72.5%): PEG 400 (13.75%) and shark liver oil (13.75%). The optimal formula of S-SNEDDS icariin with the adsorption method to solid carriers requires aerosol 200 as much as 783±28.86mg per 1mL. S-SNEDDS icariin has characteristics with an average emulsification time of 12.88±0.26 seconds, transmittance value of 98.08±0.94%, droplet size 171.8±8.9 nm, zeta potential -35.2±1.1 mV, flow speed less than 10 seconds, resting angle 35.159°. Dissolution of S-SNEDDS icariin is better than icariin without S-SNEDDS. S-SNEDDS icariin has a higher effectiveness as an aphrodisiac compared to pure icariin.

Acknowledgments

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ENHANCEMENT OF ICARIIN APHRODISIAC EFFECT BY SOLID-SNEDDS METHOD USING SHARK LIVER OIL PHASE

Septiana Indratmoko[1]*, Laela H. Nurani[2], Iis Wahyuningsih[2]

[1] Department of Pharmacy, University of Al-Irsyad Cilacap, Cilacap 53225, Indonesia,
[2] Department of Pharmacy, University of Ahmad Dahlan, Yogyakarta 55185, Indonesia
**Corresponding author: indratmoko86@gmail.com*

Abstract

The Epimedium brevicornu Maxim plant contains icariin, a flavonoid compound known for its aphrodisiac effects. However, icariin has low solubility and bioavailability. This study aims to find the best formula for S-SNEDDS icariin, its physical properties, characteristics, and aphrodisiac activity. Using the S-SNEDDS (Solid-Self Nanoemulsifying Drug Delivery System) method with shark liver oil phase is expected to increase the solubility and bioavailability of icariin. The optimum formula used is tween 80 (72.5%): PEG 400 (13.75%) and shark liver oil (13.75%). The optimal formula of S-SNEDDS icariin with the adsorption method to solid carriers requires aerosol 200 as much as 783±28.86mg per 1mL. S-SNEDDS icariin has characteristics with an average emulsification time of 12.88±0.26 seconds, transmittance value of 98.08±0.94%, droplet size 171.8±8.9 nm, zeta potential -35.2±1.1 mV, flow speed less than 10 seconds, resting angle 35.159°. The dissolution test of S-SNEDDS icariin is better than icariin instead of S-SNEDDS. S-SNEDDS icariin dose 50 mg/KgBW has a better aphrodisiac effect than pure icariin 100 mg/kgBW.

Keywords: aphrodisiac, icariin, shark liver oil, S-SNEDDS

1. Introduction

Sexual dysfunction disorders include reduced libido, abnormal ejaculation and erectile dysfunction (1). Erectile dysfunction has a considerable impact on men's psychology and quality of life, 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 treating erectile dysfunction (4). However, synthetic phosphodiesterase type 5 inhibitor compounds (PDE5) cause some side effects such as headaches, priapism, redness and visual impairment (5).

Recently, compounds of plant origin are increasingly being studied for their safety in treating sexual dysfunction (6). One of them is the epimedium plant. The main content in epimedium plants is icariin (7). Icariin has various pharmacological effects such as anti-osteoporosis (8), cardiovascular protection (9), anti-tumor (10), anti-inflammatory (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, and 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 aglycon (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), liposome (19), transfersome (20), self-emulsifying (21), Self-Nanoemulsifying (22), and solid-SNEDDS (23).

Solid-self nanoemulsifying drug delivery system (S-SNEDDS) is an isotropic mixture of oils, surfactants, cosurfactants, drugs and carrier matrix that form nanoemulsions when meeting the

water phase (24). S-SNEDDS spread easily within the gastrointestinal tract, and the digestive motility of the stomach and intestines provides the agitation necessary for emulsion systems (25).

In this study, the development of icariin nanoparticles made by the S-SNEDDS method was carried out. 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 (26). Shark liver oil can dissolve icariin better than other oils because bottled fish oil 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. The resulting icariin S-SNEDDS preparations were subsequently carried out to characterize physical properties and test aphrodisiac activity in rat test animals.

