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Optimization *of Self Nanoemulsifying Drug Delivery System* of Mature Green Betel Leaf Fraction with D-Optimal Method and Antifungal Activity *of Candida albicans*

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A R T I C L E I N F O

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A B S T R A C T

The purpose of the study was to see the most active fraction in providing activity, the influence and optimal proportion of oleic acid, tween 80, and propylene glycol on characterization and the optimal formula that has increased activity. The Fractionation Process uses water, ethyl acetate, and n-hexane as solvents. Activity tests use concentration variations of 0.4%, 0.8%, 1.6%. SNEDDS formulation uses 15% -20% oleic acid, 55% -60% tween 80, and 20% -25% propylene glycol and is tested for characterization and optimization using D-optimal. The optimum formula was tested for antifungal activity. The results of the antifungal activity test showed that the ethyl acetate fraction had the most active activity. Characterization showed that an increase in oleic acid provided an increase in emulsification time, particle size, polydispersion index, zeta potential and a decrease in drug loading. The increase of tween 80 and propylene glycol resulted in a decrease in emulsification time, particle size, polydispersity index, zeta potential, and an increase in drug loading. The optimum formula at the ratio of oleic acid:tween 80:propylene glycol = 16.1:58.9:25 with an emulsification time of 70 seconds, particle size of 186.98, polydispersity index of 0.331, zeta potential of -32.02, and drug loading of 74.3%. The optimum formula resulted in an increase in DDH of 1.3 mm.

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INTRODUCTION

Oral candidiasis is one of the infectious diseases that has increased dramatically over the last three decades and causes increased morbidity and mortality in immunocompromised patients. The most common cause of this infection is the fungus *Candida albicans*. This species of fungus can become pathogenic due to a weakened immune system (Bakhtiar*i et al*., 2019). The use of synthetic antifungals has side effects that are quite disruptive to the patient's condition, such as severe side effects, narrow antifungal spectrum, poor penetration into certain tissues, and the emergence of resistant fungi (Triyuliani, Darwis and Sariyanti, 2023). Therefore, antifungal therapy with fewer side effects is needed for effective management of candidiasis infections, thus encouraging researchers to conduct research on the use of antifungals from herbal plants, namely betel leaves.

Many studies have been conducted previously on the antifungal activity of betel leaves against *Candida albicans* . Ethanol extract of mature betel leaves and young betel leaves with a concentration of 1000 μg/mL have antifungal activity agains*t Candida albicans* with inhibition zones of 22 mm and 20 mm, respectively (Sivareddy *et al*., 2019). Other studies also show that ethanol extract and ethyl acetate fraction of betel leaves with a concentration of 1 mg/mL have strong antifungal activity against *Candida albicans* with inhibition zones of 21 mm and 24.33 mm, respectively (Phumat *et.*, 2017Z). Having strong therapeutic activity but low solubility can limit its use (Tagrida *et al*., 2021). Some of these limitations can be overcome by encapsulating betel leaf fractions into a colloidal carrier system.

Self-Nanoemulsifying Drug Delivery System has several advantages, namely it can transport lipophilic drugs such as betel leaves, is thermodynamically and kinetically stable, can be administered in various ways, including oral, topical, parenteral, and transdermal, and can increase the solubility and bioavailability of betel leaves (Suyal, Kumar and Jakhmola, 2023). Optimization can be done using *D-optimal Mixture in Design Expert 12*. This method was chosen because it is practical and fast because it does not involve determining the formula by trial *and error* (Hidayati, Saptarini and Kuncahyo, 2022). Oleic Acid is a lipid that is widely used in nanoparticle synthesis because it has non-toxic properties and can dissolve synthetic and herbal drugs and can increase drug absorption in the intestine (Kassem et al., 2016). Tween 80 is often used as a surfactant in SNEDDS preparations because it has good solubility, and is less affected by changes in pH and ionic strength and can cause the formation of a more stable emulsion (Kassem et al., 2016). Propylene Glycol was chosen as a cosurfactant because it can help dissolve hydrophilic surfactants and drugs in an oil base and has good emulsifying properties (Ke et al., 2016) . This study aims to determine the most active fraction in providing activity, and the influence and optimal proportion of Oleic Acid, Tween 80, and Propylene Glycol on characterization.

LITERATURE REVIEW

Green Betel Leaf Activity

Betel (*Piper betle* L.) is generally a dioecious plant *including* a type of climbing or climbing plant with a soft stem (not woody) that leans on other tree trunks or grows on trellises that are deliberately made for its vines. This plant is widely planted in India, Bangladesh, Pakistan, Malaysia, Indonesia and several countries in Southeast Asia, to produce leaves used for chewing betel with areca nut, soda lime or gambir and its fruit with or without tobacco. *Piper betle* contains several compounds, namely 0.8–1.8% essential oil consisting of chavicol, chavibetol (phenolic betel) and allylpyrrocatechol (hydroxychavicol) (Rahmawati *et al*., 2020).

Based on previous studies, ethanol extract of betel leaves with a concentration of 1000 μ g/mL has strong antifungal activity against *Candida albicans* with an inhibition zone of 22 mm (Sivareddy *et al*., 2019). Other studies also show that ethanol extract and ethyl acetate fraction of betel leaves with a concentration of 1 mg/mL have strong antifungal activity against *Candida albicans* with inhibition zones of 21 mm and 24.33 mm, respectively (Phumat *et al*., 2017). This is because the highest levels of hydroxicavicol can be produced by extraction using semi-polar solvents (Zamakshshari *et al*., 2021) . Mature green betel leaves have a higher content than younger green betel leaves due to the maximum plant metabolism process (Sivareddy *et al* ., 2019) .

H1: The ethyl acetate fraction of betel leaves is able to provide the strongest antifungal activity against *Candida albicans.*

Self NanoEmulsifying Drug Delivery System

Self-nanoemulsifying Drug Delivery System (SNEDDS) is an isotropic mixture of natural or synthetic oils, surfactants and cosurfactants that have the unique ability to form fine oil-in-water (O/W) nano emulsions under mild agitation followed by the addition of aqueous media (Divate et al., 2021). The characterization of SNEDDS itself is a particle size with a value of 50-1000 nm, a polydispersity index of 0.3-0.7, a zeta potential of ≤ -30 or ≥ 30, and a drug loading of ≥ 50%.

