Indonesian Journal of Cancer Chemoprevention, June 2023
ISSN: 2088–0197
e-ISSN: 2355-8989

In Silico and *In Vitro* Study *Selaginella doederleinii* Herb Extract as An Antineoplastic on MCF-7 Cells and Formulation Development of Nano Effervescent Granule

Chaessy Yori Anggraini[1], Triana Arum Kusumaningtyas[1], Melisa Junianandа[1], Dhecella Winy Cintya Ningrum[1], Rifki Febriansah[1*], Adi Hermawansyah[2]

[1]School of Pharmacy, Faculty of Medicine and Health Science, Universitas Muhammadiyah Yogyakarta, Indonesia
[2]Cell and Tissue Culture Laboratory, Faculty of Medicine and Health Sciences, Universitas Muhammadiyah Yogyakarta, Indonesia

Abstract

Breast cancer, the leading cause of cancer-related deaths in women with 685,000 deaths in 2020. Exploring natural compounds with minimal side effects has emerged as a potential treatment. However, utilizing natural substances faces challenges, such as poor bioavailability, requiring technologies like nanotechnology to enhance absorption. This study focuses on evaluating Ethanol Extract of *Selaginella doederleinii* (EESD) as an anticancer against MCF-7, both *in silico*, *in vitro* methode and develop a formulation of EESD nanoparticle effervescent granules. This study commenced with extraction, *Gas Chromatography-Mass Spectrometry* (GC-MS) identification, *in silico* studies, namely bioinformatics and molecular docking, 3-(4,-5-dimethylthiazo-2-yl)-2,5-diphenyltetrazolium bromide (MTT) Assay tests on MCF-7, and the formulation of nanoparticle preparations. EESD was extracted using the maceration method, resulting in an extract weighing 103.5 grams with a 6.9% yield. GC-MS identified three major compounds—cyclopentadecanoic, 2-hydroxy; hexadecanoic acid; and 9-octadecanoid, methyl ester. Bioinformatics revealed interactions with specific protein targets, and molecular docking indicated hexadecanoic acid's superior binding to TP53, surpassing paclitaxel at -8.7 kcal/mol. This suggests its potential to modulate TP53, impacting P53's role in impeding cancer cell growth. EESD exhibited an IC_{50} of 215µg/mL, signifying moderate cytotoxicity. In formulating nanoparticle effervescent granules, five formulas were transformed into nanoparticles and underwent organoleptic, pH, granule dissolution, and water content evaluations. Formula I is the formula that best meets the criteria with a pH of 6.55, granule dissolution <5 minutes, and water content <4%. The research results indicate that EESD shows anticancer activity against MCF-7 and this study has successfully developed a formula of nanoparticle from EESD in effervescent granule form.

Keywords: *Selaginella doederleinii, breast cancer, co-Cemotheraphy, MCF-7 Cell, in silico.*

Submitted: November 21, 2023
Revised: December 13, 2023
Accepted: December 14, 2023
Published online: December 27, 2023
*Corresponding author: rifki.febriansah@umy.ac.id

INTRODUCTION

Cancer is one of the most challenging diseases to treat and can lead to death. This disease is estimated to increase yearly until 2030 (Stewart and Wild, 2014). The most common type of cancer in women is breast cancer, accounting for 30% of all cancers in women, with 14% of these cases resulting in death (Haryanti and Widiyastuti, 2017). In 2020, breast cancer affected 2.3 million women worldwide, leading to 685,000 deaths. This cancer is a prevalent occurrence and affecting women in every country across the world (Anderson, *et al*., 2021).

One strategy for treating cancer is chemotherapy. The goal of chemotherapy is to kill cancer cells, but it has several side effects on the body's normal cells. One of these effects is disruption to the spinal cord, which plays a crucial role in the body's immune system (Sari, *et al*., 2019). Unfortunately, current chemotherapy agents have limitations, such as resistance, side effects, and inadequate efficacy, leading to therapeutic inefficiency. Therefore, there is a need to develop more effective and efficient chemopreventive agents.

Finding chemopreventive compounds with minimal side effects involves exploring natural ingredients, especially plants (Haryanti and Widiyastuti, 2017). Indonesia has many herbal plants with the potential to be chemopreventive agents. *Selaginella doederleinii* has shown activity as a chemopreventive agent and holds potential for development into a new anticancer herbal formula. In previous studies, it was found that dichloromethane and ethyl acetate extracts of *Selaginella doederleinii* have exhibited promising cytotoxic activity against cancer cells (Li, *et al*., 2020). Additionally, the ethanol extract of *Selaginella doederleinii* has shown inhibitory effects on the proliferation of T47D breast cancer cells (Risdian, *et al*., 2011). The studies have indicated that this plant has potential as an anticancer agent against various cancer cells. However, testing the activity of ethanol extracts from *Selaginella doederleinii* on breast cancer cells has not been extensively conducted, especially on MCF-7 breast cancer cells, which is one of the types of breast cancer in women. Therefore, further research is needed regarding the potential anticancer properties of *Selaginella doederleinii* against MCF-7 breast cancer cells.