2. Material and Methods

Biomaterials and Chemicals

Icariin (Liftmode, USA), standard icariin (Sigma-Aldrich 96%), aquades (Bratachem, Indonesia), PEG 400 (Bratachem, Indonesia), Tween 80 (Bratachem, Indonesia), Shark liver oil (Bumi Wijaya), aerosil 200 (Aloin Labora), 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 change in weight 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. From the stock solution, concentrations of 6 ppm, 8 ppm, 10 ppm, 12 ppm, and 14 ppm, were made. Precision was determined by applying 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.

S-SNEDDS Formula Optimization

Making S-SNEDDS begins with the selection of the most optimal Liquid-SNEDDS formula. The most optimal liquid-SNEDDS are then solidified into S-SNEDDS. Making solid SNEDSS based on the publication of Buya et al (2020), using the adsorption onto solid carriers method using aerosil 200. SNEDDS liquid icariin is inserted into a solid carrier namely aerosil 200 in a mortar until a non-sticky powder is formed.

Characterization of SNEDDS Icariin

1. *Emulsification Time Test*
   A total of 1 part of S-SNEDDS was dissolved in 500 mL aquadest using a magnetic stirrer at a speed of 500 rpm at room temperature while calculating the time to achieve emulsification using a stopwatch (27).
2. *Turbidity Test*

The determination of turbidity is carried out using the results of emulsification time. The emulsion that has been obtained is measured absorption using a UV-VIS spectrophotometer at a wavelength of 650 nm with an aquadest blank (28).

3. *Particle size characteristics of S-SNEDDS icariin*
   There are two parameters to determine the characterization of droplet size of icariin nanoemulsions, namely droplet size and droplet size distribution with a Particle Size Analyzer tool and zeta potential measurement. The test was carried out by taking a preparation of S-SNEDDS icariin as much as 2 g dissolved in 100 mL water, the mixture was homogenized using vortex. Then measured droplet size and droplet size distribution.
4. *Flow Speed Test*
   A total of 100 g of icariin powder was flowed on the flowability tester test kit. It is noted the time it takes for the powder to flow. The powder flow speed is said to be good when it has a flow time of ≤ 10 seconds.
5. *Break Angle*
   A total of 100 g of powder is input in the flowability tester test equipment, then cultured for flow and recorded radius (r) and height (h). The angle of rest is calculated using the formula:

$$tg\alpha = \frac{h}{r} \qquad (1)$$

A stationary angle value of <40° indicates that the granule flows easily (29).

Icariin Release Rate in S-SNEDDS System

Drug release or dissolution tests are carried out to determine the rate of release of the active substance using a disolution 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 S-SNEDDS Icariin

The S-SNEDDS icariin aphrodisiac test was performed using 25 male white rats and 25 female white rats. The rats were divided into five treatment groups, namely the negative control group (aquadest), negative control (S-SNEDDS base), positive control (Sildenafil 50 mg), S-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 observations made were coitus activities between male and female rats (30).

Data Analysis

Quantitative data on the activity of coitus obtained were analyzed using the oneway anova method then continued with the LSD (Lease Significant Different) test.

3. Results and Discussion

Standard curve, Accuracy, and Precision of Icariin

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 value of the correlation coefficient 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 ranging from 98.13% to 103.00%, this is in line with the provisions of the percentage of recovery analytes in the sample in the range of 95-105%. 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%.

S-SNEDDS Formula Optimization

The optimum composition of the formula can be seen in Table 1 with a ratio of the composition of tween 80: PEG 400: Shark liver oil which is 72.5%: 13.75%: 13.75% which produces the highest percentage of Transmittance of 97.3±0.77%, the fast emulsification time is 12.48±0.82 seconds and the smallest particle size is 17.82±0.62 nm.

Table 1. *Material Composition, Transmittance Results and Emulsification Time Base L-SNEDDS*

Run	Comparison in %			Transmittance (%)	Emulsification time (second)	Particle Size (nm)
	Tween 80	PEG 400	Shark liver oil			
1	70	15	15	82.7±0.32	18.5±0.53	28.43±0.44
2	72.5	13.75	13.75	97.3±0.77	12.48±0.82	17.82±0.62
3	75	12.5	12.5	87.2±0.21	16.3±0.56	22.57±0.48