The effect of oleic acid is that the higher the concentration of oleic acid, the emulsification time, particle size, polydispersity index, zeta potential and drug loading will increase. The effect of tween 80 is that the higher the concentration, the emulsification time, particle size, polydispersity index, and zeta potential will decrease, while the drug loading will be higher. The higher the propylene glycol, the emulsification time, particle size, polydispersity index, and zeta potential will decrease, while the drug loading will be higher (Kanwal *et al* ., 2019; Sulkhan *et al* ., 2019; Tungadi *et al* ., 2021). D-optimal Mixture is an optimization method used to determine the optimum formula for a mixture of materials with a proportion of the total amount of a different material must be 1 (100%). The materials or factors used in optimization consist of at least two different materials (Hidayat *et al*., 2020).

Making samples into the SNEDDS delivery system can increase activity due to the influence of the optimum formula drug loading percentage and changes in particle size to nano form and large particle surface contact area, thereby increasing the number of secondary metabolites that easily penetrate fungal cells (Anwer *et al* ., 2021; Luhurningtyas *et al* ., 2021; Zafar *et al* ., 2022).

METHODOLOGY

Tools and Materials

Tools used in study This includes: vessels maceration, blender (Philips), funnel separate, desiccator (chemical nitrate), scales analytical (Ohaus), Buchner funnel (NefMedSupply), evaporator (IKA RV10 Digital V), apparatus glass, ultrasonicator TUE-500, UV-Vis Spectrophotometer (Specord 200), micropipette (Drago Lab), Zetasizer Malvern (Zetasizer Nano -Zs 90, MPT-Z, UK).

The materials used in this study were betel leaf powder, 70% ethanol (Onemed), n-hexane (Merck), ethyl acetate (Merck), Oleic Acid (hblt), Tween 80 (Merck), Propylene Glycol, *Candida albicans* fungus , *paper disc* (Oxoid), Nystatin 100000 IU (Novell), *Follin ciocalteu reagent* (Merck), gallic acid (sigma-Aldrich), NaOH 1%, NaCl (Otsu), demineralized water (Brataco), SDA media.

The Course of Research

1. Preparation and Characterization of Powder

Betel leaves that have been determined and taken from the city of Salatiga, are sorted manually, washed using running water, drained, then dried using an oven at a temperature of 50 ^{0} C. The simplicia is then blended and then sieved using sieve number 40. The results in the form of dry powder are subjected to characterization tests, namely drying shrinkage tests, total ash content, acid-insoluble ash content, water-soluble essence content and ethanol-soluble essence content.

2. Preparation and Characterization of Extracts

Put 2 kg of dry betel leaf powder into the macerator, add 30 L of 70% ethanol. Soak for the first 6 hours while stirring then let stand for 18 hours. Separate the macerate by filtration. Repeat the extraction process at least once with the same type of solvent and the volume of solvent is half the volume of the solvent in the first extraction. Collect all the macerate then evaporate with a rotary evaporator until a thick extract is obtained.

3. Fractionation

10 Ethanol extract of betel leaves was fractionated using 75 ml of water, ethyl acetate, and n-hexane solvents. Fractionation was carried out 3 times and evaporated until thick.

4. Identification of Candida albicans

Identification of fungi consists of 3 tests, namely macroscopic tests, microscopic tests using lacto phenol catton blue, and biochemical tests using lactose, maltose, glucose, and sucrose media.

5. Antifungal activity test of betel leaf extract and fraction

Put 60 ml of SDA medium into a petri dish then scratch the inoculated test fungus evenly on the media. Then make a 6 mm well on the media using a cork borer then add ethanol extract, n-hexane fraction, ethyl acetate fraction, water fraction, negative control (DMSO) and positive control (Nystatin 100 IU) as much as 50 μL, incubate at 37ºC for 24 hours then observe the inhibition zone formed.

6. SNEDDS Formulation of Green Betel Leaf Fraction

The optimization stages of SNEDDS betel leaf fraction were carried out based on several Tables I and II:

Table I. SNEDDS Fraction Formula Leaf Betel

Formula	Concentration
Betel leaf fraction	1.6%
Oleic Acid	15% - 20%
Tweens 80	55% - 60%
Propylene Glycol	20% - 25%

Table II. D-Optimal Mixture Design

Oleic Acid (%)	Tween 80 (%)	Propylene Glycol (%)
15	60	25
19.17	57.92	22.92
19.17	59.17	21.67
16.67	59.17	24.17
17.5	57.5	25
17.5	57.5	25
20	55	25
15	60	25
17.5	60	22.5
19.17	56.67	24.17
20	60	20
17.92	59.17	22.92
20	55	25
20	60	20
20	57.5	22.5
20	57.5	22.5

The SNEDDS formula of betel leaf fraction begins with mixing Oleic Acid, Tween 80, and Propylene Glycol using a magnetic stirrer for 10 minutes at 300 rpm. After the SNEDDS is formed, the betel leaf fraction that has been dissolved with DMSO is added little by little until saturation is achieved (room temperature conditions 25 ± 1 ^{o}C). The mixture is centrifuged *at* 5000 rpm for 45 minutes. The SNEDDS drug supernatant results are stored in microtubes and protected from exposure to sunlight at room temperature. The supernatant results are characterized.

7. SNEDDS Characterization of Green Betel Leaf Fraction

Emulsification time characterization was carried out by dispersing one mL of SNEDDS rapidly in 100 mL of distilled water using *a magnetic stirrer* with a magnetic rotation of 100 rpm while calculating the time for the SNEDDS preparation to dissolve completely (forming a nanoemulsion). Characterization of particle size, polydispersity index and zeta potential using a Malvern

Zetasizer. Drug *loading* was carried out by measuring the total phenol content using a UV-Vis spectrophotometer with a gallic acid comparator at a wavelength of 649 nm.

