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Original Article

OPTIMIZATION OF SELF NANO-EMULSIFYING DRUG DELIVERY SYSTEM (SNEDDS) FORMULA OF COMBINED 70% ETHANOLIC EXTRACT OF BENALU BATU (*BEGONIA MEDICINALIS*) HERBS AND KELOR (*MORINGA OLEIFERA* L.) LEAVES USING *SIMPLEX LATTICE DESIGN* METHOD

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ABSTRACT

Objective: This research aims to perform Self Nano-Emulsifying Drug Delivery System (SNEDDS) formulation of combined ethanolic extract of benalu batu (*Begonia medicinalis*) herbs and kelor (*Moringa oleifera*) leaves, determine the optimal concentration based on physicochemical characteristics as well as the phytochemical contents and *in vitro* anticancer activity.

Methods: Surfactant and co-surfactant concentrations were determined by Design Expert v.13 software with Simplex Lattice Design (SLD) method. The phytochemical contents were measured by using a UV-Vis spectrophotometer, and the inhibition activity on HeLa cancer cells was tested by using the MTT method.

Results: Design Expert with the SLD method produces five design formulas. The most optimal SNEDDS formula based on the SLD method was formula 5, which contains a combination of extract of benalu batu herbs and kelor leaves with a concentration ratio of 1:1 (100 mg: 100 mg), 12% Virgin Coconut Oil (VCO), 64% tween 80, and 16% propylene glycol. The optimal formula has the characteristics of an emulsification time of 39.30±3.055 seconds, a transmittance percentage of 92.25%±0.004, a particle size of 14.43 nm±0.306 with a Polydispersity Index (PI) of 0.237, pH of 4.70±0.031 and viscosity of 355 cps±2.6. It also contains a total phenolic content of 5.517±0.382 mg/g GAE, total flavonoids of 8.501±0.695 mg/g QE, and total saponins of 17.991±0.052 mg/g EE. In addition, it also possesses a high percentage of cell death of Hela cancer, which is 84.334% at a concentration of 200 µg/ml.

Conclusion: Formula 5 has the potential for anticancer activity with good characteristics as SNEDDS formula.

Keywords: *Begonia medicinalis*, *Moringa oleifera*, SNEDDS, *Simplex lattice design*, HeLa

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INTRODUCTION

Benalu batu (*Begonia medicinalis*) and kelor (*Moringa oleifera*) are popular plants in Central Sulawesi for traditional medicine. *B. medicinalis* is one of the plants widely used by the Wana tribe in North Morowali and is empirically used to treat various diseases such as cancer, asthma, and gout [1]. It was reported to contain 2-O-β *glycopyranosil cucurbitacin* D, a cucurbitacin-type saponin compound, with cytotoxic activity against HCT-116 and MCF-7. Its methanol extract also has a high cytotoxic effect on cervical cancer cells (HeLa) [2, 3]. *Moringa oleifera* L. is one of the plants cultivated for medicinal purposes. Moringa plants have been reported to have anti-inflammatory, antioxidant, antiulcer, anticancer, antihyperlipidemia, antidiabetic, antiasma, hepatoprotective, and antihypertensive properties. Most of these reported bioactivities were attributed to the presence of flavonoids as bioactive compounds [4, 5]. The combination of these plants is expected to have a synergetic effect on particular diseases. Our previous study showed that the combination of 70% ethanol extract of *B. medicinalis* and *M. oleifera* has an effect as an immunomodulator with the parameter of macrophage phagocytosis activity and induction of IFN-α and TNF-γ cytokines in Wistar male white rats (*Rattus norvegicus*). The highest activity was found at a combination dose of 100:100 mg/Kg BW (1:1) [6]. However, the low solubility of these combined extracts has been considered for its development as a pharmaceutical preparation.

The rationale for developing medication technology consists of three main factors: creating an effective system, reducing toxic effects during application, and accepting the system by patients. The Biopharmaceutical Classification System (BCS) is one of the most widely applied scientific classification systems of drug substances based on their permeability and solubility. Two important factors govern the speed and scope of oral drug absorption: water solubility and intestinal permeability. The solubility, dissolution, and permeability of drugs or active substances in the gastrointestinal tract are important parameters that control drug absorption and bioavailability. The aqueous solubility of drugs is important for oral delivery. It is related to the design and development process of drug preparation. The solubility of the drug will correlate with the absorption rate of the drug to be absorbed and produce therapeutic effects. Drugs or active substances with low solubility will be bound to plasma proteins, rapidly distributed and metabolized by the liver [7].