A suitable formulation is necessary to process natural ingredients into a dosage form readily accepted by the public, considering the current lifestyle preference for fast food preparations. Efforts to increase public interest and practicality in consuming herbal medicines include formulating them into effervescent granule dosage forms (Setiana and Kusuma, 2018).

In the utilization of natural ingredients as a formulation, the active components of natural substances face challenges in penetrating the lipid membrane of body cells due to their large molecular size and low water solubility, leading to poor absorption and bioavailability (Ajazuddin and Saraf, 2010). One technology capable of reducing the particle size of an active component of natural substances is nanoparticle technology. Nanotechnology is currently being developed and is becoming a trend in improving the quality of functional food products. Nanotechnology can be developed as a delivery agent for active substances in medicinal products to regulate the release rate, increase solubility, and enhance absorption in the body due to its smaller particle size. This research tested the anti-breast cancer activity of the ethanol extract of *Selaginella doederleinii* (EESD) *in vitro,* and *in silico* to obtain research data supporting the development of new drugs.

MATERIALS AND METHODS

Selaginella doederleinii was collected in Bantul, Yogyakarta, ethanol 70% (One Med, East Java, Indonesia), MCF-7 cultured cells (Cell Culture Laboratory Collection, Universitas Muhammadiyah

Yogyakarta, Indonesia), aquadest (Brataco Chemical, Jakarta, Indonesia), Phosphate Buffered Saline (PBS) (Sigma Aldrich, St. Louis Missouri, USA), Roswell Park Memorial Institute (RPMI) (Gibco, Invitrogen, USA), Fetal Bovine Serum (FBS Qualified, Gibco, Invitrogen USA) 10% v/v (Sigma Aldrich), Dimethyl sulfoxide (DMSO) (DMSO 99,5% pro GC, Sigma Aldrich Chemie GmBH, Steinheim, Germany), 3-(4,-5-dimethylthiazo-2-yl)-2,5-diphenyltetrazolium bromide (MTT) Reagent 0.5 mg/mL (Sigma Aldrich), MTT 5 mg/mL in culture medium (Sigma Aldrich), Penicillin-streptomycin 1.5% v/v (Gibco), Fungizone 0.25% (Gibco), Sodium Dodecyl Sulfate (SDS) stopper reagent in HCl 10% (Merck, Darmstadt, Germany), Trypsin-EDTA 0.25% (Gibco), chitosan (Sigma Aldrich), STPP (Merck), citric acid (Merck), tartaric acid (Merck), PVP (Merck), sodium benzoate (Merck), sodium bicarbonate (Merck), essence (Brand, Indonesia), aspartame (Sigma Aldrich), and lactose (Merck).

Extraction

Selaginella doederleinii is washed and then dried for seven days. Afterward, it is pureed using a blender and sifted. The *Selaginella doederleinii* simplicia powder is macerated using a 70% ethanol solvent (1:10) for seven days. The maceration results are then thickened into an extract using a water bath (Laksana, 2000).

Identification of Compounds Using the Gas Chromatography-Mass Spectrometry (GC-MS) Method

The identification of compounds using the Gas Chromatography-Mass Spectrometry (GC-MS) method involved weighing 2 grams of *Selaginella doederleinii* extract samples in an Eppendorf tube. The weighed sample was then dissolved in a solvent. Subsequently, 1 µl of the sample was injected into a gas chromatography instrument and analyzed using GC-MS (Novilda and Marcellia, 2022).

STITCH-STRING Bioinformatics Test

The STITCH bioinformatics method is executed on the website http://stitch.embl.de/. The outcomes of the STITCH analysis yield direct target proteins (DTPs). Subsequently, DTPs are examined for protein interactions using the STRINGdb database (http://string-db.org/) until indirect target proteins (ITPs) are identified. Download the data for both DTPs and ITPs, then synchronize it with the breast cancer regulatory genes obtained through the PubMed gene database. Proceed with the analysis using the Venny 2.1 web tool to create a Venn diagram. Visualize the results of the selected target proteins with Cytoscape v3.8.2 to identify the top 10 based on the degree score. The two proteins with the highest degree scores are selected for further analysis through molecular docking (Saputri, *et al*., 2022).