8. Optimum Formula Determination and Verification

The determination of the optimum formula is carried out by analyzing the results of the particle size characterization test, zeta potential, polydispersity index, emulsification time, and drug *loading using the Design Expert Version* 12 application with D-Optimal Mixture where the characterization test data is subjected to analysis and must show significance.

9. Optimum Formula Antifungal Activity Test

Put 60 ml of SDA medium into a petri dish then scratch the inoculated test fungus evenly on the media. Then make a well in the media using a cork borer then insert the SNEDDS formula, negative control (SNEDDS base) and positive control (nystatin) as much as 50 mg, incubate at 37ºC for 24 hours then observe the inhibition zone formed.

Data Analysis

Analysis of activity test results using *Two Way Anova*, optimization of SNEDDS formula using *Design Expert* 12 software, and comparative analysis of optimum formula and ethyl acetate fraction using *Kruskal Walis*.

RESEARCH RESULT

Preparation and Characterization of Powder

11 kg of betel leaf plants were dried to obtain 2.5 kg of simplex with a yield of 22.73%. The results of the powder characterization can be seen in Table III:

Table III. Powder Characterization Results

Test Type	**Results (%)**	**Condition**	**Source**
Drying shrinkage	9.17±0.29	<10%	Indonesian Herbal Pharmacopoeia
Total ash content	5.04 ± 0.25	<14%	Indonesian Medical Material
Acid insoluble ash content	1 ± 0.05	<1.1%	Indonesian Herbal Pharmacopoeia
Water soluble content	22.7 ± 1.53	>20.8%	Indonesian Herbal Pharmacopoeia
Ethanol soluble extract content	26 ± 1	>17.6%	Indonesian Herbal Pharmacopoeia

Preparation and Characterization of Extracts

The extraction process using 2000 grams of green betel leaf powder and 30 L of 70% ethanol solvent for 2 days produced a thick extract of 497 grams, thus obtaining an extract yield of 24.85%, where the yield meets the requirements of

> 10% (Ministry of Health of the Republic of Indonesia, 2017) . The results of the extract characterization can be seen in Figure I and Table IV:

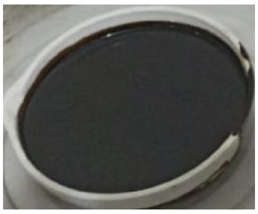

Figure II. Thick Extract of Mature Green Betel Leaves

Table IV. Extract Characterization Results

Test Type	Results (%)	Condition	Source
Water content	9.4 ± 0.3	<10%	Indonesian Herbal Pharmacopoeia
Total ash content	1.62 ± 0.15	<10%	Indonesian Medical Material
Acid insoluble ash content	0.098 ± 0.08	<0.1%	Indonesian Herbal Pharmacopoeia

Fractionation

The fractionation method used is liquid-liquid extraction with polar water solvents, semi-polar ethyl acetate, and non-polar n-hexane (Herdiana and Aji, 2020). The fraction yield can be seen in Table V:

Table V. Results yield faction

No	Types of Fractions	Yield (%)	SD
1	Water fraction	22.36	± 0.55
2	Ethyl acetate fraction	55.21	± 2.02
3	n-hexane fraction	3.48	± 0.61
	Total :	81.05	

Identification of Candida albicans

The macroscopic test results showed that the colonies on the solid medium were slightly protruding from the surface of the medium, were yellowish white in color, had a yeasty odor, and the surface of the colonies was wet. The test results can be seen in Figure II:

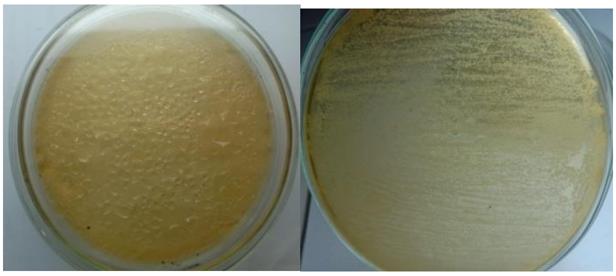

Figure II. Identification in a way macroscopic

The results of the microscopic test showed the presence of pseudohyphae and chlamydospores. The colonies were stained blue as in Figure III:

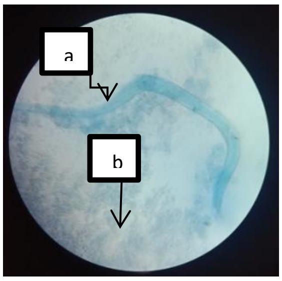

Figure III. *Pseudohyphae* (a), *Chlamydospores* (b)

Biochemical tests using sugar media, namely glucose, lactose, maltose, and sucrose filled with Durham tubes to see the ability of fungi to ferment sugar into acid and gas bubbles. The test results can be seen in Figure IV and Table VI:

Figure IV. Results of fermentation test

Table VI. Results identification fermentation

Media	Results
Lactose	F-/G-
Maltose	F+/G+
Sucrose	F+/G+
Glucose	F+/G+

Information :
F+ : Fermentation occurs
G+ : Formation of gas in the Durham tube
F- : no fermentation occurs
G-: no gas is formed in the Durham tube.

Antifungal Activity of Betel Leaf Extract and Fractions

Antifungal activity testing was carried out using the well diffusion method because it is easier to measure the area of inhibition zones formed because the fungus is active throughout the media. The results of the antifungal activity test of betel leaf extracts and fractions can be seen in Table VII:

Table VII. Results test activity antifungal extract and faction

Concentration	Group					
	control +	control -	ethanol extract	water fraction	ethyl acetate fraction	n-hexane fraction
0.4%	23	0	6.8	6.7	9.2	7.7
0.8%	23.3	0	7.5	7.2	11	9.2
1.6%	23.3	0	8.2	7.8	13.3	10.7

Characterization of SNEDDS

The D-optimal Mixture design shows 16 formula runs with various concentrations of Oleic Acid, Tween 80, Propylene Glycol with a total concentration of 100%. The eleven formulas from this software have different compositions with 5 formulas being replicated. This aims to minimize the possibility of errors that may occur. In this study, a thick, dark brown, and clear SNEDDS preparation was obtained. The results of the SNEDDS characterization test can be seen in Table VIII.

