Pharmacogn J. 2022; 14(2): 369-373
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com

Research Article

Formulation and Characterization of Meniran (*Phyllanthus Niruri Linn*) Extract Nanoparticle on Antibacterial Activity Against *Salmonella Pullorum*

Sri Hidanah, Emy Koestanti Sabdoningrum*, Sri Agus Sudjarwo

ABSTRACT

This study aims to examine the results of meniran extract (*Phyllanthus niruri* Linn.) on antibacterial activity. *Salmonella Pullorum* is capable of causing huge economic losses. The misuse of antimicrobials has resulted in the evolution of multidrug-resistant strains. Meniran has potential as an antibacterial because it contains many bioactive components such as alkaloids, flavonoids, tannins and saponins. Nanoparticles help in the bioavailability of plant extracts. The research was conducted by making a meniran extract nanoparticles formulation with ionic glass method using chitosan and TPP sodium with a dose difference of 5%, 10% and 20%. Each dose of meniran nanoparticles then were characterized by PSA, SEM and TEM. The result on PSA showed that size range from 192.67 nm to 385.16 nm and 5% meniran extract nanoparticles have the best homogeneity and stability. EE value showed that the increase in the dose was directly proportional to the increase in the EE value. The result on SEM showed that the overall production of nanoparticle samples, it looks like they are nano-sized. The result on TEM showed small sample morphology with a good distribution. After that, the antibacterial activity test was then carried out using the MIC and MBC tests. The results showed that 5% of meniran extract nanoparticles had the best antibacterial activity against *Salmonella Pullorum*.

Key words: *Phyllanthus niruri*, Nanoparticle, *Salmonella Pullorum*.

Sri Hidanah, Emy Koestanti Sabdoningrum*, Sri Agus Sudjarwo

Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, INDONESIA.

Correspondence

Emy Koestanti Sabdoningrum

Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, INDONESIA.

E-mail: emykoestanti10@gmail.com

History

- Submission Date: 02-02-2022;
- Review completed: 26-02-2022;
- Accepted Date: 08-03-2022.

DOI : 10.5530/pj.2022.14.47

Article Available online

http://www.phcogj.com/v14/i2

INTRODUCTION

Salmonella Pullorum is a bacteria that causes pullorum disease, a systemic disease that can cause huge economic losses. Antimicrobials have played an important role in the control of Salmonella, misuse of antimicrobials has resulted in the evolution of multidrug-resistant strains and made prevention and treatment more difficult.[1]

Indonesia is a tropical country that has wealth of various types of plants that have the potential as drugs which used by its population from generation to generation.[2-9] Meniran (*Phyllanthus niruri* Linn) is a type of herb that has medicinal properties and has potential as an antibacterial because it contains many bioactive components such as alkaloids, flavonoids, tannins and saponins.[10] This shows that meniran has the potential as an antibacterial against *Salmonella Pullorum* in form of nanoparticles.

The constraint in the use of plant extract is its low solubility in the digestive tract so that their absorption in blood plasma is low.[11] The use of nanoparticle preparations in assisting the bioavailability of plant extracts has been developed in recent years. Small particles have higher stability and bioavailability than conventional drug delivery systems, this is because of their smaller size, bioactive compounds are more easily absorbed directly by cells.[12,13] The characterization of nanoparticles consisted of particle size using Particle Size Analyzer (PSA) and particle morphology using Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM).

In this study, we conducted a study to obtain the results of formulation and charactarization of meniran (*Phyllanthus niruri* Linn.) extract nanoparticle on antibacterial activity against *salmonella pullorum*.

MATERIALS AND METHODS

Extraction of meniran (*Phyllanthus niruri* Linn.)

Meniran was macerated using 96% methanol solvent. The mixture was then soaked for 3 days and filtered. Maceration was conducted and clear colored filtrate was obtained. The filtrate obtained was concentrated with a rotary evaporator at 50°C.

Meniran (*Phyllanthus niruri* Linn.) extract nanoparticle formulation using ionic glass method

0.625 g, 1.25 g and 2.5 g of chitosan powder were dissolved in 100 mL 1% acetic acid with magnetic stirring for 30 minutes. Then the chitosan solution was filtered using filter paper and sonicated for 10 minutes.

The TPP solution was prepared by dissolving 0.125 g, 0.25 g, and 0.5 g of TPP sodium powder in 25 mL, 50 mL and 100 mL aquabidest (respectively).