Molecular Docking Test

The retrieval of protein structures was conducted through the Protein Data Bank (PDB) (www.rcsb.org). Proteins and ligands were prepared using DS Visualizer. The original ligand is sourced from the target protein file. Test compounds or ligands can be downloaded via PubChem (http://pubchem.ncbi.nlm.nih.gov). Conformations were selected with RMSD values ≤2.5 Å. The docking results were visualized using DS Visualizer (Saputri, *et al*., 2022).

Cytotoxic Test using the 3-(4,-5-dimethylthiazo-2-yl)-2,5-diphenyltetrazolium bromide (MTT) Assay Method

Tools were sterilized by autoclaving for 20 minutes at 121°C under 15 lb pressure. Complete Media (CM) consists of RPMI, FBS 10%, Fungizone 0.5%, and penicillin-streptomycin 2%. MCF-7 cells were cultured in a tissue culture flask until confluent, then harvested and counted using a hemocytometer. The EESD test solution was prepared as a stock with a concentration of 105

μg/mL in DMSO, with a concentration of <0.2%. Subsequently, the stock solution was diluted to create a concentration series of 62.5; 125; 250; 500; 1000 μg/mL in culture medium. Cells and samples were then prepared for the MTT cytotoxic assay. The cells were distributed into 96-well plates, washed, and incubated with reagents. Living cells reacted with MTT to form purple formazan crystals, which were then measured using an ELISA reader at 595 nm (CCRC, 2017).

Development Nano Particle Extracts

The method employed for nanoparticle synthesis in this study is the ionic gelation method. A 0.2% chitosan solution, totaling 200 mL, was prepared over 24 h using a magnetic stirrer set at 500 rpm. Simultaneously, a 100 mL solution of 0.1% TPP was created. Additionally, 40 mg of extract was dissolved in 10 mL of 70% ethanol. This extract solution was introduced into 50 mL of chitosan on a magnetic stirrer set at 500 rpm for 15 minutes. Subsequently, it was continuously dripped with 10 mL of TPP solution and left on the magnetic stirrer for 1 h (Wijayadi dan Rusli, 2019).

Particle Size Analyzer Test

Nanoparticle colloids produced from EESD were characterized using a PSA tool to determine both particle size and polydispersity index (particle size distribution) (Harahap, 2012).

Effervescent Granule Preparations

Effervescent granules were produced by placing *Selaginella doederleinii* extract in oven at 45°C for 72 h. The solidified extract was then ground until no crystals remained. The effervescent granules were created using the wet granulation method, which involves a separate granulation process for the acid and base components. The materials utilized in this research include nano extract as an active ingredient, aerosol as a lubricant, sodium benzoate as a preservative, sodium bicarbonate as a base component, PVP as a binder, flavoring agents, citric acid, and tartaric acid as acid components, aspartame as a sweetener, and lactose as an additional ingredient. Effervescent granule formulations were developed with five different variations to determine the optimal formulation.

Evaluation Test of Effervescent Granule Preparations

Effervescent granules of NEESD underwent various evaluation tests including organoleptic test, pH analysis, water content and dissolution time test. An organoleptic test for color, shape, smell, and taste of NEESD; pH analysis, involving the dissolution of seven grams in 200 mL of distilled water and subsequent pH measurement; a water content test, conducted by placing one gram in a water content tester at 105°C for 10 minutes; and a dissolution time test, where seven grams were dissolved in 200 mL of distilled water, with the time calculated (Elisabeth, *et al*., 2018).

RESULTS

Extraction

The extraction process employed the maceration method, resulting in a thick, dark green extract weighing 103.5 grams with a yield value of 6.9%. Identification of Compounds using the GC-MS.

Identification of Compounds Using the Gas Chromatography-Mass Spectrometry (GC-MS) Method

The results were obtained in the form of a chromatogram, as shown in Figure 1. This chromatogram displays peaks representing the compounds present in EESD, along with the corresponding percent area. This information indicates the concentration of the compounds in the sample, with higher percent areas suggesting higher concentrations.

Indonesian Journal of Cancer Chemoprevention, June 2023
ISSN: 2088–0197
e-ISSN: 2355-8989