In contrast, active substances with high solubility will be limitedly distributed and metabolized by the kidneys. Therefore, solubility plays a vital role in the pharmacokinetic phase. According to BCS, one of the techniques to increase the solubility of active substances is to modify the physical drug delivery systems where particle size is reduced [8]. One of them is nanoparticles. Nanoparticle distribution is a formulation of dispersed particles in nanometres or thousandths of a micron. This ability of nanoparticles usually increases the bioavailability of poorly soluble drugs in the systemic circulation, such as quercetin and repaglinide [9, 10].

Self Nano-Emulsifying Drug Delivery System (SNEDDS) is commonly used in the development of natural material preparation formulations [11]. SNEDDS is an oil or fat nanoparticle-based formulation composed of an anisotropic mixture of oil, surfactant, and co-surfactant phases, which, when in contact with gastric juices, will spontaneously form a nano-emulsion [12]. The development of SNEDDS following trials is very time-consuming and in effective cost. This approach combines various excipients (factors) in different ratios. Therefore, a suitable experimental design is required to optimize the SNEDDS formulation [13]. Design Expert, a statistical method software, can be used for formula optimization and also to interpret the factors involved in the experiment. One of the standard mixture designs that is widely used for optimization is Simplex Lattice Design (SLD) [14, 15]. This first study used design

expert software with the SLD method to optimize the SNEDDS formula of combined ethanol extract of *B. medicinalis* and *M. oleifera.* The evaluation of total phenolic, total flavonoids, total saponins, and *in vitro* anticancer activity was also reported.

MATERIALS AND METHODS

Materials

Begonia medicinalis was obtained from Toddopuli, Soyojaya District, North Morowali, and *Moringa oleifera* was obtained from Sibedi Village, Sigi Regency. Plants were deposited and identified by Ramadanil (Professor in Botany) at the Laboratory of Plant Biosystematic, Department of Biology, Faculty of Mathematics and Natural Sciences, Tadulako University, with the specimen number BM12010624 and MO13010624, respectively. Ethanol (pro analysis), tween 80, propylene glycol, gallic acid, folin-ciocalteau reagent, quercetin, escin, vanillin, sulfuric acid, aluminium chloride ($AlCl_3$), potassium acetate, and sodium carbonate were purchased from Sigma Aldrich. Distilled water and Virgin Coconut Oil (VCO) from a local store in Palu, Central Sulawesi.

Extraction

B. medicinalis (leaves and stem) and *M. oleifera* (leaves) were put into a vessel and dissolved with 70% ethanol solvent for three days in a room protected from light and occasionally stirring, then filtered using a flannel filter. Then, re-maceration was carried out up to 4 times. The liquid extracts obtained were then collected and evaporated using a rotary evaporator at a temperature of 40–45 °C.

Solubility test in oil, surfactants, and co-surfactants

The test was conducted by mixing 100 mg of *B. medicinalis* herb extract and 100 mg of *M. oleifera* leaf extracts, then mixed into the carrier (*corn* oil, *olive oil, virgin* coconut oil, tween 80, propylene glycol) and stirred using a magnetic stirrer for 10 min.

Determination of formula concentration using design expert

Determination of formula concentration was carried out using Design Expert software using the Simple Lattice Design (SLD) method. The low and high values of each component were determined based on the literature and entered into SLD [15, 16].

SNEDDS formulation

Ethanol extracts of *B. medicinalis* herb and *M. oleifera* leaf were each weighed 100 mg and dissolved with distilled water. After that, the extract solution was mixed with VCO and stirred with a magnetic stirrer at 500 rpm for 15 min (Mixture A). Propylene glycol was then added to mixture A while stirring with a magnetic stirrer for 15 min at 500 rpm (Mixture B). After that, mixture B was dripped with Tween 80 while stirring with a magnetic stirrer at 500 rpm for 45 min until the mixture became homogeneous. After that, the formula sample was sonicated for 45 min.

