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Original Research Article

Synthesis of Nanoherbal from Ethanol Extract of Indonesian Fern *Selaginella plana* and Antibacterial Activity Assay

Suyatno Sutoyo[1]*, Amaria Amaria[1], I Gusti M. Sanjaya[1], Rusly Hidayah[1], Devy P. Sari[1], Nabella Dwitarani[1], Farida D. Oktavia[1], Nurrulhidayah A. Fadzlillah[2]

[1]*Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Negeri Surabaya, 60231, Indonesia*
[2]*International Institute for Halal Research and Training (INHART), International Islamic University Malaysia (IIUM), Gombak 53100, Kuala Lumpur, Malaysia*

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ABSTRACT

Selaginella plana is one of the ferns that grows widely in Indonesia and has been used by the people as food, ornamental plants and traditional medicine. The preparation of *S. plana* extract in the form of nanoparticles can overcome the weakness of the extract, namely the low water solubility, bioavailability, stability, and absorption so as to increase its efficacy as a herbal medicine. Research related to the synthesis of nanoherbal from *Selaginella plana* as an antibacterial had never been reported. This study aims to synthesize nanoherbal from the ethanol extract of *S. plana* and test for its antibacterial activity. Extraction was carried out by maceration method, synthesis of nanoherbal by ionic gelation method, and antibacterial assay by disc diffusion method. The synthesized nanoherbal were characterized by FT-IR spectrophotometer, zetasizer nano, and scanning electron microscopy (SEM). The results showed that nanoherb of formula-1 (F-1) had a particle size in nanoparticle range (701.54±67.72 nm) and had a zeta potential of +499.3±78.00 mV. The shift on the stretching vibration of the O-H group (3370.12 cm^{-1}), the bending vibration of the N-H (1563.55 cm^{-1}), and the appearance of the vibration of the phosphate group (1083.86 cm^{-1}) supported the formation of nanoherbal in F-1. The F-1 nanoherbal has very strong antibacterial activity against *E. coli*, *S. dysentriae*, and *S. aureus* as well as strong antibacterial activity against *B. subtilis*. It showed stronger antibacterial activity than the ethanol extract of *Selaginella plana*. Thus the F-1 nanoherbal has the potential to be developed as an antibacterial agent.

Keywords: Antibacterial activity, Ethanol extract, Ionic gelation, Nanoherbal, *Selaginella plana*

Introduction

Selaginella plana (*S. plana*) is one of the fern which becomes Indonesia's biological wealth. This plant is spread in Southeast Asia, in Java it grows to a height of approximately 750 above sea level, generally on the moist and protected riverbank and on the slopes of ravines. *S. plana* has been known and used by people as food (vegetables), ornamental plants, and as traditional medicine. It has been used for postpartum care, treating wounds and bleeding, menstrual disorders, increasing the body immunity, as well as a postnatal tonic.[1-3] Traditionally, *S. plana* has been used to treat cancer, respiratory infections, liver disorders, urinary tract infections, fractures and rheumatism.[4] The *Selaginella* species on the island of Java has been found to contain secondary metabolites of the flavonoid group, with the highest content of biflavonoid compounds.[5] Biflavonoid compounds from ferns of the *Selaginella* genus have various bioactive properties, including antioxidants, anticancer, antibacterial, antiviral, antifungal, antimalarial, and anti-inflammatory properties.[3,6] As a phenolic compound, biflavonoid had been separated from several species of ferns in the Selaginellaceae family, namely amentoflavone, robustataflavone, ginkgetin, kayaflavone, podocarpus flavone, and hinokiflavone.[7-12]

*Corresponding author. E mail: suyatno@unesa.ac.id
Tel: +6285645385502

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The herbal active compounds in the form of extracts have the disadvantage of low water solubility so that their bioavailability is low and functional properties decrease during processing and storage. Preparation of herbal extracts into nano size or so called nanoherbal is able to overcome the poor water solubility of active substances that are difficult to dissolve, improve poor bioavailability, modify drug delivery systems so that drugs can go directly to specific areas, increase the stability of active substances from environmental degradation (enzymatic decomposition, oxidation, hydrolysis), improve absorption of a macromolecular compound, and reduce the irritating effect of active substances on the gastrointestinal tract.[13,14] The shape and size of the particles is one of the factors that affect the effectiveness of the drug, because the particle size is very influential in the process of solubility, absorption and distribution of drugs. In the nano size, the surface contact area of the particles becomes larger which can increase the number of active substances that interact so that the biological activity is stronger.[15]