Table VIII. Results test SNEDDS characterization

AO:T80:PG	WE(s)	UP (nm)	IP	ZP (mV)	LO (%)
15:60:25	65	187,414	0.3	-30.6	73
19.17:57.92:22.92	80	204,573	0.46	-33.2	62
19,17:59,17:21,67	78	201,109	0.46	-33.1	66.5
16.67:59.17:24.17	68	188,025	0.39	-31.4	73.3
17.5:57.5:25	75	196,302	0.44	-32.7	69
17.5:57.5:25	76	197,823	0.43	-32.9	70.2
20:55:25	91	225,241	0.53	-39.8	51
15:60:25	66	186,139	0.33	-30.7	73.3
17.5:60:22.5	70	190,012	0.38	-31.6	82.5
19.17:56.67:24.17	82	206,891	0.46	-33.5	59
20:60:20	86	208,192	0.48	-35.4	56
17.92:59,17:22.92	73	194,501	0.4	-31.9	71.7
20:55:25	92	223,179	0.52	-39.2	50
20:60:20	85	210,047	0.47	-34.6	57.2
20:57,5:22,5	89	214,868	0.49	-37.7	54.1
20:57,5:22,5	90	215,179	0.5	-38.4	53.1

Note: AO : Oleic Acid
T80 : Tween 80
PG : Propylene Glycol

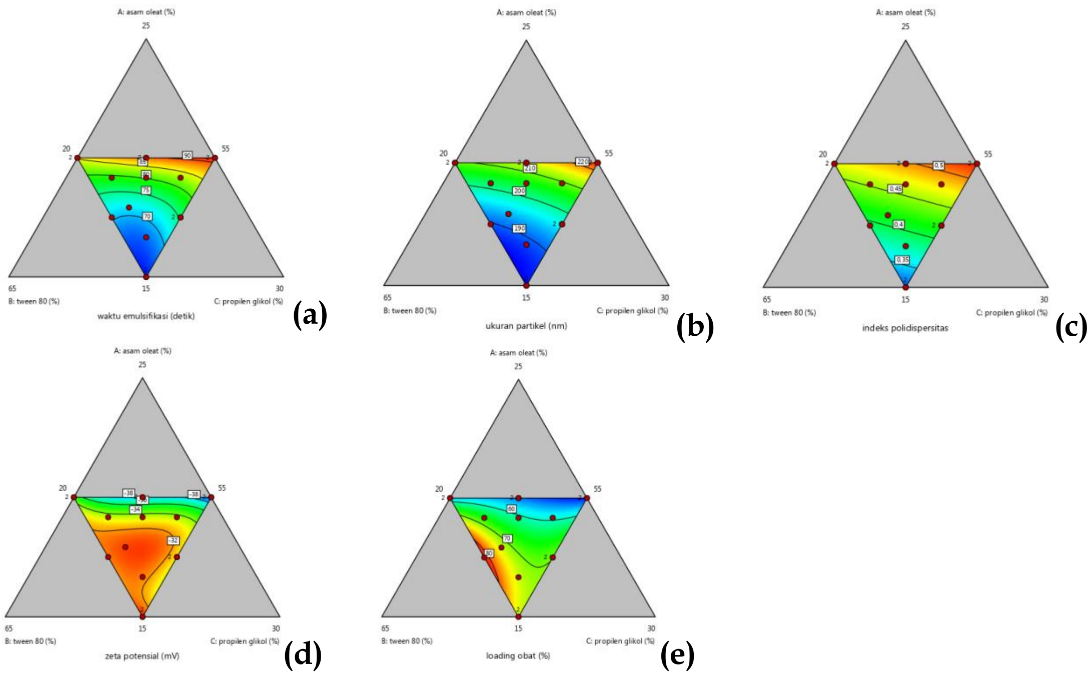

Figure V. Contour *plots of* emulsification time (a), particle size (b), polydispersity index (c), zeta potential (d), drug *loading (e)*

Determination of Optimum Formula and Verification

Optimization of SNEDDS formulation was carried out using numerical optimization techniques based on the desirability approach. The results of determining the optimum formula can be seen in Table IX:

Table IX. Results determination of optimum formula

AO	T 80	PG	WE	UP	IP	ZP	LO	Desirability	
16.1	58.9	25	68.9	188.66	0.361	-33	71.6	0.584	Selected

The optimum formula obtained was then re-verified. The verification results can be seen in Table X:

Table X. Verification of SNEDDS formula optimization data

Parameter	D-Optimal Mixture prediction results	Verification results
Emulsification time (s)	68.9	70
Particle size (nm)	188.66	186.98
Polydispersity index	0.361	0.331
Zeta potential (mV)	-33	-32.02
loading (%)	71.6	74.3

Optimum Formula Antifungal Activity

The optimum formula of SNEDDS betel leaf fraction was tested for antifungal activity to see the difference in activity before and after formulation. The results of the effectiveness test can be seen in Table XI:

Table XI. Results Test Activity Antifungal Preparation SNEDDS

Group	DDH (mm)
K (+)	23.7±0.58
K (-)	6.7±0.29
FO	21.3±0.58
EF [FO - K(-)]	14.6±0.76

Note: K (+) = Control (+)
K (-) = Control (-)
FO = Optimum Formula
EF = Fraction Encapsulation

DISCUSSION

Fractionation

The data in Table V above shows that there is 18.95% of the fractional yield lost. These data are in accordance with previous research by (Herdiana and Aji, 2020) where the highest yield is in the ethyl acetate fraction because the semi-polar nature causes the withdrawal of compounds from the extract to be greater because it is able to attract semi-polar compounds and a few polar and non-polar compounds such as phenolic compounds which are the largest content of green betel leaves, tannins, and flavonoids (Susanti et al., 2017) while the lowest yield is in the n-hexane fraction. This is because the amount of essential oil compounds which are non-polar compounds based on several studies is ± 0.8% -1.8%. While the yield of the water fraction is lower, it may be because the amount of polar compounds has been fractionated by the semi-polar ethyl acetate solvent (Herdiana and Aji, 2020; Rahmawati, Mujahid and Widiyastuti, 2020).