Emulsification time test

To determine the self-emulsification time, the formula was mixed with 900 ml of Simulated Gastric Fluid (SGF) (pH 1.2) and continuously stirred in the dissolution tester. The stirring speed was kept constant at 50 rpm, and the temperature was maintained at 37±0.5 °C. The self-emulsification time was visually observed [17].

Percentage of transmittance test

The percentage of transmittance test was carried out by observing the clarity of the emulsion formed at the previous stage using a UV-Vis spectrophotometer by measuring the absorption at a wavelength of 650 nm. If the percentage result of the sample is close to the percentage of distilled water, which is 100%, it can be assumed that the nano-emulsion droplet size has been nano-sized [18, 19]. The SNEDDS formula was taken as much as 1 ml and then added with aquabidest to a volume of 50 ml. The mixture was stirred with a magnetic stirrer for 1 minute and then measured using a UV-Vis spectrophotometer [20].

Particle size analysis (PSA)

PSA measurements were carried out to determine the size and distribution of sample particles using the Horiba Scientific SZ 100 test equipment. A total of 1.0 ml of formula sample was taken to be measured on the PSA device [21].

Determination of the optimal formula

Design Expert software was used to determine the optimum SNEDDS formula. The effect of oil, surfactant, and co-surfactant were evaluated on the response, and the results were statistically significant if p-value<0.05. Verification between predicted and observed values was performed by using simplex lattice design with analytical software, and there was no statistically significant difference if *p-value*>0.05 [20].

pH measurement

The pH measurement of each formula was carried out using a pH meter. The pH meter electrode was inserted into 10 ml of SNEDDS formula, and then the number shown by the pH meter was recorded.

Viscosity test

Viscosity testing was conducted using a viscometer (Brookfield, USA). The samples of the SNEDDS formula were taken as much as 50 ml. The SNEDDS sample was put into a beaker, and the sample must be ensured to be free of bubbles and spread evenly on the surface of the beaker. Next, the beaker containing the sample was placed on the viscometer, and then the viscometer was turned on and set at a speed of 100 rpm. Finally, the viscosity value was recorded.

Determination of total phytochemical compounds in SNEDDS formula sample preparations

Total phenolics

About 1 ml of each formula was taken, and 0.4 ml of Folin-Ciocalteu reagent was added. Then, shaken until homogeneous and allowed to stand for 4-8 min. After that, 4 ml of a 7% Na_2CO_3 solution was added, shaken until homogeneous, and aquabidest to a volume of 10 ml. After that, it was left for 2 h at room temperature. Next, the absorbance of each sample was measured using a UV-Vis spectrophotometer [6]. The experiment was performed in triplicate

Total flavonoids

Each formula was taken as much as 1 ml, then mixed with 0.2 ml 10% $AlCl_3$, 0.2 ml 1M potassium acetate, and 5.6 ml aquabidest. The mixture was incubated for 30 min at room temperature. After that, the absorbance of each sample was measured using a UV-Vis spectrophotometer. The experiment was performed in triplicate [6].

Total saponin

About 25 µl** of each formula was taken (also pipetted ethanol as a blank) and mixed with 250 µl** of vanillin (8 g/100 ml ethanol). Then, 2.5 ml of sulfuric acid (72%) was added. After that, the mixture was heated at 60 °C for 15 min. Then, it was put in cold water for 5 min, and the absorbance of each sample was measured using a UV-Vis spectrophotometer. The experiment was performed in triplicate [6].

Anticancer test of SNEDDS formula with micro tetrazolium (MTT) method

Anticancer testing of the SNEDDS formula and combined extracts (1:1) was carried out by propagating HeLa cells first. After which, 10 mg of extracts and formula were weighed and dissolved in 100 µl of Dimethyl Sulfoxide (DMSO). After that, dilutions were made using a culture medium to make extract solutions with concentrations of 250 µg/ml. Cells with a concentration of $1x10^4$ cells/µl were distributed into wells (96-well plate) with 100 µl in each well and incubated for 24 h in a 5% CO_2 incubator. After incubation, the remaining media in the plate was removed, and 100 µl of the test extract solution and formula were added to each well.