In this study, nanoherbal were synthesized by ionic gelation method using chitosan and sodium tripolyphosphate (Na-TPP) as crosslinking reagents. The positively charged amine group of chitosan interacts with the negative charge of Na-TPP to form complexes with sizes in the nanoparticle range.[16-18] Chitosan has specific properties, namely the presence of bioactive, biocompatible, chelating, antibacterial and biodegradable properties. In the form of micro or nanoparticles, chitosan has many advantages, namely stability during use, high surface area, and can be used as a matrix for various types of drugs.[19] The principle of nanoparticle formation in the ionic gelation method is the occurrence of electrostatic interactions between positively charged chitosan amino groups and negatively charged Na-TPP polyanions to form a three-dimensional intramolecular structure.[18,20]

Research related to the synthesis of herbal nanoparticles from fern extract of *S. plana* as an antibacterial agent has never been reported. In

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this paper, we will report on the synthesis of nanoherbal from the ethanol extract of the fern *S. plana* using the ionic gelation method and the evaluation of its antibacterial activity.

Materials and Methods

Materials

The dried powder of *S. plana*'s aerial parts, ethanol (80%, p.a.), acetic acid (Merck), sodium tripolyphosphate (Merck), tween-80 (Merck), chitosan (Sigma-Aldrich), dimethyl sulfoxide (DMSO) (Merck), tetracycline (Sigma-Aldrich), nutrient broth, nutrient agar, *E.coli, S. dysentriae*, *B. subtilis*, and *S. aureus.*

Preparation of plant extract

Samples of *S. plana* (5 kg) were obtained from the Kletak forest, Nongkojajar, Pasuruan, East Java, Indonesia on 18 April 2021. Before the further investigation, the sample was identified at Purwodadi Botanical Garden of Indonesian Institute of Sciences on 20 April 2021 with the voucher number of B-3085/III/KS.01.03/4/2021. Furthermore, the sample is cleaned of attached dirt, then let to dry for 12 days at room temperature. The dried sample was milled into a fine powder that was ready for extraction (1,154 g).

The dried powder of *S. plana'*s aerial part (1000 g) were macerated with ethanol 80% (3 L) for 24 hours, then filtered using a Buchner funnel. The ethanol extract obtained was evaporated in vacuo using rotary vacuum evaporator (Buchi R-300). The resulting concentrated extract was dried in freeze dryer (Martin Christ Alpha 1-2 Ldplus) for 16 hours to yield a dark green solid (49.14 g).[21]

Synthesis of nanoherbal from ethanol extract of S. plana

Chitosan solutions of 0.5%, 0.75%, 1.0% were prepared by weighing 0.5 g, 0.75 g, and 1.0 g, respectively, then dissolved with 5% acetic acid solution (v /v) to 100 mL and stirred with a magnetic stirrer until dissolved. While a 0.5% sodium tripolyphosphate (Na-TPP) solution was prepared by weighing 0.1 g, 0.15 g, and 0.2 g, respectively. Then it was dissolved with distilled water up to 20 mL, 30 mL, and 40 mL, respectively, then stirred with a magnetic stirrer (Heidolph) until dissolved. In this study, three formula of nanoherbal were prepared, namely F-1, F-2, and F-3. The composition of each formula is presented in Table 1.

A total of 100 mL of 0.5% (F-1), 0.75% (F-2) and 1.0% (F-3) chitosan solution were put into an Erlenmeyer flask, respectively. Tween-80 (1 mL) was added to each chitosan solution and stirred using a homogenizer (Heidolph) at 1000 rpm for 15 minutes. After that, 10 mL of solution containing 0.1 g of *S. plana*'s ethanol extract in ethanol was added to each chitosan solution and stirred using a homogenizer (Heidolph) at 1400 rpm for 120 minutes. Into the 0.5%, 0.75% and 1% chitosan solutions, 20 mL, 30 mL, and 40 mL of 0.5% Na-TPP were added, respectively, and then homogenized at 1400 rpm for 150 minutes. The obtained mixture was allowed to stand for 24 hours and then dried in freeze-dryer (Martin Christ Alpha 1-2 Ldplus) for 28 hours to obtain dry nanoherbal. The fuctional groups of the resulting nanoherbal were determined using FTIR spectrophotometer (Shimadzu FTIR-8400S), particle size and zeta potential was done using Zetasizer Nano ZS (Malvern) and the surface morphology was determined using scanning electron microscopy (SEM) (FEI Inspest S50).[22,23]

