



Green-Synthesis Of Silver Nanoparticles Based On Bioreductor Key Orange Extract As Antibacterial

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*Silver nanoparticles are nano-sized particles ranging from 1-100 nm which are commonly used as antifungal and antibacterial agents in various products. In this study, silver nanoparticles were synthesized from a natural bioreductant, namely key orange peel extract. The silver nanoparticle phase which was successfully synthesized and then XRD test was carried out was cubic in shape with the highest peak at $2\theta : 38.1959^\circ; 44.4038^\circ; 64.5491^\circ$. The lattice parameter of silver nanoparticles is $a=b=c= 4.0861$. The resulting crystal structure of silver nanoparticles is cubic with a crystallite size of 39.84 nm, in addition to XRD test, XRF test was conducted to determine the main constituent components of silver nanoparticles, while the main component of synthesized silver nanoparticles was Ag of 97.66%. Antibacterial activity of silver nanoparticles was indicated by the formation of inhibition against the growth of *Staphylococcus aureus* bacteria with a clear zone formed around 12.091 mm. Therefore, the antibacterial inhibition formed from the silver nanoparticles synthesized in this study can be classified as a strong antibacterial property.*

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INTRODUCTION

Nanotechnology is a field of physics, chemistry, biology and engineering that has attracted attention in the research world in recent years. Nanotechnology that is being developed at this time is nanoparticles because of the very wide application of nanoparticles both from agriculture to the biomedical world [1]. Nanoparticles are a type of nanotechnology that has nano-sized particles ranging from 1-100 nm. The nanoparticles that have attracted the most attention are metal nanoparticles, various types of metal nanoparticles that have been synthesized at this time are gold, silver, iron, zinc and metal oxide nanoparticles [2]. Of the several types of nanoparticles, silver nanoparticles are the type of nanoparticles that are often studied by scientists today. The application of silver nanoparticles can be used as antifungal and antibacterial in various products such as socks, wet wipes, food storage containers and others [3].

There are several nanoparticle synthesis techniques such as precipitation, hydrothermal techniques, thermolysis, photolysis, sonolysis, sol-gel and polyol [4]. One of these techniques still have drawbacks because they can cause enormous environmental pollution and harmful by-products. Therefore, at this time the green-synthesis nanoparticles is a topic that is seriously studied. The green-synthesis nanoparticle method is a nanoparticle synthesis method that uses natural ingredients such as plant extracts as the bioreductant agent. This method is considered clean, non-toxic and more environmentally friendly than conventional nanoparticle synthesis methods [5].

Factors that affect particle size in the green-synthesis include the type of bioreductant, reaction time, and solution temperature [6]. Natural reducing agents can be found in plants that contain secondary metabolites such as terpenoids, flavonoids, and tannins which have antioxidant activity [7]. In this study, key orange peel extract (*Citrus x microcarpa bunge*) was used as a natural bioreductant in the preparation of silver nanoparticles. Key oranges are a fruit that is widely used in various food products and the presence of this plant is very abundant in the Bangka Belitung Islands. The main content of key oranges is steroids, tannins, flavonoids, alkaloids, terpenoids and saponins [8]. The compound is a phenolic compound which theoretically has reducing properties so that the key citrus plant can be used as a bioreductant in the synthesis of silver nanoparticles [9]. The characteristics of nanoparticles, provide many environmentally friendly benefits and are suitable for pharmaceutical and other biomedical applications because they do not use toxic chemicals. So this research will also examine the antibacterial activity of the synthesized silver nanoparticles.

METHODS

Extraction of Key Orange

The key orange peels used in this study were collected from Bangka Regency, Bangka Belitung Islands. The key orange peel is cleaned and then dried by drying in the sun. The dried key orange peel is blended until smooth and then stored in a clean container at room temperature. A total of 5 grams of finely chopped key orange peel was dissolved in 150 mL of distilled water then stirred for 30 minutes and brought to a boil, the boiling extract was filtered and the residue was taken and then stored at 4° C.

Synthesis of Silver Nanoparticles

We used AgNO₃ as a source of silver nanoparticles in this research. A total of 12 grams of silver AgNO₃ dissolved in 400 mL of distilled water and stirred until homogeneous. The solution was added with key orange peel extract with a ratio of AgNO₃ with key orange extract of 10:1 then titrated using NaOH to pH 7 and stirred for 48 hours. The synthesized solution was aged for 24 hours then filtered and dried.

Antibacterial Test

Antibacterial activity was tested qualitatively using the disc paper method [10]. The initial step of antibacterial testing using the paper disc method is to make nutrient agar (NA) media as a medium for bacterial growth. All equipment and NA media were sterilized by autoclaving. Next, the NA medium was put into a petri dish and test tube. This bacterial test was carried out using *S. aureus* which was applied in a zig-zag manner into the media that had been made, the paper disc was immersed in a solution of silver nanoparticles and placed into a petri dish that had been filled with media and bacteria. After that, the media was incubated for 24 hours. The inhibition of the test was determined by measuring the width of the clear zone formed around the paper disc.