L-SNEDDS icariin is converted into S-SNEDDS icariin using the adsorption method to solid carriers by mixing aerosil 200 into SNEDDS icariin then stirring until homogeneous. This method is the simplest method also has advantages including better uniformity of granule size and the drug can be adsorbed up to 70% w/w with suitable carriers. The manufacture of S-SNEDDS icariin is carried out using adsorption techniques to solid carriers where aerosil 200 used as an absorbent has the property of being able to absorb L-SNEDDS formulations. The addition of aerosil is done little by little until the desired powder period is obtained. After the addition of aerosil 200 obtained a powder of pale yellow color. In 1 mL L-SNEDDS icariin requires 783.33 mg of aerosil 200 so that L-SNEDDS icariin can be absorbed until a powder period is formed. Data on the results of the addition of aerosil 200 can be seen in Table 2.

Table 2. *Addition of Aerosil 200 per 1 mL L-SNEDDS Icariin*

Adsorben	R1	R2	R3	Average ± SD
Aerosil (mg)	800	750	800	783.33± 28.86

The addition of aerosil 200 as an adsorbent can increase the ability to bind high moisture, the ability to overcome the stickiness of particles to each other so as to minimize friction that occurs between particles and maintain good flowability (31).

Characteristics of S-SNEDDS Icariin

1. Emulsification Time S-SNEDDS Icariin

Emulsification time is the time it takes for S-SNEDDS to form nanoemulsions starting when the initial 1 part of S-SNEDDS is dripped until the consistency of the nanoemulsion is formed when it encounters a liquid medium in the presence of mild agitation (32). An emulsification time test is performed to determine how fast the S-SNEDDS formula forms an emulsion. An S-SNEDDS formula is good when emulsification occurs rapidly in less than 1 minute with visually clear or

transparent observations (33). Emulsification time testing performed 3 replications on the dissolution tester tool on aquuadest media and artificial gastric fluid (AGF) media shown in Table 3.

Table 3. *Emulsification Time S-SNEDDS Icariin (15 mg)*

Replication	***Aquadest (second)***	***AGF (second)***
1	*13,05*	*17,53*
2	*12,57*	*18,12*
3	*13,01*	*18,87*
Avarage ± SD	*12,88 ± 0,26*	*18,17 ± 0,67*

From the results of the emulsification time test, it was found that the emulsion formation time on aquadest media was 12.88± 0,26 seconds and on AGF media nanoemulsions were formed at 18.17± 0,67 seconds so that it can be said that S-SNEDDS icariin meets good criteria because the time needed is less than 1 minute (34).

2. Turbidity S-SNEDDS Icariin

The turbidity test aims to determine the level of clarity of the preparation, where a good transmittance value is close to 100%. The measurements were made using UV-Vis spectrophotometry at a wavelength of 650 nm. In testing, aquades are used as a blank solution or comparison because aquades are neutral and minimize interference with S-SNEDDS components when taking data.
The test was carried out using a solution that had passed the emulsification time test stage, taken 5 mL. Vortex 30 seconds and measured absorption at a wavelength of 650 nm with an aqueous blank to determine the level of clarity. Turbidity test results can be seen in Table 4.

Table 4. *Turbidity S-SNEDDS Icariin*

Replication	***Transmittance (%)***
1	*97,03*
2	*98,34*
3	*98,87*
Avarage ± SD	*98,08 ± 0,94*

From the data obtained, the transmittance value of S-SNEDDS icariin meets the requirements, which is close to 100%, with the average replication value obtained at 98.08± 0,94 %, which is the value of water transmittance.

3. Droplet Size and Zeta Potential of Icariin S-SNEDDS Nanoemulsion

In the particle size characteristics test, there are 2 parameters: droplet size measurement and zeta potential test. Droplet size or droplet size is important in making S-SNEDDS because it determines the size of droplets produced to determine the rate of drug release and absorption (35). Meanwhile, the Polydispersity Index (PI) value measures the molecular mass distribution in a particular sample, which is used as a parameter of uniformity and reliability of nanoemulsion manufacturing methods. There is also a zeta potential test to determine the characteristics of nanoemulsion preparations. The zeta potential value is a stability parameter of a system in which globules are dispersed through the presence of opposite forces between

particles with the same charge when close together. Data on droplet size, PI, and zeta potential can be seen in Table 5.