Furthermore, it was incubated again in a 5% CO_2 incubator for 24 h. As a positive control, a culture medium containing only cancer cells without the addition of the extract solution was used. At the end of incubation, the culture medium was removed from the wells. In each well, 100 µl of culture medium and 10 µl of MTT solution (5 mg/ml) were added. Cells were re-incubated for 4 h in a 5% CO_2 incubator. Living cells will react with MTT to form formazan crystals that are

dark purple-blue. The MTT reaction was stopped with 100 µl of SDS solution (10% in 0.01N HCl). The plate was shaken on a shaker for 10 min and then incubated at room temperature in a dark room for one night. The absorbance of each well was read with an ELISA reader at a wavelength of 595 nm [2].

RESULTS AND DISCUSSION

Solubility test

The solubility test of the combined extracts from *B. medicinalis* herb and *M. oleifera* leaves yields visually striking results, highlighting a distinct preference for solubility within VCO. This preference becomes evident as the extracts effortlessly dissolve in VCO under 5 min. In contrast, when introduced to olive oil and corn oil, a noticeable disparity emerges, with the extracts showing reluctance to dissolve. This reluctance is discernible by the presence of residual extract sediment settling at the bottom of the vessel after agitation. These findings seamlessly align with prior research that reinforces VCO as the optimal solvent for achieving maximum drug solubility among the scrutinized oil samples [22].

The outcomes of the extract solubility test involving tween 80 and propylene glycol underscored the extract's swift dissolution within the surfactant and co-surfactant matrix. Evidently, the extract seamlessly dissolved in both agents in under 5 min, and crucially, no residue accumulated upon amalgamation with the specified surfactant and co-surfactant.

The selection of VCO as an oil phase, tween 80, and propylene glycol in the SNEDDS formula, apart from being based on the solubility test results, also contains fatty acids. The dominant fatty acid in VCO is lauric acid (>47%), which is a Medium-Chain Fatty Acid (MCFA). A high percentage of MCFA in the oil phase is preferred for nano-emulsion formulations to achieve stability [20]. VCO is widely chosen as the oil phase in nano-emulsion formulations because it is easier to emulsify and can produce nanometer-sized preparations. In addition, VCO is also safe for oral consumption [23]. Meanwhile, tween 80 is chosen as a nonionic surfactant based on its properties, which are less affected by pH changes in ionic strength and are generally considered safe and biocompatible [24]. Tween 80 forms a larger self-emulsifying area with long-chain (ethyl oleate) and medium-chain (miglyol812) oils [25]. The interaction between olive oil, tween 80, and propylene glycol with the highest value is propylene glycol with a value of-4513.94, meaning that the concentration of propylene glycol can reduce the transmittance value [26]. The higher concentration of surfactants can reduce the particle size. Propylene glycol helps surfactants reduce the surface tension between oil and water, therefore reducing the particle size. The upper and lower limits of each oil component, surfactant, and co-surfactant were obtained based on the literature stating that the selection of oils, surfactants, and co-surfactants that showed maximum solubility were selected as independent variables with the levels selected for optimization, namely VCO at 12%, Tween at 80-60%, and propylene glycol at 12%-22% [20]. Determination of formula concentration using Design Expert version 13, using SLD method, showed five formulas with varying concentrations of surfactants and co-surfactant. The five formulas recommended by the software are different without any replicated formulas to reduce the possibility of errors (table 1).

Table 1: Composition of SNEDDS formula

Material	Formula				
	F1	F2	F3	F4	F5
B. medicinalis (mg)	100	100	100	100	100
M. oleifera (mg)	100	100	100	100	100
Aquadest (ml)	2	2	2	2	2
VCO (ml)	7.2	7.2	7.2	7.2	7.2
Tween 80 (ml)*	40.8	39.6	37.2	36.0	38.4
Propylene glycol (ml)*	7.2	8.4	10.8	12	9.6

*Recommended by design expert

Characterization of SNEDDS formula

The characterization of SNEDDS formulas can be seen in table 2. Consistently, across all formulas, our observations yield highly favorable results, with emulsification occurring in less than one minute. This assessment of emulsification time provides valuable insights into the seamless formation of SNEDDS within the body. This swift emulsification process is made possible by the synergistic action of surfactants and co-surfactants, which work in harmony to establish an oil-water interface layer rapidly. The inclusion of co-surfactants plays a pivotal role in reducing droplet size and subsequently shortening emulsification time. These co-surfactants create interstitial spaces between surfactant molecules, imparting a lightweight and fluid-structure characterized by enhanced fluidity, thus expediting the formation of nano-emulsions [19].