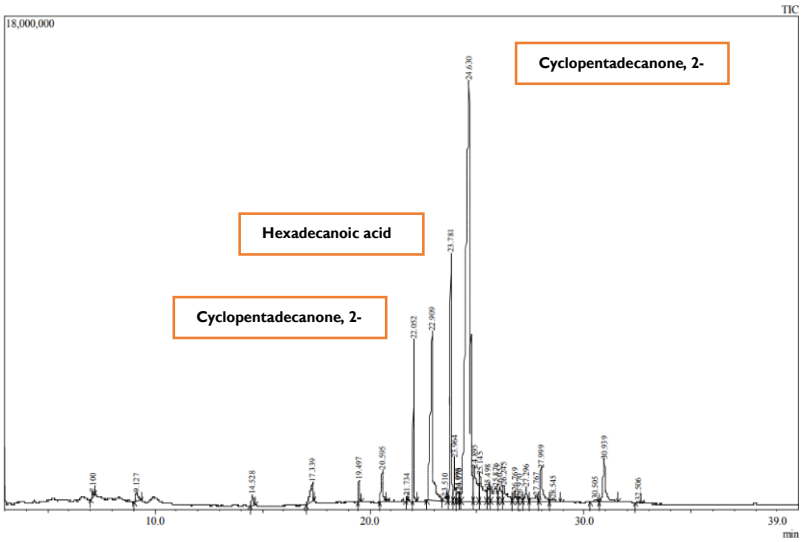

Figure 1. EESD chromatogram with GC-MS.

Based on the chromatogram, the analysis revealed the presence of 30 compounds in EESD, with three major compounds exhibiting the largest percent area. The detailed results of this analysis are presented in Table 1.

Table 1. Analysis of EESD chemical components using GC-MS.

Peak#	R.Time	Area%	Name
1	22.909	12.53	Hexadecanoic acid (CAS)
2	23.781	7.77	9-Octadecenoic acid, methyl ester, (E)- (CAS)
3	24.630	47.67	Cyclopentadecanone, 2-hydroxy-

STITCH-STRING Bioinformatic Test

Based on searches conducted on NCBI, 5,566 genes associated with the proliferation of breast cancer cells were identified. The compounds hexadecanoic acid and octadecanoic acid in *Selaginella doederleinii* are known to interact with 399 and 146 proteins, respectively, as Direct Target Proteins and Indirect Target Proteins. Subsequently, an analysis was performed using a Venn diagram, and the results were visualized using Cytoscape based on the degree score. The following represents a visualization of the top 10 target proteins for each compound.

Table 2. Visualization of results based on degree score.

No	Hexadecanoic Acid		Octadecanoic Acid	
	Protein	Degree Score	Protein	Degree Score
1	**TP53**	80	**PPARG**	27
2	**TLR4**	59	**INS**	25
3	TGFB1	56	SREBF1	22
4	SREBF1	52	SCARB1	21
5	SCARB1	60	SREBF2	19
6	PPARGC1A	45	SCD	19
7	PPARG	70	LEP	14
8	PPARA	61	MC4R	11
9	INS	56	PPARGC1B	10
10	IL6	48	NR1H3	27

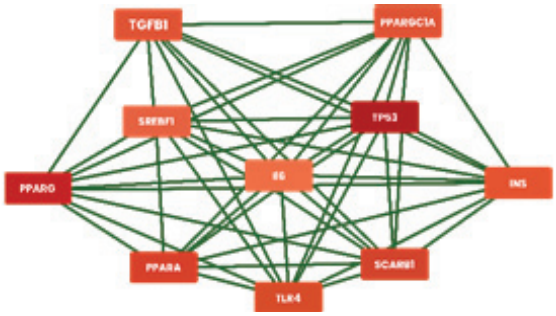

Figure 2. Visualization of top 10 degree score hexadecanoic acid

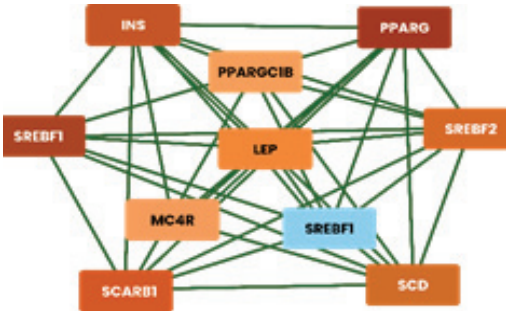

Figure 3. Visualization of top 10 octadecanoic acid.

After obtaining the visualization of results based on the highest degree score, the target proteins were selected for analysis using molecular docking. The hexadecanoic acid compound was docked with TP53 and TLR4, while the octadecanoic acid compound was linked to the PPARG and INS proteins.

Molecular Docking Test

This method involved linking the ligand in the test compound with the breast cancer protein target. The test results revealed that each compound interacted with its respective protein targets, namely hexadecanoic acid-TP53, hexadecanoic acid-TLR4, octadecanoic acid-PPARG, and octadecanoic acid-INS. The proteins that have been expressed were subsequently compared with the reference compound, Paclitaxel. The choice of Paclitaxel as the reference compound stems from its frequent utilization as the primary treatment drug for breast cancer. Furthermore, the mechanisms by which Paclitaxel acts, leading to the inhibition of tumor growth, can operate at various biological levels (Abu Samaan, *et al.*, 2019). The docking results are presented in Table 3.