Table 1: Composition of nanoherbal formula F-1, F-2, and F-3

Materials	F1	F2	F3
Ethanol extract of *S. plana*	0.1 g	0.1 g	0.1 g
Chitosan solution 0.50%	100 mL	-	-
Chitosan solution 0.75%	-	100 mL	-
Chitosan solution 1.00%	-	-	100 mL
Na-TPP solution 0.5%	20 mL	30 mL	40 mL
Tween-80	1 mL	1 mL	1 mL

Antibacterial activity assay of nanoherbal from ethanol extract of S. plana

In the study the materials tested for antibacterial activity were nanoherbal from ethanol extract of *S. plana*, ethanol extract of *S. plana* (763 ppm), chitosan, positive control (tetracycline 500 ppm), and negative control (aquadest and dimethyl sulfoxide). Meanwhile, the bacteria used consisted of gram-negative bacteria (*E.coli, S. dysentriae*) and gram-positive bacteria (*B. subtilis, S. aureus*).

The agar media that has been autoclaved at 121^{o}C for 15 minutes was cooled to 40^{o}C. A total of 100 µL of test bacteria that have been cultured in a test tube, pipetted and inoculated on agar media was then poured into petri dishes, and allowed to harden. Paper discs with a diameter of 6 mm after being immersed in the test solution were taken with tweezers and then placed into a petri dish, then incubated for 24 hours at 37^{o}C. The diameter of the inhibition zone was obtained from measuring the clear zone diameter around the paper disc using a caliper.[24,25]

Results and Discussion

Synthesis of nanoherbal from ethanol extract of S. plana

Three nanoherbal formulas were synthesized from the ethanol extract of the fern *S. plana*, with variations in the mass of chitosan, namely 0.5% (F-1), 0.75% (F-2) and 1.0% (F-3). Meanwhile, sodium tripolyphosphate of 0.5% was used as a crosslinker agent to increase the stability of nanoherbal. The results of the synthesis showed that the three nanoherbal formulas were golden yellow colloidal liquids (Figure 1). Furthermore, each nonaherbs formulas was dried in a freeze dryer for 28 hours to produce greenish-white solid nanoherbal (Figure 2). FT-IR spectrum measurements were carried out to identify the type of functional group and the changes that occurred in the wave number of the functional group. The FT-IR analysis of ethanol extract of *S. plana*, chitosan, and nanoherbal of F-1, F-2, F-3 resulted data as shown in Figure 3. The absorption peaks of the hydroxyl group (3284.2 cm^{-1}), carbonyl (1741.2 cm^{-1}), and aromatic C=C double bond (1563.92 cm^{-1}) in the infrared spectrum of the ethanol extract of *S. plana* support the presence of the phenolic compound in the extract. The infrared spectra of the three nanoherbal formulas indicated that the nanoherbal from ethanol extract of *S. plana* was synthesized using the ionic gelation method. This is indicated by a shift in the absorption band of the stretching vibration of the hydroxyl group, the bending vibration of the N-H group, and the emergence of new peaks from the vibration of the phosphate group (P=O).

Figure 1: The colloidal liquids of nanoherbal formulas from ethanol extract of *S. plana*

Figure 2: The solid of nanoherbal formulas from ethanol extract of *S. plana*

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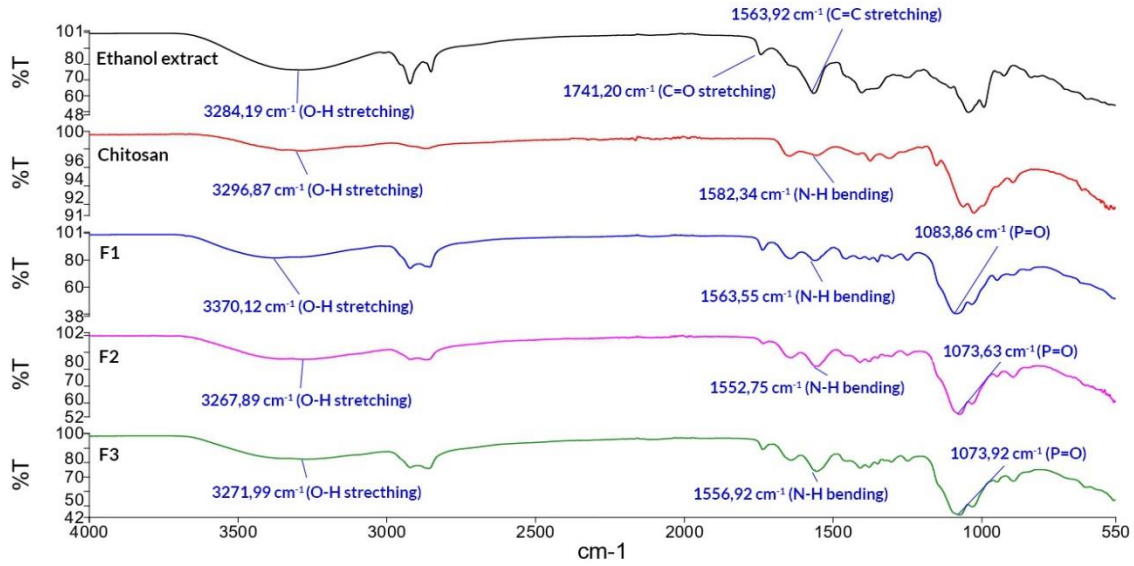