Meniran extract doses 5%, 10% and 20% were prepared by dissolving 0.125 g, 0.25 g and 0.5 g of extract in 2.5 mL 96% ethanol, respectively.

Nanoparticles synthesis was carried out by adding 1 mL Tween 80 to the chitosan solution and stirred at 1000 rpm for 10 minutes. Then 5%, 10% and 20%

Cite this article: Hidanah S, Sabdoningrum EK, Sudjarwo SA. Formulation and Characterization of Meniran (*Phyllanthus Niruri Linn*) Extract Nanoparticle on Antibacterial Activity Against *Salmonella Pullorum*. Pharmacogn J. 2022;14(2): 369-373.

doses extract solutions were added and stirred for 30 minutes. Then the TPP solution was dripped and stirred for 3 hours at a speed of 1000 rpm.

The design for nanoparticles synthesis can be seen in the table below.

CHITOSAN	TPP	EXTRECT	Stirring speed (rpm)	Stirring time (hour)
0.625 g / 100 mL	0.125 g / 25 mL	0.125 g / 2.5 mL	1.000	3
1.25 g / 100 mL	0.25 g / 50 mL	0.25 g / 2.5 mL	1.000	3
2.5 g / 100 mL	0.5 g / 100 mL	0.5 g / 2.5 mL	1.000	3

Each sample was then characterized using PSA, SEM dan TEM tests.

Meniran (*Phyllanthus niruri* Linn.) extract nanoparticle characterization using Particle Size Analyzer (PSA)

The examination was carried out based on the Dynamic Light Scattering (DLS) method using the Zetasizer Nano ZS (Malvern Instruments). PSA was used to characterize the size of the nanoparticles. Each sample (maniran extract nanoparticles 5%, 10% and 20% doses) was dissolved in 3 ml of acetic acid. Then the solution is put into a tube with a maximum height of 15 mm. The diameter distribution of the samples was measured using the VASCO Nano Particle Analyzer.

Meniran (*Phyllanthus niruri* Linn.) extract nanoparticle characterization using Scanning Electron Microscopy (SEM)

SEM was used to characterize the morphology of the nanoparticles, the meniran extract nanoparticle powder was placed on the butt using a double-sided tape. The powder is then given a conductive electric with a thin layer of platinum beam from the coating for 30 seconds at pressure below 2 Pa and current strength of 30 mA. The image is taken at an electron voltage of 15 kV at the desired magnification.

Meniran (*Phyllanthus niruri* Linn.) extract nanoparticle characterization using using TEM

Transmission Electron Microscopy (TEM) is used to characterize the morphology of nanoparticles by placing the sample on a very thin gold grid, then in a vacuum tube the electrons will go to the sample which will analyze the sample size at a magnification of 500-10000x.

Test of *Salmonella Pullorum* antibacterial activity with dilution method

This test consisted of the Minimum Inhibitory Concentration (MIC) test and the Minimum Bactericidal Concentration (MBC) test. The MIC procedure was started using 18 test tubes for 3 treatments and 6 repetition for each treatment. Meniran extract nanoparticles at 5%, 10% and 20% doses were added 3 ml each into a test tube, then added 3 ml of *Salmonella Pullorum* (1 x 10^8 CFU/ml) for each concentration and then incubated at 37°C for one day. Observations of cloudy and clear at each dose that had been planted with *Salmonella Pullorum* were observed.

Five plates of Nutrient Agar (NA) media were used to perform the MBC test and each plate was divided into 3 treatments. The results of the MIC test were then planted on NA media and then at 37°C, the media was incubated for one day. The results can be observed by the presence or absence of the growth of *Salmonella Pullorum* colonies on NA media.

RESULTS AND DISCUSSION

The results of the meniran (*Phyllanthus niruri* Linn.) extract nanoparticle using the ionic glass method are shown in Figure 1. The meniran (*Phyllanthus niruri* Linn.) extract nanoparticle formulation using the ionic glass method was then characterized by PSA, SEM and TEM. The results of PSA characterization are shown in Table 1.

PSA results showed that there were differences in particle size at different doses with a size range from 192.67 nm to 385.16 nm. This shows that the sample being examined is in the form of nanoparticles because a formula can be called a nanoparticle if it has size in the range of 1-1000 nm.[14] The more the number of compound compositions in a nanoparticle formulation, the larger the particle size formed.