Table 3. Docking results with protein targets.

Test Compound	Target Proteins	RMSD Value	Docking Score	Conformation
Hexadecanoic Acid	**TP53**	1.192	**-8.7**	4
	TLR4	1.239	-5.3	8
Octadecanoic Acid	PPARG	1.170	- 8.2	3
	INS	1.717	-5.4	3
Paclitaxel	TP53	1.985	- 7.2	6
	TLR4	2.306	- 8.2	8
	PPARG	2.227	-7.4	9
	INS	2.381	-5.1	7

Indonesian Journal of Cancer Chemoprevention, June 2023
ISSN: 2088–0197
e-ISSN: 2355-8989

Cytotoxicity Test using the 3-(4,-5-dimethylthiazo-2-yl)-2,5-diphenyltetrazolium bromide (MTT) Assay

The results of the EESD cytotoxic test on MCF-7 cells revealed an IC_{50} value of 215 µg/mL, indicating the concentration at which 50% of the MCF-7 cells were inhibited. According to IC_{50} characteristics ranging from 100 to 1000 µg/mL, EESD has the potential to be moderately cytotoxic in inhibiting cancer cells (Rahmawati, *et al*., 2023).

Table 4. Cytotoxic activity test results.

Consentration (µg/mL)	% Cell Viability
1000	6.75
500	19.89
250	34.58
125	49.17
62.5	64.84
31.25	79.68

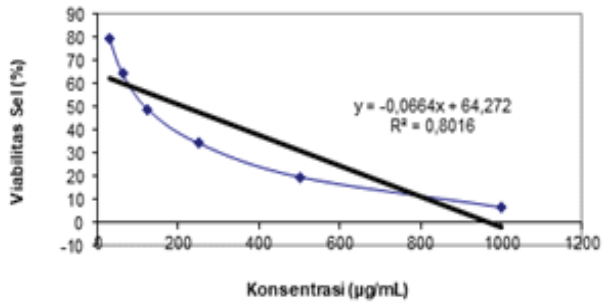

Figure 4. Viability of MCF-7 in EESD.

Nanoparticles and Particle Size Analyzer (PSA) Test

Based on the results of measurements using the PSA test, the average particle size and particle distribution results were obtained in Table 5.

Table 5. Particle size analyzer test results.

	Particle Size	Particle Distribution
	330	0.0677
	451	0.1993
	410	0.1331
Average Score	**397±61.54**	**0.1334±0.06**

Effervescent Granule Preparations

To create effervescent granules, five different formulation variations were developed to determine the optimal formulation.

Table 6. Effervescent granule formulation.

Material	Treatment (%)				
	F1	F2	F3	F4	F5
Nano Extract	1.5	1.5	1.5	1.5	1.5
Aerosil	1	1	1	1	1.5
Sodium Benzoate	1	1	1	1	1
Sodium Bicarbonate	33	33	28	28	33
PVP	5	5	5	5	5
Flavoring agent	qs	qs	qs	qs	Qs
Citric Acid	8	26	4	25	8
Tartric Acid	26	8	25	4	26
Aspartame	5	5	5	5	5
Lactose	ad 50 mL				

Evaluation Test of Effervescent Granule Preparations

Preparative evaluation tests are conducted to assess the quality and capabilities of the preparation based on its characteristics. In this research, the preparative evaluation tests encompassed organoleptic assessments, pH tests, water content measurements, and dissolution time analyses. The results of the evaluation tests for the effervescent granule preparations are presented in Table 7.

Table 7. Evaluation test results of effervescent granule preparations.

Formula	Organoleptic Test			pH	%Water content	Dissolution Time
	Color	Smell	Taste			
I	**Homogeneouse**	**Essence**	**Sweet**	**6.55**	**1.67**	**3.33 minutes**
II	Homogeneouse	Essence	Sweet	6.06	2.34	450 minutes
III	Homogeneouse	Essence	Sweet	4.56	1.51	3.22 minutes
IV	Homogeneouse	Essence	Sweet	3.98	1.49	4.16 minutes
V	Homogeneouse	Essence	Sweet	5.29	1.88	4.02 minutes

DISCUSSION

Breast cancer is the most prevalent cancer among women, with multifactorial causes. The utilization of herbal plants as chemotherapy agents represents one solution to mitigate the side effects of therapy. One plant demonstrating potential as an anti-breast cancer agent is *Selaginella doederleinii*. Based on GC-MS test results, EESD contains 30 active compounds. The top three compounds, based on the highest percentage area, are cyclopentadecanoic, 2-hydroxy; hexadecanoic acid; and 9-octadecanoic acid, methyl ester. Among these major compounds, it is noteworthy that cyclopentadecanoic, 2-hydroxy, is a constituent of essential oils. Several studies have indicated that the essential oil content in a plant possesses the potential for significant cytotoxic activity against breast cancer. (Udin, 2013). In addition, minor compounds in EESD, such as hexadecanoic acid methyl ester, also display antioxidant activity (Belakhdar, *et al*., 2015). Another compound, tetradecanoic acid, also demonstrates antioxidant activity (Krishnamoorthy and Subramaniam, 2014).