Figure 3: FT-IR spectra of *S. plana*'s ethanol extract, chitosan, nanoherbal of F-1, F-2, F-3

The stretching vibration of the hydroxyl group in chitosan shifted from 3296.87 cm-1 to 3370.12 cm^{-1} (F-1), 3267.89 cm^{-1} (F-2), and 3271.99 cm^{-1} (F- 3) in nanoherbal from ethanol extract of *S. plana*. This was caused by the interaction between the hydroxyl group in chitosan and the hydroxyl group of the phenolic compounds in the ethanol extract of *S. plana*. The bending vibration of the NH group in chitosan shifted from 1582.34 cm^{-1} to 1563.55 cm^{-1} (F-1), 1552.75 cm^{-1} (F-2), and 1556.92 cm^{-1} (F- 3) in nanoherbal from ethanolic extract of *S. plana*. This is caused by the occurrence of cross-linking between the NH_2 group of chitosan and the phosphate group in Na-TPP and the interaction with the hydroxyl group of phenolic compounds in the ethanol extract of *S. plana*. The appearance of absorption bands at 1083.86 cm^{-1} (F-1), 1073.63 cm^{-1} (F-2), 1073.92 cm^{-1} (F-3) in the nanoherbal from ethanol extract of *S. plana* due to vibrational peak of the phosphate group showed that there had been a cross-linking between chitosan and phosphate groups in Na-TPP.[26,27]

Particle size of nanoherb from ethanol extract of S. plana

The three nanoherbal formulas from ethanol extract of S. plana was determined its particle size using the DLS (Dynamic Light Scattering) method by the Zetasizer Nano ZS (Malvern). Determination of particle size was carried out 5 times for each formula and the results were presented in Table 2. Table 2 showed that the nanoherbal of F-1 had a particle size of less than 1000 nm, namely 701.54±67.72 nm. Meanwhile, the nanoherbal of F-2 and F-3 had a particle size of more than 1000 nm, namely 1291.8±183.78 nm and 1394.6±28.40 nm, respectively. Nanoparticles used as drug carriers have diameters between 1-1000 nm.[20,28] Thus, among the three synthetic nanoherbal formulas that fullfilled the requirements as nanoparticles was F-1 because its particle size was less than 1000 nm. Particle size in the form of nanoparticles affect the ease of entry of these materials into cells. The smaller the particle size, the easier it is to enter the cell and the higher its absorption in the body.[29] The particle size of the nanoherbal increased with the increase in the amount of chitosan in the nanoherbal formula. However, nanoherbal containing 0.5% chitosan and 0.5% Na-TPP have particle sizes in the nanoparticle category.[22]

Zeta potential of nanoherb from ethanol extract of S. plana

Zeta potential is a parameter of electric charge between colloidal particles. The zeta potential value is generated from the potential difference between the electric charge on the Stern layer and the diffuse layer of colloidal particles. Zeta potential measurement is needed to determine the nanoparticles stability with respect to aggregation. The stability of nanosystem is affected by the Van der Waals attraction and the electrostatic repulsion force. The high repulsion force among nanosystem particles will increase the zeta potential value which can lead to stability of a nanosystem.[30]

The zeta potential value of nanoherbal from ethanol extract of *S. plana* was determined using the Zetasizer Nano (Malvern). The determination of the zeta potential was carried out three times for each formula and the results were shown in Table 3.