Identification of Candida albicans

Macroscopic and microscopic tests show that the fungus is *Candida albicans*. Based on Figure IV and Table VI, it can be seen that there is a color change from red to yellow in the fermentation reaction of sucrose, maltose, and glucose media. *Candida albicans* can ferment sucrose, maltose, and glucose media to produce acid with the help of *phenolic red 1% indicator. Candida albicans* can also produce gas bubbles in sucrose, maltose, and glucose media as evidenced by the presence of gas bubbles in the Durham media tube. This change is caused by a change in pH (Sulistyawati, Wiryosoendjojo and Puspawati, 2019). *Candida albicans* cannot form acid and gas in lactose media because there is no color change from red to yellow and no gas bubbles are formed in the Durham media tube. The results of this biochemical test are in accordance with previous research conducted by (Sulistyawati, Wiryosoendjojo and Puspawati, 2019).

Antifungal Activity of Betel Leaf Extract and Fractions

The data in table VII shows that all experimental groups experienced increased activity along with increasing concentration. The results of the two-way anova test showed a significant difference with a significant value of <0.05. Control + (Nystatin) has very strong activity as an antifungal with an inhibition zone of 23 mm. This is because the control + used is a synthetic compound that has been tested for its activity accurately and has been circulating on the market. Control + is the first choice in the treatment of oral candidiasis with a working mechanism that is to damage the *Candida albicans cell membrane* (DiPiro et al., 2017). Ethanol extract and water fractions have moderate activity as antifungals because they have DDH values between 5-10 mm. The ethyl acetate and n-hexane fractions have DDH values> 10 mm so they are considered to have strong activity as antifungals, but the ethyl acetate fraction shows a higher DDH value than the n-hexane fraction, which is 13 mm. The results of statistical analysis illustrate that there is a significant difference between the ethyl fraction and all other groups. This is because the active compound hydroxykavicol has semi-polar properties so that it will be more attracted to semi-polar solvents such as ethyl acetate. Hydroxykavicol works by changing the structure of the cell membrane which results in disruption of the permeability barrier of the membrane structure (Zamakshshari et al., 2021).

Characterization of SNEDDS

Emulsification time describes the lipid formulation spontaneously forming a fine dispersion when introduced into a medium with the condition of less than 1-2 minutes (Huda and Wahyuningsih, 2018; Tungadi et al., 2021) . The results of the emulsification time test in this study showed that the formula was successful in forming SNEDDS. The lower the emulsification time, the more it shows the desired SNEDDS characteristics so that drug absorption will increase (Tungadi, 2021). The results of the emulsification time test were analyzed using *software Design Expert* obtained the response results with the following equation $Y = 235.945A + 165.597B + 137.511C - 405.43AB - 437.52AC - 343.92BC$. The equation shows that the coefficient value of component A (Oleic Acid) is greater than components B (Tween 80) and C (Propylene Glycol), meaning that Oleic Acid has a greater effect on increasing emulsification time compared to Tween 80 and Propylene Glycol. Conversely, Tween 80 has a major effect on reducing emulsification time. This is due to the difference in oil and water phases which results in high surface tension, thus inhibiting water penetration in forming nanoemulsions spontaneously (Winarti et al . , 2018) . The coefficient value of component BC is greater than AB and AC, meaning that the combination of Tween 80 and Propylene Glycol has a major effect on increasing emulsification time. This is in line with previous research by (Camelia et al., 2021) showing that the use of Tween 80 and Propylene Glycol can significantly reduce emulsification time. The good emulsification ability of Propylene Glycol and Tween 80 causes excessive diffusion from the water phase into the oil phase, resulting in significant interface disruption (Nasr et al . , 2016). *The contour plot* above shows that the increase in emulsification time is due to the high concentration of Oleic Acid, while the low emulsification time is obtained from increasing the concentration of Tween 80 and Propylene Glycol. Tween 80 and Propylene Glycol have better emulsification and solubility capabilities, allowing rapid dispersion when in contact with biological fluids (Ke et al., 2016; Ujilestari *et al.*, 2018) . The use of nonionic surfactants with high HLB values will accelerate the formation of o/w nanoemulsions in polar media (Artanti *et al.*, 2022) .

The smaller the particle size, the more it shows the desired SNEDDS characteristics (Khan *et al.*, 2015; Kanwal *et al.*, 2019) . A good nanoemulsion preparation has a particle size of <1000 nm (Suyal *et al.*, 2023) . The particle size of all SNEDDS formulations shows that the formula has met the requirements as a nanoemulsion preparation. The results of the particle size test were analyzed using *software Design Expert* obtained the response results with the following equation Y = 306.5A + 184.186B + 208.67C-144.73AB-134.37AC-38.21BC. The equation shows that the coefficient value of component A (Oleic Acid) is greater than components B (Tween 80) and C (Propylene Glycol), meaning that Oleic Acid has a greater effect on increasing particle size compared to Tween 80 and Propylene Glycol. Conversely, Tween 80 reduces particle size. The coefficient value of component BC is greater than AB and AC, meaning that the combination of Tween 80 and propylene glycol has a major effect on increasing particle size. High concentrations of oil can cause phase separation, thereby increasing particle size and making the preparation less stable (Ke *et al.*, 2016). *The contour plot* above shows that the increase in particle size is due to the high concentration of Oleic Acid. Meanwhile, the low particle size is obtained from increasing the concentration of Tween 80 and Propylene Glycol which is caused by having a high dissolving ability (Tungadi et al., 2021) . The use of surfactants with high concentrations can also reduce interfacial tension, because the surfactant will cover the oil droplets when emulsified in the digestive tract so that the globules formed including in category *nano -sized* (Tungadi *et al.*, 2021) .