Based on *in silico* tests using bioinformatics methods, ten proteins show the highest degree scores for hexadecanoic acid and octadecanoic acid. Subsequent analysis using the Molecular Docking method revealed that each compound interacts with its target protein, specifically between hexadecanoic acid-TP53, hexadecanoic acid-TLR4, and octadecanoic acid-PPARG and octadecanoic acid-INS. Following this, a comparison was made with the drug paclitaxel, which was linked to four target proteins. Almost all the docking scores for the comparison drug were lower than those for the hexadecanoic and octadecanoic acid compounds. The hexadecanoic acid compound targeting the TP53 protein exhibited the highest binding affinity with a docking score of -8.7 kcal/mol compared to the chemotherapy comparison drug paclitaxel. The TP53 gene encodes the P53 protein, which plays a pivotal role in preventing damaged cells from developing into cancer. The P53 protein is widely recognized for its crucial involvement in apoptosis, DNA repair, and cell growth regulation. Its mechanism induces cells to stay in the G1 phase and promotes apoptosis in response to DNA damage. This dual function, preventing cell division and facilitating cell self-destruction, underscores the significance of the P53 protein as a major antineoplastic factor, safeguarding cells from potentially fatal damage (Moningka, 2019).

The results of the MTT Assay cytotoxic test indicate that EESD has an IC_{50} value of 215 µg/mL. Based on the IC_{50} characteristic range of 100-1,000 µg/mL, EESD exhibits the potential for moderate cytotoxicity in inhibiting cancer cells (Rahmawati, *et al*., 2023).

After that, EESD was formulated into using the ionic gelation method. The purpose of creating nanoparticles is to address issues with drugs, such as enhancing the solubility of the active substance, improving bioavailability, modifying the drug delivery system, and increasing the stability of the active substance. The principle behind forming

nanoparticles through the ionic gelation method involves dripping the dispersed oil or organic phase with liquid droplets to create nanoparticles. The procedure entails mixing two liquid phases: one phase containing chitosan and another phase containing multivalent anions (Mohanraj and Chen, 2007).

NEESD was subsequently analyzed using a Particle Size Analyzer (PSA). The data reveals that the nanoparticles derived from the ethanol extract of *Selaginella doederleinii* plants have a nanoparticle size of 397 nm, falling within the <1000 nm range. The particle distribution value is close to 0 and >0.5. The results indicate that the NEESD possess favorable characteristics, with a particle size distribution that tends to be homogeneous. This makes them a suitable delivery system for the active substance of a drug into the body. Small particle sizes contribute to a larger surface area, accelerating the absorption process (Mulwandari, 2022).

Effervescent granule formulations are pharmaceutical preparations created by combining acid and base compounds, which react to release carbon dioxide (CO_2) and produce foam when added to water (H_2O). In this study, granules were produced using the wet granulation method, with the principle of wetting the granule mass using a binding solution until a specific level of wetness was achieved (Lannie Hadisoewignyo and Achmad Fudholi, 2013). Effervescent granule formulations were developed using five different formulation variations to determine the optimal formulation.

The preparation evaluation tests included organoleptic assessments, pH tests, water content measurements, and dissolution time assessments. Organoleptic tests were conducted to assess the product's acceptability using human senses as the primary tool. This test comprised color, smell, and taste evaluations. Based on the results of the preparation evaluation presented in Table 7., the organoleptic tests indicated that all five formulations exhibited consistent color, smell, and taste. Another test performed was the pH test. This test aimed to measure the acidity level of effervescent solutions using a pH meter. The variation concentration of the acid source in the five formulations determined the pH value of each formula. A desirable pH value for effervescent granule formulations is in the range of 6-7. The pH test results in this study revealed that Formula I demonstrated the optimal pH compared to the other formulations. These findings suggest that the ratio of acid and base significantly influences the pH value generated by the preparation. However, no significant differences were observed among the pH test results of the five formulations. It is important to highlight that all other formulations also adhere to the specified parameter range of 6-7. Nevertheless, the preference for Formula I as the formulation with the optimal pH is based on its proximity to neutrality, signifying its suitability for safe consumption within the human body.