From Table 3 it could be stated that the three nanoherbal formulas had zeta potential value greater than + 30 mV. Nanoparticles with zeta potential value less than -30 mV and greater than +30 mV had higher stability, because they had a force to prevent particle agglomeration. Thus, the three nanoherbal formulas had stable properties or were not easy to agglomerate or flocculate so that they were not easy to settle.[30]

Based on the zeta potential value, herbal nanoparticles of formula F-1 that fullfilled the requirements as nanoparticles had a zeta potential value of more than + 30 mV, namely +499.3±78.00 mV so that it had stable properties.[18,23,30]

The surface morphology of nanoherbal from ethanol extract of S. plana

The nanoherbal of F-1, F-2 and F-3 were analyzed using scanning electron microscopy to obtain data of surface morphology. The SEM images of the third nanoherbal are shown in Figure 4. Based on the Figure 4, the shape of the three nanoherbal particles tendend to be spherical. The particle size of F-1 appeared to be the smallest, while the F-3 particle had the largest size. This data supported the particle size of F-1, F-2, F-3 obtained by the Zetasizer Nano ZS. Particles of F-3 tended to clump together while particles of F-1 and F-2 were separated from each other.[27]

Antibactial activity of nanoherbal from ethanol extract of S. plana.

Based on the results of particle size measurements using azetasizer nano, it was known that among the three nanoherbal formulas that fullfilled the requirements as nanoparticles was the nanoherbal of F-1 with a particle size of 701.54±67.72 nm. Furthermore, the antibacterial activity of F-1 was determined using the disc diffusion method against 4 types of bacteria consisting of 2 types of gram-negative bacteria, namely *E. coli* and *S. dysentriae*, and 2 types of gram-negative bacteria, namely *S. aureus* and *B. substilis*. The samples tested for antibacterial activity were the liquid of nanoherbal F-1, ethanol extract solution of the fern *S. plana* (763 ppm), chitosan solution, positive control (tetracycline 500 ppm), and negative control (aquades and dimethyl sulfoxide). The results of the antibacterial activity assay were presented in Table 4.

In the antibacterial activity test using the disc diffusion method, the strength of the antibacterial activity is indicated by the diameter of the inhibition zone or the clear zone.

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Table 2: Particle size of nanoherbal from ethanol extract of *S. plana*

Nanoherbal formulas	Particle size of nanoherbal (nm)					
	1	2	3	4	5	Average
F-1	780.7	702.0	640.4	628.4	756.2	701.54 ± 67.72
F-3	1302.0	1488.0	1411.0	1250.0	1008.0	1291.8 ± 183.78
F-2	1351.0	1406.0	1421.0	1382.0	1413.0	1394.6 ± 28.40

Table 3: Zeta potential of nanoherbal from ethanol extract of *S. plana*

Nanoherbal formulas	Zeta potential (mV)			
	1	2	3	Average
F-1	+421	+500	+577	+499.3 ± 78.00
F-2	+409	+440	+491	+446.7 ± 41,40
F-3	+375	+403	+368	+382.0 ± 18.52

If the diameter of the clear zone of 5 mm or less, 5-10 mm, 10-20 mm, and 20 mm or more are categorized as weak, moderate, strong, and very strong, respectively.[24,31] Based on Tabel 3, nanoherbal F-1 had very strong antibacterial activity against *E.coli*, *S. dysentriae*, and *S. aureus* as well as strong antibacterial activity against *B. subtilis*. Meanwhile, the ethanol extract of the fern *S. plana* showed strong antibacterial activity. against *E. coli* and *S. dysentriae*, and moderate antibacterial activity against *S. aureus* and *B. subtilis*.Tetracycline showed strong antibacterial activity against *E. coli*, *S. dysentriae*, and *B. subtilis*, as well as antibacterial activity which was very strong against *S. aureus*.