Table 5. *Droplet Size, Polydispersity Index, and Zeta Potential of Icariin S-SNEDDS Nanoemulsion Zeta*

Replication	***Droplet Size (nm)***	***Polydispersity Index (PI)***	***Zeta Potential (mV)***
1	*162,2*	*0,475*	*-34,7*
2	*173,6*	*0,491*	*-34,5*
3	*179,8*	*0,483*	*-36,5*
Avarage ± SD	*171,8±8,9*	*0,484±0,008*	*-35,2±1,1*

The results of the droplet size test on S-SNEDDS icariin using the Particle Size Analyzer (PSA) tool obtained size of 171.8±8.9 nm, the value of the nanoparticle droplet size is in the range of 20-200 nm so that the droplet size value of S-SNEDDS icariin is said to meet the range of requirements, where the smaller the droplet size, the faster the absorption, the solubility increases, and the pharmacological effect is faster. The Polydispersity Index (PI) value obtained an average value of 0.484±0.008 PI value below 1 or close to zero, which means that the distribution is good and said to be homogeneous.
The zeta potential measurement of S-SNEDDS icariin is an average of -35.2±1.1 mV, and no flocculation occurs. Zeta potential values of more than ± 30 mV provide nanoemulsion droplet stability in the system so that flocculation does not occur. The negative charge from the droplet surface occurs due to free fatty acids derived from the S-SNEDDS component, namely surfactants, co-surfactants and shark liver oil containing fatty acids (36).

4. Icariin S-SNEDDS Flow Speed

The flow velocity test of S-SNEDDS icariin is performed to determine the characteristics of granules that are qualified to be produced to become oral solid preparations. Granules from S-SNEDDS icariin are expected to have a good flow speed so that the capsule preparation size can be uniform when filling into capsules. The granule is said to have a good flow speed. If the flow speed is not less than 10g/second or 100 g of granule the flow time is not more than 10 seconds. The flow velocity test results can be seen in Table 6.

Table 6. *Icariin S-SNEDDS Flow Speed*

Replication	***Flow speed (seconds)***
1	*10*
2	*9,25*
3	*9,27*
Avarage ± SD	*9,50 ±0,42*

A good flow speed is less than 10 seconds; the data obtained is the average flow speed carried out in 3 replications, which is 9.50±0.42 seconds.

5. S-SNEDDS Icariin Break Angle

The rest angle test is a fixed angle between the cone-shaped particle pile and the horizontal plane when the powder flows into the tool. The higher the granule cone, the higher the resting angle. The values of the break angle can be seen in Table 7.

***Table 7.** Rest angle of S-SNEDDS Icariin*

Repication	***Rest angle (º)***
1	*33,663*
2	*34,837*
3	*36,979*
Avarage ± SD	*35,159 ± 1,681*

From the stationary angle test data in Table 7, it is known that the average test result is 35.16°± 1.681, where this value meets the stationary angle requirement of <40°, and it can be said that the granule flows well (29).

Icariin Release Rate in S-SNEDDS base

Dissolution is releasing drug compounds from preparations and dissolving them in their solvent media. The dissolution test describes the speed of release and dissolution of the active substance in the preparation. The importance of dissolution tests because a drug's availability depends on the substance's ability to dissolve into the solvent medium before being absorbed into the body.

The results of determining icariin levels based on the dissolution test of S-SNEDDS, icariin in capsules and icariin without S-SNEDDS in capsules can be seen in Figure 1.