The next test is the water content test. The amount of water in the effervescent preparation will affect the results of the chemical reaction between the acid and base components (Anova, *et al*., 2016). The results of evaluating the water content of effervescent powder in this research ranged from 2.34% to 1.49%, with the best percent water content being 1.67% in Formula IV. All preparation evaluation results in the % water content test have met the criteria for powder to be packaged in sachets, namely a maximum of 3%.

The following preparation evaluation test is the dissolution time test. In an effervescent preparation, solubility is the most important material property. An effervescent reaction will not occur if the components do not dissolve and disintegrate quickly. The more water that is absorbed and meets the material, the more optimal the reaction will be. The ease of absorption of water molecules is due to the formation of hydrogen bonds during the reaction (Supriyanto, *et al.*, 2013). The soluble preparation evaluation test results in this research showed that Formula I was the formula that dissolved and reacted the fastest. However, all preparation evaluation

test results in this research have met the official requirements stated in the British Pharmacopoeia (2001) with a dissolution time of less than 5 minutes at a temperature of 15-25°C.

Based on the entire preparation evaluation test, Formulation I effervescent granules are the most optimal formulation with a refreshing, sweet taste that masks the extract's taste and melon essence aroma. It has a neutral pH of 6-7, so it does not irritate the stomach. Granule dissolution time is <5 minutes. The water content in the appropriate formulation is <4%. However, during the process of making granules, the acid component is challenging to form and clumps and has high humidity. The water content of effervescent granules can be influenced by the humidity of the production room; the relative humidity in the room is 25%, and the temperature is ±25°C (Bangu, 2018).

CONCLUSION

Based on compound identification tests using GC-MS, the EESD is known to contain three major compounds, namely cyclopentadecanoic, 2-hydroxy; hexadecanoic acid, and 9-octadecanoic acid, methyl ester, which are known for their antioxidant and anticancer potential. The molecular docking test results showed that each compound interacts with its respective protein target. Notably, the hexadecanoic acid compound, targeting the TP53 protein, exhibits the best binding affinity with a docking score of -8.7 kcal/mol, surpassing the chemotherapy comparison drug paclitaxel.

In addition, in the MTT Assay cytotoxic test, EESD demonstrated an IC_{50} of 215 µg/mL, indicating its potential as a moderately cytotoxic agent in inhibiting MCF-7 cancer cells. Furthermore, EESD, characterized as nano-sized, possesses a PSA value that meets the specified parameters, specifically 397 nm, with a particle distribution value close to 0 and >0.5. Moreover, based on the comprehensive evaluation of nano-effervescent preparations, Formula-I emerges as the best formula meeting all parameter characteristics and exhibiting the most optimal evaluation result values.

ACKNOWLEDGMENT

The author wishes to extend sincere gratitude to Universitas Muhammadiyah Yogyakarta (UMY), Pharmacy Studies Program and the laboratory staff of the Faculty of Medicine and Health Science of UMY for their invaluable support in facilitating this research.

REFERENCES

Abu Samaan, T.M., Samec, M., Liskova, A., Kubatka, P., and Büsselberg, D., 2019, Paclitaxel's Mechanistic and Clinical Effects on Breast Cancer, *Biomolecules,* **9**(12), 789.

Ajazuddin, and Saraf, S., 2010, Applications of novel drug delivery system for herbal formulations, *Fitoterapia,* **81**(7), 680–689.

Anderson, B.O., Ilbawi, A.M., Fidarova, E., Weiderpass, E., Stevens, L., Abdel-Wahab, M., and Mikkelsen, B., 2021, The Global Breast Cancer Initiative: a strategic collaboration to strengthen health care for non-communicable diseases, *Lancet Oncol.,* **22**(5), 578–581.

Anova, I.T., Kamsina, K., and Hermianti, W., 2016, Formulasi Perbandingan Asam Basa Serbuk Effervescent dari Coklat Bubuk, *J. Litbang Ind.,* **6**(2), 99-106.

Bangu, A.I., 2018, *Formulasi dan Evaluasi Granul Effervescent Ekstrak Etanol Daun Jambu Biji (Psidium guajava L.), Essay,* Politeknik Kesehatan Kemenkes Kupang, Kupang.

Belakhdar, G., Benjouad, A., and Abdennebi, E.H., 2015, Determination of some bioactive chemical constituents from *Thesium humile* Vahl., *J. Mater. Environ. Sci.*, **6**(10), 2778-2783.

CCRC, 2017, *Protokol In Vitro,* Cancer Chemoprevention Research Centre, Yogyakarta, Website: https://ccrc.farmasi.ugm.ac.id/protokol-uji-in-vitro/.