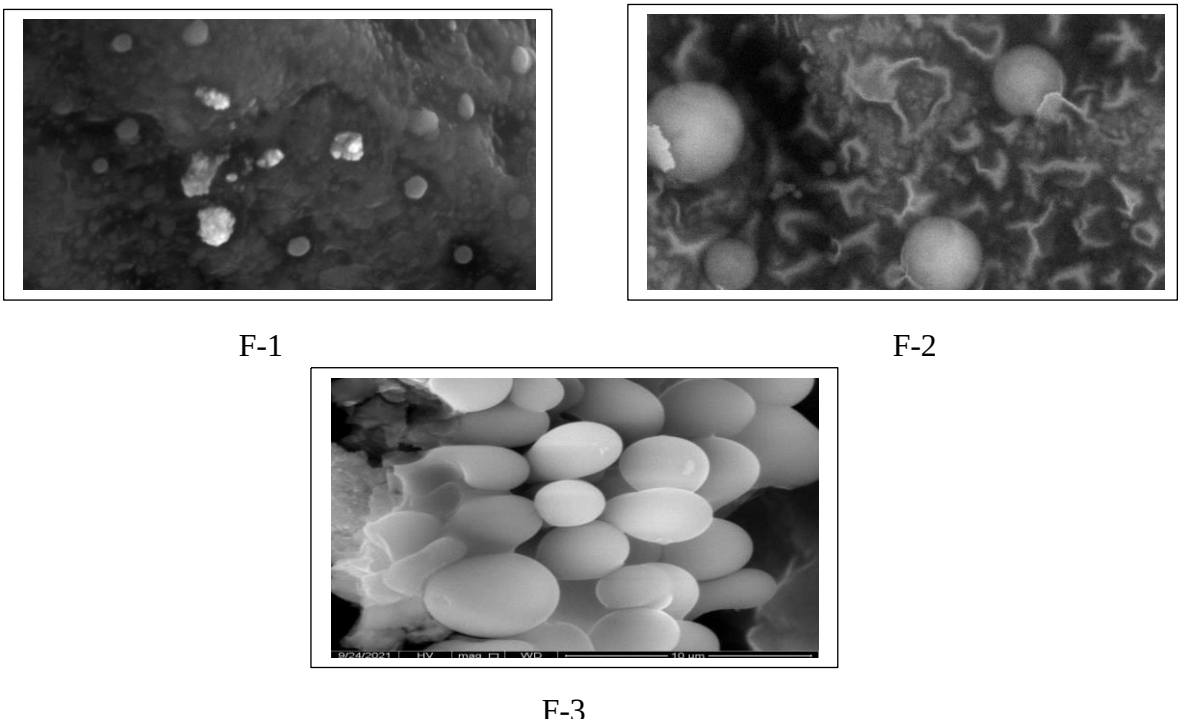

Figure 4: Scanning electron microscopy images of nanoherbal of F-1, F-2, F-3

Table 4: Antibacterial activity assay of the nanoherbal F-1 and ethanol extract of *S. plana*

Sample	Average diameter of the inhibition zone (mm)			
	E. coli	*S. dysentriae*	*S. aureus*	*B. subtilis*
Nanoherbal F-1	20.43 ± 2.28	23.10 ± 2.50	20.33 ± 3.61	18.96 ± 2.57
Ethanol extract of *S. plana*	10.26 ± 2.05	10.26 ± 1.80	9.90 ± 2.16	9.26 ± 0.91
Chitosan	14.03 ± 3.59	14.11 ± 1.04	14.85 ± 1.78	13.45 ± 1.88
Tetracycline	10.25 ± 1.07	19.87 ± 1.37	28.85 ± 2.60	19.60 ± 1.19
Aquadest	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
DMSO	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00

The antibacterial activity of nanoherbal F-1 against *E. coli* and *S. dysentriae* was higher than that of the tetracycline. Meanwhile, the antibacterial activity of nanoherbal F-1 against *S. aureus* and *B. subtilis* was lower than that of tetracycline. The antibacterial activity of the ethanolic extract of *S. plana* against *S. dysentriae*, *S. aureus* and *B. subtilis* was lower than that of tetracycline, while its antibacterial activity against *E.coli* was equivalent to that of tetracycline. Therefore the nanoherbal F-1 has the potential to be developed as an antibacterial agent.

The content of phenolic compounds, especially flavonoids in the ethanol extract of the fern *S. plana* supports its antibacterial activity.[3,11,12] Flavonoid compounds inhibit bacterial growth by damaging cell membrane, inactivating enzymes, binding to adhesins, and inhibition of nucleic acid synthesis. The presence of hydroxyl group in flavonoids affect the antibacterial activity. The position of hydroxyl groups at C-5, C-6, and C-7 in the ring A of flavonoid can increase its antibacterial activity.[32,33]

The ethanol extract of *S. plana* made from the nanoherbal F-1 showed an increase in the antibacterial activity of *E. coli*, *S. dysentriae*, *B. subtilis*, and *B. subtilis*. In the nano size, the number of isolated active substances will increase due to the larger the surface contact area of the particles so that the antibacterial activity is stronger.[15] Particles in nano size have the ability to penetrate the intercellular spaces, either through diffusion or opsonification.[14,29,34] The presence of chitosan polymer in the nanoherbal F-1 also supported its antibacterial activity because it had the ability to inhibit bacterial growth. Chitosan attaches to the surface of bacterial cells to form a polymer membrane that can prevent the entry of nutrients into the cell so that the cell will die. Besides that chitosan with low molecular weight can enter the cell and cover the cell. The positive charge of chitosan will bind to negative charge on bacterial surfaces such as lipopolysaccharides, so that it will cause damage to permebility of cell membrane, cell leakage and cell death.[35]