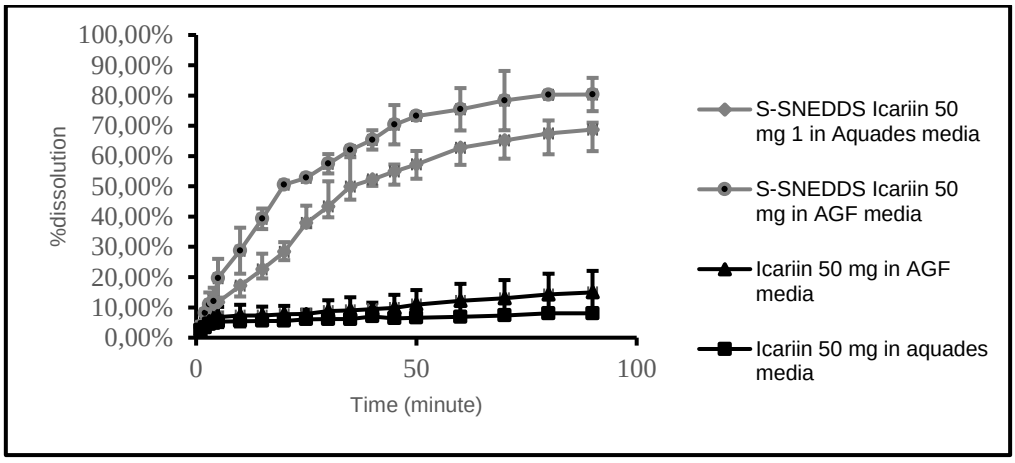

***Figure 1.** Dissolution of S-SNEDDS Icariin and Icariin in Aqueous and AGF Media*

From the dissolution test results for determining icariin levels in aqueous media, it can be seen that icariin levels in S-SNEDDS preparations increase with increasing sampling time. Levels of icariin not formulated in the form of S-SNEDDS have increased slowly. S-SNEDDS Icariin has a better solution than Icariin without S-SNEDDS in Aqueous Media and AGF with a significant differentiation ($p < 0.05$).

Aphrodisiac Test S-SNEDDS Icariin

S-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 ($p < 0.05$). S-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 2.

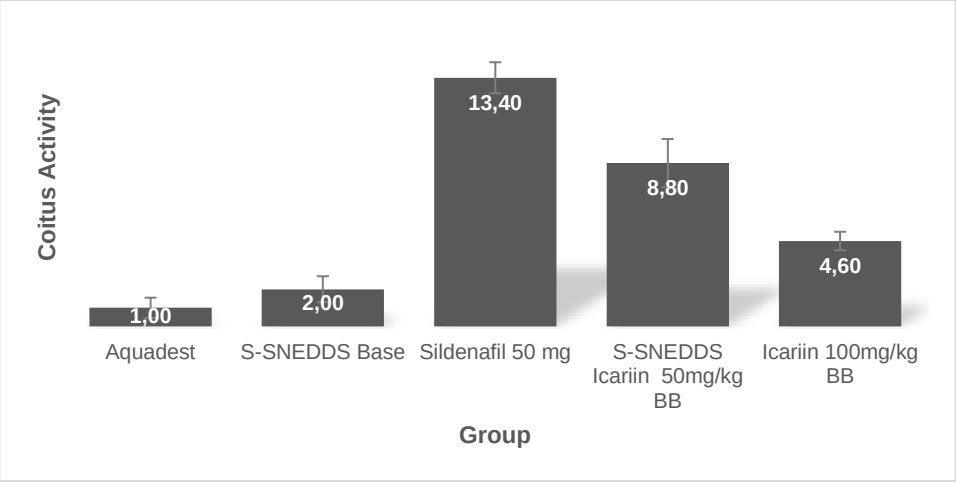

Figure 2. *Coitus activity of test animals between treatment groups*

S-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 ($p < 0.05$). S-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 2.

Icariin is a flavonoid group compound derived from the epimedium plant. This icariin has a role in reducing the contraction of the smooth muscles of the corpus cavernous by increasing levels of cyclic guanosine monophosphate (cGMP) to inhibit the formation of phosphodiesteration enzyme type 5 (PDE5) (37). cGMP serves to increase blood flow to smooth muscles in the penis area so that it can cause an increase in erection. In addition, flavonoids have a role in increasing dihydro levels of epiandrosterone, which can increase testosterone levels and encourage sexual activity (38).

4. Conclusion

The optimum formula used is tween 80 (72.5%): PEG 400 (13.75%) and shark liver oil (13.75%). The optimal formula of S-SNEDDS icariin with the adsorption method to solid carriers requires aerosol 200 as much as 783±28.86mg per 1mL. S-SNEDDS icariin has characteristics with an average emulsification time of 12.88±0.26 seconds, transmittance value of 98.08±0.94%, droplet size 171.8±8.9 nm, zeta potential -35.2±1.1 mV, flow speed less than 10 seconds, resting angle 35.159°. Dissolution of S-SNEDDS icariin is better than icariin without S-SNEDDS. S-SNEDDS icariin has a higher effectiveness as an aphrodisiac compared to pure icariin.

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