The polydispersity index shows the particle size distribution. The polydispersity index value which is getting closer to zero indicates a homogeneous particle distribution. Homogeneous particle distribution tends to be physically stable.[15] Based on the results, the polydispersity index that is closest to zero is the 5% meniran extract nanoparticles compared to the other two doses so the 5% meniran extract nanoparticles have the best homogeneity.

Zeta potential is used as a parameter to determine stability. Very small nanoparticles and low polydispersity index showed a high zeta potential value (above 30 mV) indicating a fairly stable particle.[16] The three samples of meniran extract nanoparticles showed high zeta potential values. The highest zeta potential value was shown by meniran extract (*Phyllanthus niruri* Linn.) nanoparticles at a dose of 5%. This indicates that the 5% dose of meniran extract nanoparticles (*Phyllanthus niruri* Linn.) is the most stable compared to other doses.

The amount of material, namely meniran extract, which was adsorbed in the chitosan nanoparticles was determined by calculating the Entrapment Efficiency (EE) using a microplate reader as an alternative to UV-VI spectro. The results of the EE value showed that the increase in the dose was directly proportional to the increase in the EE value. This shows that the higher the dose, the greater the material adsorbed in the nanoparticles.

The results of SEM examination with a magnification of 5 m showed the surface morphology and nanoparticle size as shown in Figure 2.

The results of Scanning Electron Microscopy (SEM) showed that from the overall production of nanoparticle samples, it looks like they are nano-sized. Scanning Electron Microscopy (SEM) is a test performed to observe the surface structure of the sample. SEM is able to show the sample area can be seen in a sufficiently large focus. SEM has the advantage of a relatively wide magnification range and the resulting image appears in three dimensions, making it easier for researchers to analyze.[17] The scattering pattern created by the interaction of the sample with the electron beam provides information about the size, shape, texture and composition of the sample.[18] SEM results of meniran (*Phyllanthus niruri* Linn.) extract nanoparticles showed an amorphous morphology and small particle size morphology.

The results of the Transmission Electron Microscopy (TEM) test are shown in Figure 2. Transmission Electron Microscopy (TEM) is a microscope that can magnify objects up to two million times using electrostatics and electromagnetics, which can form images with very good resolution.[19] The results of the TEM test showed small sample morphology with a good distribution.

Meniran extract nanoparticle activity test against *Salmonella Pullorum* bacteria using the MIC test is shown in Table 2. Meniran extract nanoparticle activity test against *Salmonella Pullorum* bacteria using the MBC test is shown in Table 3.

MIC and MBC tests showed that the nanoparticles of meniran extract were able to inhibit the growth and kill *Salmonella Pullorum* bacteria at doses of 5%, 10% and 20%. In Indonesia, herbal plants are abundant and often used as the main treatment option in rural areas.[20-23] Meniran (*Phyllanthus niruni* Linn.) is a type of herb that has medicinal properties

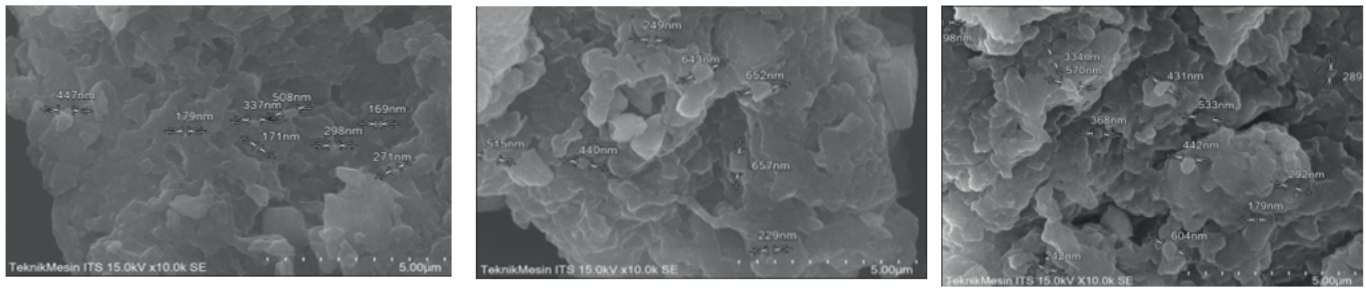


Figure 1: Morphology of meniran (*Phyllanthus niruri* Linn) extract nanoparticles using SEM test. (A) 5%, (B) 10%, (C) 20%.