The polydispersity index is one of the parameters for testing nanoemulsion preparations that describes the degree of non-uniformity of particle size distribution. The polydispersity index value varies from 0.3-0.7 (Rasoanirina *et al.*, 2020). The polydispersity index value in the SNEDDS preparation of green betel leaf fraction indicates that the preparation has a homogeneous particle size distribution. The results of the polydispersity index test were analyzed using *software Design Expert* obtained the response results with the following equation Y = 0.674A + 0.262B + 0.373C. This equation shows that the coefficient value of component A (Oleic Acid) is greater than components B (Tween 80) and C (Propylene Glycol), meaning that Oleic Acid has a greater effect on increasing the polydispersity index than Tween 80 and Propylene Glycol. Tween 80 has a major effect on reducing the polydispersity index. This is because Tween 80 can reduce the interfacial tension between layers of oil in water so that it can produce nanoemulsions that have a very wide particle size distribution (Sapiun et al., 2023). This is in line with previous research by (Camelia *et al.*, 2021) showing that increasing the concentration of Tween 80 can reduce particle size and reduce the polydispersity index value significantly . Another study by (Shabrina and Khansa, 2022) showed that the use of Tween 80 can reduce particle size and reduce the polydispersity index value. *The contour plot* above shows that the increase in polydispersity index is due to the high concentration of Oleic Acid. While the low polydispersity index is obtained from the increase in the concentration of propylene glycol and Tween 80.

Zeta potential is also a very important factor in characterizing emulsification efficiency. The zeta potential value indicates the stability of a preparation. Zeta potential indicates the repulsive force between adjacent and similarly charged particles in a dispersion system. Preparations with low potential values show an attractive force that exceeds the repulsive force, causing the dispersion to break and flocculate. Conversely, colloidal systems with high zeta potential values (negative or positive) will be electrically stable (Kassem et al., 2016) . The results of the zeta potential test were analyzed using *software Design Expert* obtained the response results with the following equation Y = -147.519 (A) - 72.649 (B) - 93.701 (C) + 300.43AB + 324.48 AC + 210.21 BC. This equation shows that the coefficient value of component A (Oleic Acid) is smaller than components B (Tween 80) and C (Propylene Glycol), meaning that Oleic Acid has a greater effect on decreasing zeta potential while Tween 80 has an effect on increasing zeta potential. The combination of Tween 80 and Propylene Glycol has a major effect on increasing zeta potential. Oleic Acid has a side chain of fatty acid carboxylate groups so that it can reduce the zeta potential value (Kuncahyo *et al.*, 2023). *The contour plot* above shows that all factors affect zeta potential where the increase is due to the high concentration of Propylene Glycol and Tween 80. While the low zeta potential is obtained from increasing the concentration of Oleic Acid. The negative zeta potential value is due to the use of Tween 80 as a non-ionic surfactant which causes differential adsorption of hydrated hydroxyl ions (OH) and oxonium ions (H3O) (Kassem *et al.*, 2016). The electrostatic repulsion between negatively charged particles can prevent the occurrence of SNEDDS coalescence.

loading is a parameter that measures the ability of a preparation to load a drug or the level of drug contained in the preparation. The higher the drug level, the more effective the preparation is in achieving therapeutic doses because it requires less formulation. The results of the drug *loading* test are analyzed using *software Design Expert* obtained the response results with the following equation Y = -25.97A + 251.66B + 177.01C - 328.83AB - 564.62AC. This equation shows that the coefficient value of component B (Tween 80) is greater than components A (Oleic Acid) and C (Propylene Glycol), meaning that Tween 80 has a greater effect on increasing drug *loading* than Propylene Glycol and Oleic Acid. Conversely, Oleic Acid has an effect on decreasing drug *loading* . The combination of acid and Tween 80 has an effect on increasing drug *loading* . Tween 80 and Propylene Glycol are less than optimal in helping to dissolve Oleic Acid if used in large quantities so that drug *loading* decreases with increasing Oleic Acid (Nandita *et al.*, 2021). *The contour plot* above shows that all factors affect drug *loading* where the increase is due to the high concentration of Propylene Glycol and Tween 80. Low drug loading is obtained from increasing the concentration of Oleic Acid. Tween 80 and Propylene Glycol are able to help Oleic Acid dissolve the drug (Nandita *et al.*, 2021; Kuncahyo *et al.*, 2023) .

Optimum Formula Antifungal Activity

Table XI shows that control + has antifungal activity with a higher DDH value than the optimum formula. Control + used is a nytatin drop preparation that has been circulating in the market. Nystatin is a synthetic compound derived from polyenate that works by damaging the fungal cell membrane. This drug has proven antifungal activity and is the first choice drug in treating oral candidiasis (Mansourian et al., 2014; DIPIRO et al., 2017) . Control - has moderate antifungal activity with a DDH range of 5-10 mm. This is because control - which is the basis of the preparation consists of Oleic Acid, Tween 80, and Propylene Glycol where Oleic Acid and Propylene Glycol have antibacterial and antifungal properties against *Candida albicans.* Propylene Glycol is also a preservative that is often used in pharmaceutical formulations (Rowe *et al*., 2009; SINGH *et al*., 2018; Muthamil *et al*., 2020). The optimum formula provides very strong activity as an antifungal with an average DDH value of 21.3 mm so that the pure activity of the encapsulated fraction is 14.6 mm. The betel leaf fraction experienced an increase in activity after the preparation was encapsulated as evidenced by an increase in DDH of 1.3 mm. The results of the comparative analysis test of the activity of the ethyl acetate fraction before and after being formulated using *the Kruskal Wallis test* showed a significant value of <0.05, which means that there was a significant increase in activity. The increase in activity is due to the high percentage of optimum formula drug loading , which is 74.3%, and the change in particle size to nano form and the large surface contact area of the particles, thereby increasing the number of secondary metabolites that easily penetrate fungal cells (Anwer et al., 2021; Luhurningtyas et al., 2021; Zafar *et al*., 2022).