Table 2: Characterization of SNEDDS formula

Formula	Emulsification time (min)	Transmittance (%)	Particle size (nm)	Polydispersity index
F1	32.00±2.00	91.20±0.001	26.30±1.25	0.577±0.02
F2	38.00±2.00	91.20±0.001	13.43±0.40	0.166±0.07
F3	44.00±2.00	90.99±0.002	15.16±0.29	0.195±0.06
F4	57.60±1.50	92.04±0.003	13.90±0.46	0.193±0.07
F5	39.30±3.10	92.25±0.004	14.43±0.31	0.237±0.04

Data were given as mean±SD, n=3

To achieve optimal results, it is essential that the transmittance value reaches or closely approaches 100%. However, a transmittance value exceeding 80% remains acceptable, indicating the adequacy for classifying the emulsion as nano-sized within the oil-in-water (o/w) phase. The dimensions of the dispersed phase inherently influence the visual characteristics of nano-emulsions. When the nano-emulsion system features minimal globule dimensions, it can effortlessly allow the passage of a light beam without obstruction. This unobstructed flow of the light beam imparts a transparent appearance to the solution, resulting in elevated transmittance values [19, 27, 28]. Clarity, measured as a percentage of transmittance serves as a critical control parameter for the formation of SNEDDS. Visual assessment of clarity provides a qualitative measure of dispersion spontaneity. A transmittance value approaching 100% signifies that SNEDDS yields clear and transparent dispersions with droplet sizes estimated to be in the nanometer range.

Analyzing particle size stands as a critical determinant in self-emulsification, as it dictates the rate of drug release, thereby influencing both drug absorption and the stability of the resulting

emulsion [20]. The success of the SNEDDS formula becomes evident through the particle size of SNEDDS particles, typically ranging from 10 to 200 nm. This small particle size presents a larger surface area, facilitating pancreatic lipase hydrolysis and promoting enhanced drug release [20, 29].

The poly-dispersion index values for formulas 1 to 5 were as follows: 0.577, 0.166, 0.195, 0.193, and 0.237, respectively. It is worth noting that an acceptable range for the polydispersity index falls within 0 to 0.5 [30]. The polydispersity index results unequivocally indicate that the SNEDDS sample, composed of 70% ethanol extract from *B. medicinalis* herb and *M. oleifera* leaf, boasts uniformly sized nano-emulsion particles. This result is underscored by the declining polydispersity index value, signifying a greater uniformity in nano-emulsion size formation.

Optimization of SNEDDS formula

Within this formulation, the elements slated for optimization encompass surfactants and co-surfactants. When striving to pinpoint the most optimal formulation, a crucial factor comes into play: the assignment of weights. In the Design Expert software, this assigned weightage is referred to as "importance." In this section, there are several positive sign options ranging from positive one (+) to positive five (+++++). The higher the importance of the positive value of the component or response to be measured, the greater the importance of the weight chosen [12]. Optimization of response targets and importance weights on the SNEDDS formula of combined 70% ethanol extract of *B. medicinalis* herb and *M. oleifera* leaves can be seen in table 3. Based on the optimization values entered, the formula suggested by Design Expert with SLD method is two solutions (table 4).

Table 3: SNEDDS formula optimization based on SLD method

Parameters	Target	Lower limit	Upper limit	Importance
Surfactant	*In Range*	60	70	3
Co-surfactant	*In Range*	12	20	3
Emulsification time	*Minimize*	1	60	3
Transmittance	*Maximize*	80	100	3
Particle size	*Minimize*	10	200	5

Table 4: SNEDDS optimal formula solution based on SLD method.