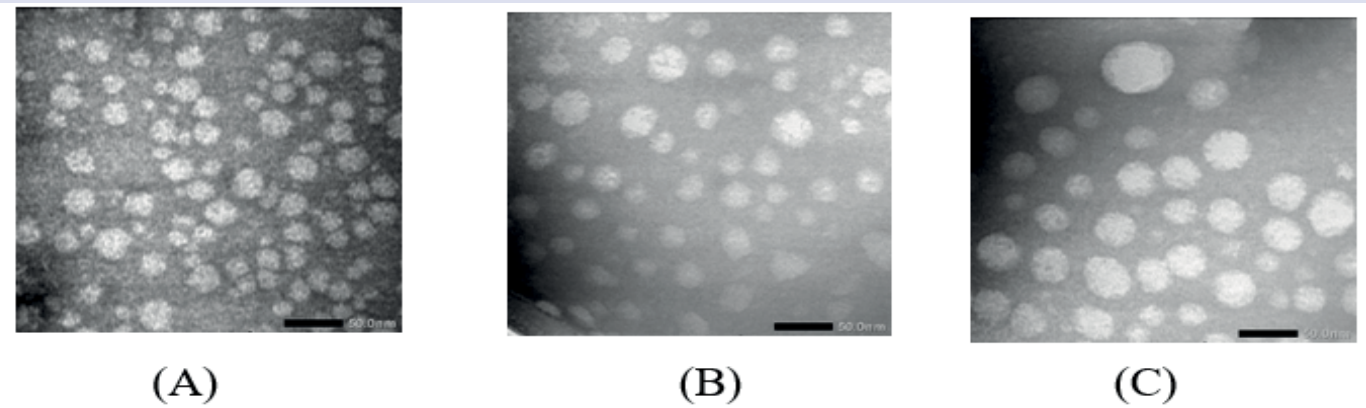


Figure 2: Results of meniran extract nanoparticles (*Phyllanthus niruri* Linn) using the TEM test. (A) 5%, (B) 10%, (C) 20%.

Table 1: Particle size of several doses meniran (*Phyllanthus niruri* Linn) extract nanoparticles.

Meniran Extract Nanoparticle Dose	Stirring Speed (rpm)	Stirring Time (hour)	Particle Size (nm)	Polidispersity Index	Zeta Potential	Entrapment Efficiency
5%	1000	3	192.67±2.90	0.37±0.01	552.33±24.34	56.22%
10%	1000	3	239.53±14.05	0.66±0.14	169.00±10.00	58.00%
20%	1000	3	385.16±10.04	0.83±0.29	119.33±2.08	61.69%

Table 2: Minimum Inhibitory Concentration (MIC) on meniran (*Phyllanthus niruri* Linn) extract nanoparticle.

Dose	Repetition					
	I	II	III	IV	V	VI
5%	-	-	-	-	-	-
10%	-	-	-	-	-	-
20%	-	-	-	-	-	-

Table 3: Minimum Bactericidal Concentration (MBC) on meniran (*Phyllanthus niruri* Linn) extract nanoparticle.

Dose	Repetition					
	I	II	III	IV	V	VI
5%	-	-	-	-	-	-
10%	-	-	-	-	-	-
20%	-	-	-	-	-	-

and has antibacterial potential because it contains many bioactive components such as alkaloids, flavonoids, tannins and saponins.[10]

The advantages of using nanoparticles are controlled size, narrow size distribution, selective and precise.[24] The antibacterial efficacy of nanoparticles is influenced by size and shape, the smaller the particle size, the stronger the effect of its antibacterial activity.

Chitosan is a natural biopolymer that is very promising to be used as a carrier in drug delivery systems. As a carrier, chitosan has been developed into nanoparticles. Among the various methods of making chitosan nanoparticles, ionic glass is a method that attracts attention because its simple process, does not use organic solvents and can be controlled easily. The principle of particle formation through this method is the occurrence of ionic interactions between

positively charged amino groups in chitosan and negatively charged polyanions to form a three-dimensional inter- and/or intramolecular network structure. The most widely used polyanion crosslinker is TPP sodium. The physical crosslinking process is able to prevent possible damage to the active ingredients that will be encapsulated in chitosan nanoparticles.[16]

CONCLUSION

Meniran extract nanoparticles at 5% dose had the antibacterial activity against *Salmonella Pullorum*.