CONCLUSION AND RECOMMENDATIONS

Ethyl acetate fraction of betel leaf has the strongest activity as an antifungal against *Candida albicans* with a DDH value of 13.3 mm. Based on the results of the study, the higher the concentration of Oleic Acid, the emulsification time, particle size, polydispersity index, zeta potential will increase and drug *loading* will decrease, while the higher the concentration of Tween 80 and Propylene Glycol, the emulsification time, particle size, polydispersity index, and zeta potential will decrease, while drug *loading* will increase. The optimum proportion of Oleic Acid, Tween 80, Propylene Glycol is at a ratio of 16.1:58.9:25 with an emulsification time of 70 seconds, a particle size of 186.98 nm, a polydispersity index of 0.331, a zeta potential of -32.02, and a drug *loading* of 74.3%. The optimum formula of SNEDDS betel leaf fraction can significantly increase antifungal activity compared to the ethyl acetate fraction of betel leaf.

ADVANCED RESEARCH

It is necessary to conduct research development by formulating SNEDDS betel leaf fraction into topical preparations to increase the delivery of active substances and accelerate the therapeutic effect.

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REFERENCES

Anwer, MK, Iqbal, M., Aldawsari, MF, Alalaiwe, A., Ahmed, MM, Muharram, MM, Ezzeldin, E., Mahmoud, MA, Imam, F., & Ali, R. (2021). Improved antimicrobial activity and oral bioavailability of delafloxacin by self-nanoemulsifying drug delivery system (SNEDDS). *Journal of Drug Delivery Science and Technology*, *64* (2457), 102572. https://doi.org/10.1016/j.jddst.2021.102572

Artanti, AN, Prihapsara, F., & Rohmani, S. (2022). Cytotoxicity Effect of Self-Nanoemulsifying Drug Delivery System from Chloroform Extract of Bay Leaf (Syzygium Polyanthum (Wight) Walp.) with Oleic Acid as a Carrier. *Indonesian Journal of Cancer Chemoprevention*, *13* (2), 94. https://doi.org/10.14499/indonesianjcanchemoprev13iss2pp94-103

Bakhtiari, S., Jafari, S., Taheri, JB, Kashi, TSJ, Namazi, Z., Iman, M., & Poorberafeyi, M. (2019). The effects of cinnamaldehyde (Cinnamon derivatives) and nystatin on candida albicans and candida glabrata. *Open Access Macedonian Journal of Medical Sciences*, *7* (7), 1067–1070. https://doi.org/10.3889/oamjms.2019.245

Camelia, FD, Nurahmanto, D. and Wisudiyaningsih, B. (2021) 'Optimization of Tween and Propylene Glycol in Self-Nanoemulsifying Drug Delivery System of VCO-Basil Leaf Oil', *e-Journal Pustaka Kesehatan*, 9(3), pp. 158–165.

DIPIRO, J.T., DIPIRO, CV, Wells, B.G., & Schwinghammer, T.L. (2017). *Pharmacotherapy Handbook* (10th ed.). McGraw-Hill Education.

Divate, M. P., Bawkar, S. U., Chakole, R. D., & Charde, M. S. (2021). Self Nano-Emulsifying Drug Delivery System: a Review. *Journal of Advanced Scientific Research* , *12* (03 Suppl 2), 1–12. https://doi.org/10.55218/jasr.s2202112301

Hidayati, R., Saptarini, O., & Kuncahyo, I. (2022). Optimization of HPMC K15M, Carbopol 940, and Propylene Glycol in the Naringenin Nanoemulgel Formula D-Optimal Mixture Design Method Optimization of HPMC K15M, Carbopol 940, and Propylene Glycol in the Naringenin Nanoemulgel Formula D-Optimal Mixture Design Metho. *Indonesian Journal of Pharmacy* , *19* (2), 236–245.

Herdiana, I., & Aji, N. (2020). Fractionation of Betel Leaf Extract and Gambir Extract and Antibacterial Test of Streptococcus mutans. *Scientific Journal of Health* , *19* (03), 100–106. https://doi.org/10.33221/jikes.v19i03.580

Kanwal, T., Kawish, M., Maharjan, R., Ghaffar, I., Ali, HS, Imran, M., Perveen, S., Saifullah, S., Simjee, SU, & Shah, MR (2019). Design and development of permeation enhancer containing self-nanoemulsifying drug delivery system (SNEDDS) for ceftriaxone sodium improved oral pharmacokinetics. *Journal of Molecular Liquids*, *289*, 111098. https://doi.org/10.1016/j.molliq.2019.111098

Kassem, AA, Mohsen, AM, Ahmed, RS, & Essam, TM (2016). Self-nanoemulsifying drug delivery system (SNEDDS) with enhanced solubilization of nystatin for treatment of oral candidiasis: Design, optimization, in vitro and in vivo evaluation. *Journal of Molecular Liquids*, *218* , 219–232. https://doi.org/10.1016/j.molliq.2016.02.081

Ke, Z., Hou, X., & Jia, X. bin. (2016). Design and optimization of self-nanoemulsifying drug delivery systems for improved bioavailability of cyclovirobuxine D. *Drug Design, Development and Therapy*, *10*, 2049–2060. https://doi.org/10.2147/DDDT.S106356

Ministry of Health of the Republic of Indonesia. (2017). *Indonesian Herbal*

Pharmacopoeia, Second Edition (II).