No	Surfactant	Co-surfactant	EmulsificationTime	Transmittance	Particle size	Desirability	
1.	64	16	39.343	91.536	13.365	0.500	*Selected*
2.	63.6	16.4	39.776	91.565	12.970	0.497	

Among the two solutions considered, the first solution corresponding to formula 5, with a surfactant composition of 64% and co-surfactant of 16%, emerges as the optimal choice. This formulation is projected to yield an emulsification time of 39.343 seconds, a percentage transmittance of 91.536, and an estimated particle size of 13.365 nm. These predictions align remarkably well with a desirability value of 0.500, signifying a notably favorable outcome. With such promising desirability results, it is anticipated that the projected optimal formulation will closely mirror the experimental test results.

This alignment is further confirmed by the observed outcomes, where the average emulsification time is recorded at 39.30±3.055 seconds, the transmittance percentage hovers around 92.25%±0.004, and the mean particle size stands at 14.43±0.306 nm (table 5).

Table 5: SNEDDS optimal formula based on SLD method

Respond	Prediction	Observation	95% Cl		95% TI	
			Low	High	Low	High
Emulsification time (s)	39.343	36	35.1092	43.5765	17.6004	61.0853
		42				
		40				
Mean±SD		39.30±3.055				
Transmitance (%)	91.536	92.25	90.6854	92.3866	86.4116	96.6604
Mean±SD		92.25±0.004				
Particle size (nm)	13.365	14.7	2.27493	24.4559	36.2251	62.956
		14.1				
		14.5				
Mean±SD		14.43±0.306				

The evaluation of the optimal formula of SNEDDS was performed by measuring the viscosity and pH values (table 6). The viscosity value obtained is 355±2.6 cps. These results are in accordance with the SNEDDS viscosity requirements, which generally range from 100 to 1000 cps [31]. This viscosity shows the characteristics of a liquid and its resistance to flow. The higher the viscosity of the preparation, the greater the force required to flow [19]. Viscosity is used as an illustration to see the ease of SNEDDS when filling into soft or hard capsules [12]. Meanwhile, the resulting pH is 4.70±0.031. This result has met the pH requirements of SNEDDS preparations, which range from 1.2 to 7.4, and did not show phase separation from nano-emulsions [32].

Table 6: pH and viscosity measurements of optimal formula

Optimal formula (Formula 5)	Replication			Mean±SD
	1	2	3	
Viscosity (cps)	356	357	352	355±2.6
pH	4.69	4.67	4.73	4.70±0.031

Phytochemical analysis

It can be seen from fig. 1 that the phytochemical contents obtained are significantly different due to the influence of different concentrations of ingredients in the five formulas. The concentration levels of phytochemical compounds contained in the optimal formula (F5) showed high total phenolics (5.517±0.382 mg/g GAE) and total flavonoids (8.501±0.695 mg/g QE). Meanwhile, the total saponins showed the lowest value (17.991±0.052 mg/g EE). Therefore, it can be assumed that the SNEDDS formulation had an influence on the chemical compositions of the formula, and the optimal formula (F5) was characterized by a high concentration of phenolics and flavonoid compounds but a low saponin concentration.

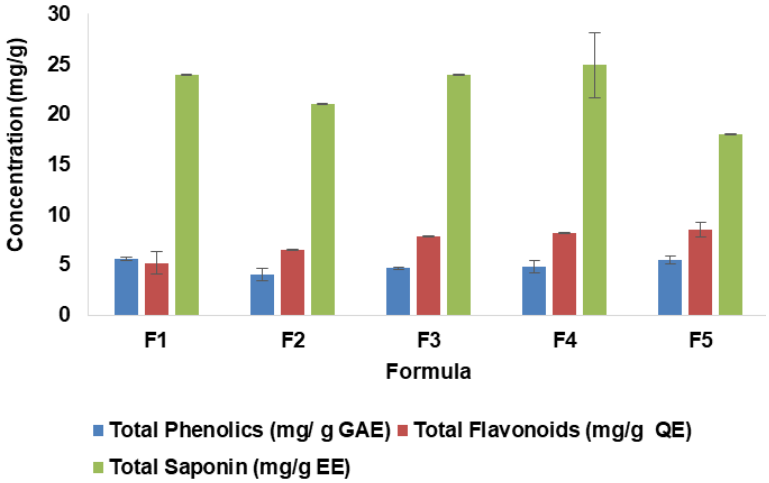

Fig. 1: Total phenolics, total flavonoid, and total saponin of SNEDDS formula. Data were presented as mean±SD, n=3