Elisabeth, V., YamLean, P.V.Y., and Supriati, H.S., 2018, Formulasi Sediaan granul Dengan Bahan Pengikat Pati Kulit Pisang Gorojo (*Musa acuminafe* L.) dan Pengaruhnya pada Sifat Fisik Granul, *Pharmacon*, **7**(4), 1–11.

Hadisoewignyo, L., and Fudholi, A., 2013, *Sediaan Solida*, XI ed. Yogyakarta: Pustaka Pelajar.

Harahap, Y., 2012, *Preparasi dan Karakterisasi Nanopartikel Kitosan dengan Variasi Asam, Thesis*, Universitas Indonesia, Jakarta.

Haryanti, S., and Widiyastuti, Y., 2017, Aktivitas Sitotoksik pada Sel MCF-7 dari Tumbuhan Indonesia untuk Pengobatan Tradisional Kanker Payudara, *Media Penelit. dan Pengemb. Kesehat.*, **27**(4), 247–254.

Krishnamoorthy, K., and Subramaniam, P., 2014, Phytochemical Profiling of Leaf, Stem, and Tuber Parts of *Solena amplexicaulis* (Lam.) Gandhi Using GC-MS, *Int. Sch. Res. Not.*, **2014**, 567409.

Laksana, A.S., 2000, *Parameter Standar Umum Ekstrak Tumbuhan Obat*, Direktorat Jendral Pengawasan Obat dan Makanan.

Li, S., Wang, X., Wang, G., Shi, P., Lin, S., Xu, D., Chen, B., *et al.*, 2020, Ethyl Acetate Extract of *Selaginella doederleinii* Hieron Induces Cell Autophagic Death and Apoptosis in Colorectal Cancer via PI3K-Akt-mTOR and AMPKα-Signaling Pathways, *Front. Pharmacol*, **11**, 565090.

Mohanraj, V.J., and Chen, Y., 2007, Nanoparticles - A review, *Trop. J. Pharm. Res.*, **5**(1), 561–573.

Mulwandari, M., 2022, *Synthesis of Silver Nanoparticles Using Citronella Oil (Cymbopogon nardus L. Rendle) to Inhibit The Growth of Lichen on Temple Stones, Thesis,* Universitas Islam Indonesia, Yogyakarta

Rahmawati, I.S., Dyanti, G.P., Widyanto, R.M., Maulidiana, A.R., Nabila, W., and Purwestri, R.C., 2023, *In Vitro* Evaluation of Cytotoxicity Effect of Ihau Fruit Extract (*Dimocarpus longan* var. Malesianus Leenh.) on MCF-7 Breast Cancer Cell Line, *Indones. J. Hum. Nutr.*, **10**(1), 59–67.

Risdian, C., Handayani, S., Udin, Z., Andriyani, R., and Angelina, M., 2011, Anticancer Activity of Ethanolic Extract of *Selaginella Plana* Hieron. on T47d Cell Line *in Vitro, Indones. J. Appl. Chem.*, **13**(2), 153.

Saputri, D., Kusumaningtyas, T., Setiadi, P., and Febriansah, R., 2022, Investigation of Moringa Leaf Compounds as Colon Anticancer Agents using Bioinformatics and Molecular Docking Methods, *Proc. Univ. Muhammadiyah Yogyak. Undergrad. Conf.*, **2**, 24–27.

Sari, S.L., Indra, R.L., and Lestari, R.F., 2019, Korelasi Persepsi Tentang Efek Samping Kemoterapi Dengan Kualitas Hidup Pasien Kanker Payudara, *J. Cakrawala Promkes.*, **1**(2), 40-47.

Setiana, I.H., Kusuma, A.S.W., 2018, Review Jurnal: Formulasi Granul Effervescent Dari Berbagai Tumbuhan, *Farmaka*, **16**(3), 100-105.

Stewart, B.W., and Wild, C.P. (Eds.), 2014, *World cancer report 2014*, International Agency for Research on Cancer, Lyon, France.

Supriyanto, Murdiati, A., and Aprilia, F., 2013, *Kajian pembuatan effervescent cokelat pada berbagai variasi jumlah asam dan basa yang digunakan terhadap sifat fisik, kimia dan sensorisnya, Proceeding*, Jember: Seminar Nasional PATPI, ISBN: 978-602-9030-49-5, pp. 71-80.

Udin, Z., 2013, Sitotoksisitas Xanthorrhizol dari Minyak Atsiri Rimoang *Curcuma xanthorrhiza* Roxb. terhadap Sel Kanker Payudara YBM-1, *Jurnal Kimia Terapan Indonesia*, **15**(1), 23-29.

Wijayadi, L.J., and Rusli, T.R., 2019, Characterized and synthesis of chitosan nanoparticle as nanocarrier system technology, *IOP Conf. Ser. Mater. Sci. Eng.*, **508**, 012143.