ACKNOWLEDGMENT

We would to thank Kementerian Pendidikan, Kebudayaan Riset dan Teknologi for the financial support. We thank Arif Nur Muhammad Ansori (http://arifnma.wixsite.com/home) for editing the manuscript.

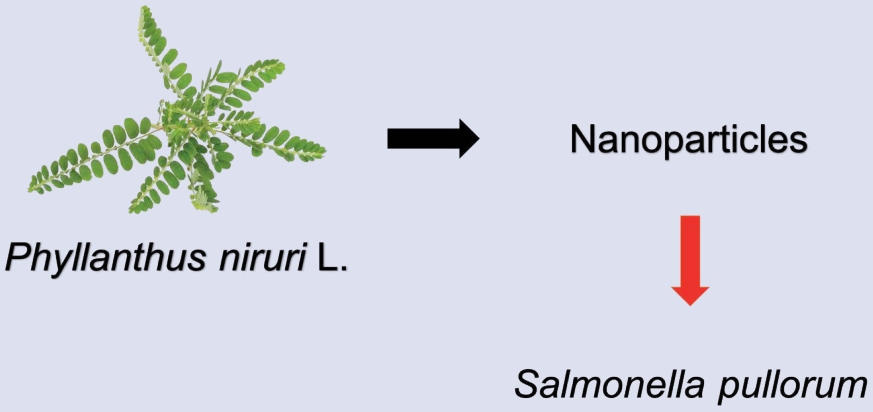
DISCLOSURE STATEMENT

No conflicts of interest.