Percentage of HeLa cell death

In this study, the anticancer test was conducted on cervical cancer cells (HeLa) by using the MTT method. As shown in fig. 2, there is a clear correlation between the concentration used and the percentage of cell death, where the higher concentrations lead to a more significant percentage of cell death. Notably, when testing the combined 70% ethanolic extract of *B. medicinalis* herb and M. *oleifera* leaves (at a 1:1 ratio) at a concentration of 250 µg/ml, the percentage of cell death remained below 50%, specifically at 44.08% (mean value). In contrast, the optimal formula (F5) exhibited a significantly higher percentage of cell death, reaching 84.33% (mean value) at a concentration of 200 µg/ml. These results demonstrate a notable increase in the percentage of cell death in HeLa cancer cells following the formulation of the combined extracts into an SNEDDS preparation.

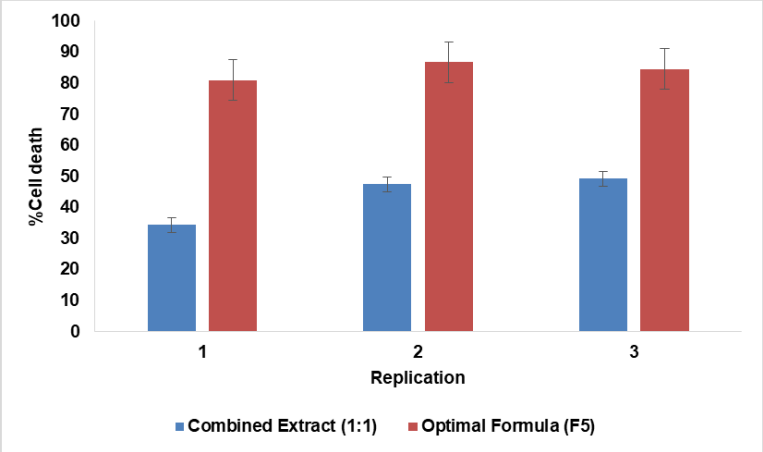

Fig. 2: Percentage of cell death of combined extract (1:1) and optimal formula (F5). Data were presented as mean±SD, n=3

These high percentages of HeLa cell death results prove that the optimal SNEDDS formula has a droplet size on a nanometer scale and has been proven to increase bioavailability and maintain drug stability [33, 34]. In addition, the death of cancer cells was also influenced by the chemical compounds contained in *B. medicinalis* herb and *M. oleifera* leaves. It is known that *B. medicinalis* herb has flavonoid and saponin compounds that show potential anticancer activity, such as flavonol and 2-O-β-glycopyranosyl cucurbitacin D. These compounds show anticancer activity by inhibiting STAT3 signaling and inducing apoptosis by inhibiting the JAK/STAT pathway. In addition, *M. oleifera* leaves also contain flavonoids that can inhibit cancer cell proliferation by inhibiting the cell cycle and inducing apoptosis [2, 35].

CONCLUSION

The optimal SNEDDS formula containing a combined 70% ethanol extract of *B. medicinalis* herb and *M. oleifera* leaves was obtained. The most optimal SNEDDS formula is formula 5, which contains 12% VCO, 64% Tween 80, and 16% propylene glycol. This optimal formula has characteristics of an emulsification time of 39.30±3.055

seconds, percentage transmittance of 92.25%±0.004, and particle size of 14.43±0.306 nm, with a polydispersity index (PI) of 0.237. The pH of the optimal formula is 4.70±0.031, and viscosity of 355±2.6 cP. s. Phytochemical analysis showed a total phenolic content of 5.517±0.382 mg/g GAE, total flavonoids of 8.501±0.695 mg/g QE, and total saponins of 17.991±0.052 mg/g EE. In addition, it also provides a higher percentage of HeLa cancer cell death of 84.334% at a concentration of 200 µg/ml.

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AUTHORS CONTRIBUTIONS

MSZ: Conceived, designed, data analysis, and finalized the manuscript. JS: Performed the experiments, performed data analysis, and drafted the manuscript. ES: Design, write, and finalize the manuscript. AS: Data analysis and review of the manuscript. AR: Data analysis and review of the manuscript.

CONFLICT OF INTERESTS

Declare none

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