Conclusion

The study concludes that nanoherbal from ethanol extract of *S. plana* with formula of F-1 had particle sizes that fullfill the requirements as nanoparticles because it has a particle size of less than 1000 nm, namely 701.54±67.72 nm. It had the zeta potential value of +499.3±78.00 mV. The occurrence of a shift in the wave number of stretching vibrations of the O-H group, bending vibration of the N-H group, as well as the emergence of a new peak of the vibration of the phosphate group (P=O) in the infrared spectrum supported the formation of nanoherbal in F-1 formula. Nanoherbal F-1 has very strong antibacterial activity against *E.coli*, *S. dysentriae*, and *S. aureus* as well as strong antibacterial activity against *B. subtilis*. Nanoherbal F-1 showed stronger antibacterial activity than the ethanol extract of *S. plana*.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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References

1. Uluk A, Sudana M, Wollenberg E. The dependence of the Dayak community on the forest around the Kayan Mentarang national park. Bogor: Cifor; 2001; 15p.
2. Harada K, Rahayu M, Muzakkir A. Medicinal plants of Gunung Halimun National Park, West Java, Indonesia. Bogor: Biodiversity Conservation Project -JICA, PHPA & LIPI; 2002; 52-57p.
3. Setyawan AD and Darusman LK. Review: Biflavonoid compounds in *Selaginella Pal. Beau* and its utilization. Biodiversitas. 2008; 9(1):64-81.
4. Bensky D, Clavey S, Stöger E. Chinese herbal medicine. Materia medica**. (**3rd ed). Seattle, W.A.: Eastland Press; 2004; 286-293p.
5. Chikmawati T, Setyawan AD, Miftahudin. Phytochemical composition of *Selaginella spp* from Java Island Indonesia. Jurnal Makara Seri Sains. 2012; 16(2):129-133.
6. Chen JJ, Duh CY, Chen JF. New cytotoxic biflavonoids from *Selaginella delicatula.* Planta Med. 2005; 71(7):659-665.
7. Yang JW, Pokharel YR, Kim MR, Woo ER, Choi HK, Kang KW. Inhibition of inducible nitric oxide synthase by sumaflavone isolated from *Selaginella tamariscina*. J Ethnopharmacol. 2006; 105(1-2):107-113.
8. Sah NK, Singh SNP, Sahdev S, Banerji S, Jha V, Khan Z, Hasnain SE. Indian herb 'Sanjeevani' (*Selaginella bryopteris*) can promote growth and protect against heat shock and apoptotic activities of ultra violet and oxidative stress. J Biosci. 2005; 30(4):499-505.
9. Su Y, Sun CM, Chuang HH, Chang PT. Studies on the cytotoxic mechanisms of ginkgetin in a human ovarian adenocarcinoma cell line. Naunyn Schmiedebergs Arch. Pharmacol**.** 2000; 362(1):82-90.
10. Swamy RC, Kunert O, Schuhly W, Bucar F, Ferreira D, Rani VS, Kumar BR, Rao AVNA.. Structurally unique biflavonoids from *Selaginella chrysocaulos* and *Selaginella bryopteris*. Chem Biodivers. 2006; 3(4):405-414.
11. Lin LC, Kuo YC, Chou CJ. Cytotoxic biflavonoids from *Selaginella delicatula*. J Nat Prod. 2000; 63(5):627-630.
12. Yu S, Yan H, Zhang L, Shan M, Chen P 1, Ding A**,** Li SFY. A Review on the phytochemistry, pharmacology, and pharmacokinetics of amentoflavone, a naturally-occurring biflavonoid. Molecules. 2017; 22(2):299.
13. Mohanraj VJ and Chen Y. Nanoparticles-A Review. Trop J Pharm Res. 2006; 5(1):561-573.
14. Rizvi SAA and Saleh AM. Applications of nanoparticle systems in drug delivery technology. Saudi Pharm J. 2018; 26 (1):64-70.
15. Kammona O and Costas K. A review recent advances in nanocarrier-based mucosal delivery of biomolecules. J Contr Rel**.** 2012**;** 161(3):781-794.
16. Bhumkar DR and Varsha BP. Studies on effect of pH on crosslinking of chitosan with sodium tripolyphosphate: A technical note. AAPS Pharm Sci Technol. 2006; 7(2):1-6.
17. Kafshgari MH, Mohammad K, Mobina K, Sahar K. Reinforcement of chitosan nanoparticles obtained by an ionic crosslinking process. Iran Polym J. 2011; 20(5):445-456.
18. Atun S, Dewi Y, Aznam N. Characterization of nanocurcuminoid from ethanol extract of *Curcuma xanthorrhiza* rhizome loaded by chitosan and alginic and its antioxidant activity test. Rasayan J Chem. 2020; 13(2):817-825.
19. Agnihotri SA, Nadagounda NM, Tejraj MA. Recent advances on chitosan based micro and nanoparticles in drug delivery. J Contr Rel. 2004; 22(100):5-28.
20. Bangun H, Tandiono S, Arianto A. Preparation and evaluation of chitosan-tripolyphosphate nanoparticles suspension as an antibacterial agent. J Appl Pharm. Sci. 2018; 8(12):147-156.