Khan, A.W., Kotta, S., Ansari, S.H., Sharma, R.K., & Ali, J. (2015). Self-nanoemulsifying drug delivery system (SNEDDS) of the poorly water-soluble grapefruit flavonoid Naringenin: Design, characterization, in vitro and in vivo evaluation. *Drug Delivery*, *22* (4), 552–561. https://doi.org/10.3109/10717544.2013.878003

Kuncahyo, I., Choiri, S., Fudholi, A., Martien, R., & Rohman, A. (2023). Development of pitavastatin-loaded super-saturated self-nano emulsion: a continued screening and optimization approach using statistical techniques. *Journal of Dispersion Science and Technology*, *44* (4), 608–617. https://doi.org/10.1080/01932691.2021.1957922

Luhurningtyas, FP, Vifta, RL, Pradana, A., & Tatengkeng, Y. (2021). *Journal of Scientific Farmako Bahari THE ACTIVITY ASSAY OF SURI CUCUMBER SEED NANOPARTICLES AS ANTIMICROBIAL AGAINST CANDIDA ALBICANS AND STREPTOCOCCUS MUTANS ARTICLE HISTORY* . 107–116.

Nandita, SP, Kuncahyo, I., & Harjanti, R. (2021). Formulation and Optimization of Furosemide Snedds With Variation Concentration of Tween 80 and PEG 400. *Journal of Fundamental and Applied Pharmaceutical Science* , *2* (1), 34–42. https://doi.org/10.18196/jfaps.v2i1.12180

Nasr, A., Gardouh, A., Ghonaim, H., Abdelghany, E., & Ghorab, M. (2016). Effect of oils, surfactants and cosurfactants on phase behavior and physicochemical properties of self-nanoemulsifying drug delivery system (Snedds) for irbesartan and olmesartan. *International Journal of Applied Pharmaceutics* , *8* (1), 13–24.

Phumat, P., Khongkhunthian, S., Wanachantararak, P., & Okonogi, S. (2017). Potential of *Piper betle* extracts on inhibition of oral pathogens. *Drug Discoveries & Therapeutics*, *11* (6), 307–315. https://doi.org/10.5582/ddt.2017.01061

Rahmawati, N., Mujahid, R., & Widiyastuti, Y. (2020). Cultivation and Benefits of Betel for Health. In *Center for Research and Development of Medicinal Plants and Traditional Medicines* .

Rasoanirina, BNV, Lassoued, MA, Miladi, K., Razafindrakoto, Z., Chaâbane-Banaoues, R., Ramanitrahasimbola, D., Cornet, M., & Sfar, S. (2020). Self-nanoemulsifying drug delivery system to improve transcorneal permeability of voriconazole: in-vivo studies. *Journal of Pharmacy and Pharmacology* , *72* (7), 889–896. https://doi.org/10.1111/jphp.13265

Rowe, R.C., Sheskey, P.J., & Quinn, M.E. (2009). *Hanbook Of Pharmaceutical Excipients Sixth Edition* (RC Rowe, PJ Sheskey, & ME Quinn (eds.); 6th ed.).

Rusminingsih, E., Susanto, H., Afifah, D.N., Martien, R., & Anas, Y. (2023). Improving the Performance of Self Nanoemulsifying Drug Delivery System of Moringa Leaves (Moringa oleifera Lam) Using Ultrasonic Emulsification Method. *PHARMACY: Jurnal Farmasi Indonesia (Pharmaceutical Journal of Indonesia)*, *1*, 1–7.

Sapiun, Z., Imran, AK, Dewi, STR, Pade, DFM, Ibrahim, W., Tungadi, R., Abdulkadir, WS, Banne, Y., Sartini, S., Permana, AD, Rifai, Y., Ysrafil, Y., & Slamet, NS (2023). Formulation and Characterization of Self Nano-

Emulsifying Drug Delivery System (Snedds) Fraction of N-Hexane: Ethyl Acetate From Sesewanua Leaf (Clerodendrum Fragrans Wild.). *International Journal of Applied Pharmaceutics*, *15* (2), 72–77. https://doi.org/10.22159/ijap.2023v15i2.46365

Sivareddy, B., Reginald, B.A., Sireesha, D., Samatha, M., Reddy, K.H., & Subrahamanyam, G. (2019). Antifungal activity of solvent extracts of Piper betle and Ocimum sanctum Linn on Candida albicans: An in vitro comparative study. *Journal of Oral and Maxillofacial Pathology* , *23* (3), 333–337. https://doi.org/10.4103/jomfp.JOMFP_167_19

Sulistyawati, D., Wiryosoendjojo, K., & Puspawati, N. (2019). Antifungal Activity Test of Ethanol Extract of Berenuk Leaves and Fruit Flesh (Crescentia cujete, Linn.) against Candida albicans ATCC 1023. *Biomedicine* , *12* (2), 217–227. https://doi.org/10.31001/biomedicine.v12i2.616

Sulkhan, AAR, Artanti, AN, Ermawati, DE, & Prihapsara, F. (2019). Optimization of Self-Nanoemulsifying Drug Delivery System (SNEDDS) of Annona muricata L. leaves chloroform extract using VCO (Virgin Coconut Oil) as an oil phase. *IOP Conference Series: Materials Science and Engineering* , *578* (1). https://doi.org/10.1088/1757-899X/578/1/012046

Susanti, NMP, Dewi, LPMK, Manurung, HS, & Wirasuta, IMAG (2017). Identification of Phenol Compounds in Green Piper betle Leaf Ethanol Extract by TLC-Spectrophotodensitometry. *Jurnal Metamorfosa* , *4* (1), 108–113.

Suyal, J., Kumar, B. and Jakhmola, V. (2023) 'Novel Approach Self Nanoemulsifying Drug Delivery System: A Review', *Advances in Pharmacology and Pharmacy*, 11(2), pp. 131–139. doi:10.13189/app.2023.110205.

Tagrida, M. *et al.* (2021) 'Liposomes loaded with betel leaf (Piper betle L.) ethanolic extract prepared by thin film hydration and ethanol injection methods: Characteristics and antioxidant activities', *Journal of Food Biochemistry* , 45(12). doi:10.1111/jfbc.14012.

Tungadi, R., Thomas, N.A., & Gobel, W.G. Van. (2021). Formulation, Characterization, and Evaluation of Drops Liquid Self Nano-Emulsifying Drug Delivery System (SNEDDS) Astaxanthin. *Indonesian Journal of Pharmaceutical Education*, *1* (3), 168–178. https://doi.org/10.37311/ijpe.v1i3.11400