REFERENCES

1. Gong J, Xu M, Zhu C, Miao J, Liu X, Xu B, *et al*. Antimicrobial resistance, presence of integrons and biofilm formation of Salmonella Pullorum isolates from eastern China (1962–2010). Avian Pathology. 2013;42(3):290-294.
2. Sudarno S, Setiorini FA, Suprapto H. Efektifitas Ekstrak Tanaman Meniran (*Phyllanthus niruri*) Sebagai Antibakteri Edwardsiella tarda Secara *In Vitro* [Effectivity of Meniran (*Phyllanthus niruri*) Extract as Edwardsiella tarda Antibacterial According *In Vitro*]. Jurnal Ilmiah Perikanan dan Kelautan. 2011;3(1):103-108.
3. Wahyuni DK, Ansori ANM, Vidiyanti F. GC-MS Analysis of phytocomponent in methanolic extracts of leaf-derived callus of Justicia gendarussa Burm of Bioscience Research. 2017;14(3):668-677.
4. Fadholly A, Ansori ANM, Jayanti S. Cytotoxic effect of *Allium cepa* L. extract on human colon cancer (WiDr) cells: *In vitro* study. Research Journal of Pharmacy and Technology. 2019;12(7):3483-3486.
5. Husen SA, Wahyuningsih SPA, Ansori ANM. Therapeutic effect of okra (*Abelmoschus esculentus* Moench) pods extract on streptozotocin-induced type-2 diabetic mice. Research Journal of Pharmacy and Technology. 2019;12(8):3703-3708.
6. Husen SA, Winarni D, Khaleyla F. Activity assay of mangosteen (*Garcinia mangostana* L.) pericarp extract for decreasing fasting blood cholesterol level and lipid peroxidation in type-2 diabetic mice. AIP Conference Proceedings. 2017;1888(1):020026-1-6.
7. Husen SA, Khaleyla F, Kalqutny SH. Antioxidant and antidiabetic activity of *Garcinia mangostana* L. pericarp extract in streptozotocin-induced diabetic mice. Bioscience Research. 2017;14(4):1238-1245.
8. Ansori ANM, Susilo RJK, Hayaza S. Renoprotection by *Garcinia mangostana* L. pericarp extract in streptozotocin-induced diabetic mice. Iraqi Journal of Veterinary Sciences. 2019;33(1):13-19.
9. Ansori ANM, Fadholly A, Hayaza S. A review on medicinal properties of mangosteen (*Garcinia mangostana* L.). Research Journal of Pharmacy and Technology. 2020;13(2):974-982.
10. Fitri I. Efektivitas antibakteri ekstrak herba meniran (*Phylanthus niruni*) terhadap pertumbuhan bakteri *Salmonella* sp. dan *Propionibacterium acnes*. JST (Jurnal Sains dan Teknologi). 2017;6(2):300-310.
11. Barragan PLA, Figueroa JJB, Duran LVR, González CNA, Hennigs C. Fermentative Production Methods. Biotransformation of Agricultural Waste and By-Products. 2016;189-217.
12. Joye IJ, Davidov-Pardo dan G, McClements DJ. Nanotechnology for increased micronutrient bioavailability. Trends in Food Science & Technology. 2014;40(2):168-182.
13. Jafari SM, McClements DJ. Nanotechnology Approaches for Increasing Nutrient Bioavailability. Advances in Food and Nutrition Research. 2017;1-30.
14. Nugroho BH, Wardhani MT, Suparmi S. Perbandingan Teknik Aerasi dan Ultrasonikasi Gelasi Ionik Nanopartikel Deksametason Natrium Fosfat. Jurnal Kefarmasian Indonesia. 2020;102-109.
15. Speed OOS, Toward ST. Optimasi Kecepatan dan Lama Pengadukan Terhadap Ukuran Nanopartikel Kitosan-Ekstrak Etanol 70% Kulit Jeruk Siam (*Citrus nobilis* L. var Microcarpa).
16. Mardliyati E, Muttaqien SE, Setyawati DR. Sintesis nanopartikel kitosan-trypoly phosphate dengan metode gelasi ionik: pengaruh konsentrasi dan rasio volume terhadap karakteristik partikel. Prosiding Pertemuan Ilmiah Ilmu Pengetahuan dan Teknologi Bahan. 2012;90:93.
17. Stadtlander CTK. Scanning Electron Microscopy and Transmission Electron Microscopy of Mollicutes: Challenges and Opportunities, Modern Research and Educations Topics in Microscopy: USA. 2007;29634.
18. Choudhary OP, Priyanka. Scanning Electron Microscope: Advantages and Disadvantages in Imaging Components. International Journal of Current Microbiology and Applied Sciences. 2017;6(5):1877-1882.
19. Tacharina MR, Ansori ANM, Plumeriastuti H. Beneficial effect of grinting grass (*Cynodon dactylon*) on the streptozotocin induced diabetes mellitus in the mice. Indian Veterinary Journal. 2020;97(4):35-38.
20. Fadholly A, Ansori ANM, Proboningrat A. Apoptosis of hela cells via caspase-3 expression induced by chitosan-based nanoparticles of *Annona squamosa* leaf extract: *In vitro* study. Indian Journal of Pharmaceutical Education and Research. 2020;54(2):416-421.
21. Husen SA, Salamun, Ansori ANM. Renal protective effects of gamma-mangostin in streptozotocin-induced diabetic mice. Indian Journal of Forensic Medicine and Toxicology. 2020;14(3):1221-1226.
22. Husen SA, Setyawan MF, Syadzha MF. A novel therapeutic effect of *Sargassum ilicifolium* alginate and okra (*Abelmoschus esculentus*) pods extracts on open wound healing process in diabetic mice. Research Journal of Pharmacy and Technology. 2020;13(6):2764-2770.
23. Husen SA, Wahyuningsih SPA, Ansori ANM. The effect of okra (*Abelmoschus esculentus* Moench) pods extract on malondialdehyde and cholesterol level in STZ-induced diabetic mice. Ecology, Environment and Conservation. 2019;25(4):S50-S56.
24. Kalishwaralal K, Ramkumarpandian DVS, Nellaiah H, Sangiliyandi G. Extracellular Biosynthesis of Silver Nanoparticles by the Culture Supernatant of *Bacillus licheniformis*. Materials Letters. 2008;62(29):4411-4413.

GRAPHICAL ABSTRACT



ABOUT AUTHORS

Emy Koestanti Sabdoningrum has conducted several research regarding the utilazation of Indonesia medicinal plants for enchancing animal productivity and health. She is one of members of Animal Husbandry Division in Faculty of Veterinary Medicine, Airlangga University, Indonesia.

Cite this article: Hidanah S, Sabdoningrum EK, Sudjarwo SA. Formulation and Characterization of Meniran (*Phyllanthus Niruri Linn*) Extract Nanoparticle on Antibacterial Activity Against *Salmonella Pullorum.* Pharmacogn J. 2022;14(2): 369-373.