21. Sutoyo S, Tukiran, Khotijah,S. Antioxydant activity of the silver nanoparticles (AgNPs) synthesized using *Nephrolepis radicans* extract as bioreductor. J Phys Conf Ser. 2021; 1747:012038.
22. Pakki E, Sumarheni S, Aisyah F, Ismail I, Safirahizdni S. Nanoparticle formulation of bawang dayak (*Eleutherine americana* (Aubl) Merr) extract with variation of chitosan tripoliphosphate (TPP) consentration. J Trop Pharm Chem. 2016; 3(4):251-263.
23. Wardana AP, Aminah NS, Fahmi MZ, Kristanti AN, Zahrah HI, Takaya Y, Choudhary MI. Nanoencapsulation of *Syzygium polycephalum* extract using folate modified κ-carrageenan as vehicles for pronounced anticancer activity. Trop J Nat Prod Res. 2020; 4(11):945-952.
24. Diastuti H, Chasani M, Suwandari S. Antibacterial activity of benzyl benzoate and crotepoxide from *Kaempferia rotunda* L. rhizome. Indo J Chem. 2020; 20(1):9-15.
25. Mostafa AA, Al-Askar AA, Almaary KS, Dawoud TM, Sholkamy EN, Bakri MM. Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases. Saudi J Biol Sci. 2018; 25:361-366.
26. Putri IA, Sundaryono A, Candra IN. Characterization of chitosan nanoparticles from sweet potato leaf extract (*Ipomoea Batatas* L.) using ionic gelation method. Alloptrop. 2018; 2(2):203-207.
27. Dogan M. Preparation of chitosan nanoparticles and characterization studies. Cumhur. Med J. 2020; 42(3):344-350.
28. Buzea C, Blandino IIP, Robbie K. Nanomaterial and nanoparticles: sources and toxicity, Biointerphases. 2007; 2:MR170– MR172.
29. Martien R, Adhyatmika A, Irianto IDK, Farida V, Sari DP. The development of nanoparticle technology as a drug delivery system. Majalah Farmaseutik. 2012; 8(1):133-144.
30. Ahdyani R, Novitasari L, Martien R, Danarti R. Formulation and characterization of timolol maleate-loaded nanoparticles gel by ionic gelation method using chitosan and sodium alginate. Int J Appl Pharm. 2019; 11(6):48-54.
31. Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. J Pharm Anal. 2016; 6(2):71-79.
32. Adamczak A, Ozarowski M, Karpinski, TM. Antibacterial activity of some flavonoids and organic acids widely distributed in plants. J Clin Med. 2020; 109(9):3-17.
33. Nugaraha AC, Prasetya AT, Mursiti S. Isolation, identification, activity test of flavonoid compounds as antibacterial from mango leaves. Indo J Chem Sci. 2017; 6(2):91-96.
34. Chakraborty K, Shivakumar A, Ramachandran S. Nano-technology in herbal medicines: A review. Int J Herb Med. 2016; 4(3):21-27.
35. Alqahtani F, Aleanizy F, Tahir EE, Alhabib H, Alsaif R, Shazly G, Alqahtani H, Alsarra A, Mahdavi J. Antibacterial activity of chitosan nanoparticles against pathogenic *N. gonorrhoea.* Int J Nanomed. 2020; 15:7877-7887.