RESULTS AND DISCUSSION

Based on Figure 1, the FTIR spectrum of the key orange peel extract can be observed that there is absorption in the wave number region of 3300 cm⁻¹ which indicates the -OH functional group [11]. According to Gurunathan, *et al.*, 2009, functional group -OH is a group that can accelerate the process of production of Ag⁺. Then there is an absorption at 2923 cm⁻¹ which indicates the CH functional group, besides that there is an absorption at 1614 cm⁻¹, 1442 cm⁻¹, 1247 cm⁻¹, and 1022 cm⁻¹, where each absorption indicates the presence of a C functional group. =C, CH₂, CH and CC. These functional groups are functional groups that indicate the presence of phenolic compounds in key orange peel.

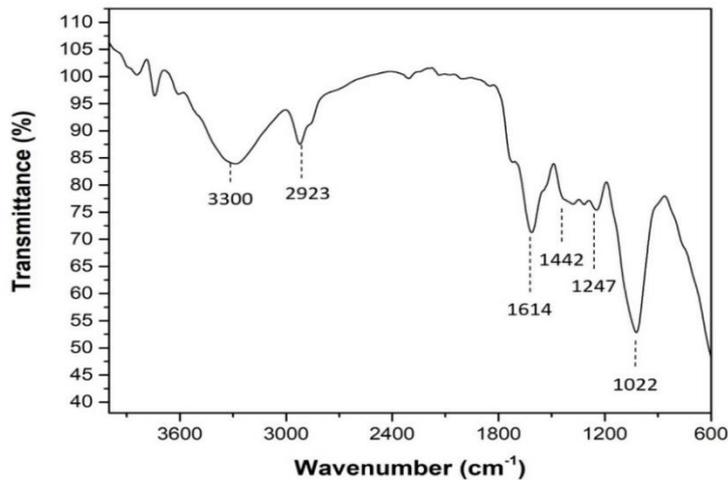


Figure 1. FTIR spectrum of Key Orange Peel Extract

In Figure 2.(b) During the mixing process of AgNO_3 solution with key orange peel extract, the color of the solution was originally yellow, after adding NaOH, the color of the solution changed to brown and gradually became gray as shown in Figure 2.(d). The color change is one indicator of the formation of silver nanoparticles because of the silver ion reduction process [13]. The synthesis of silver nanoparticles was carried out by stirring method using a magnetic stirrer for 48 hours. After the addition of NaOH, the solution which was initially yellow changed to brown and gradually became gray accompanied by a precipitate.

The synthesis of silver nanoparticles using orange peel extract is included green synthesis method which is an environmentally friendly method [14]. In this study, the source of Ag used was silver nitrate (AgNO_3) which was then mixed into a key orange peel extract solution which produced a grayish brown precipitate (Figure 3) and indicated the formation of silver nanoparticles [15].

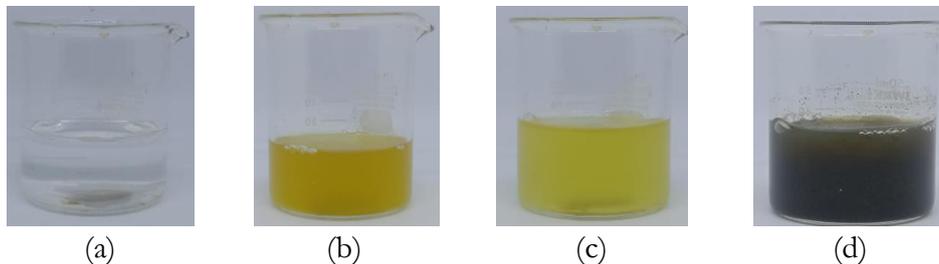


Figure 2. Color Solution (a) AgNO_3 Solution (b) Key Orange Peel Extract Solution (c) Mixed Solution (d) Solution After Addition of NaOH



Gambar 3. Nanopartikel perak

XRF characterization is used to determine the composition of the material. Based on Table 2 through the XRF test that has been carried out, it is known that the content contained in silver nanoparticles using a key orange peel bioreductant is Ag of 97.66%.

Table 2. Nanoparticle Composition data Through XRF Characterization

Element	Persentase Kandungan (%)
Si	0.14
P	0.30
Fe	1.91
Ag	97.66
Total	100.00

Based on XRD analysis, the silver nanoparticle difactogram can be seen in Figure 4. Silver nanoparticles were successfully synthesized using key orange peel extract according to [16] which produces silver nanoparticle peaks. The highest peak is located at $2\theta : 38,1959^\circ; 44,4038^\circ; 64,5491^\circ$. The silver nanoparticle lattice parameter is $a=b=c= 4.0861 \text{ \AA}$. The resulting crystal structure of silver nanoparticles is cubic with a crystalline size of 39.84 nm.

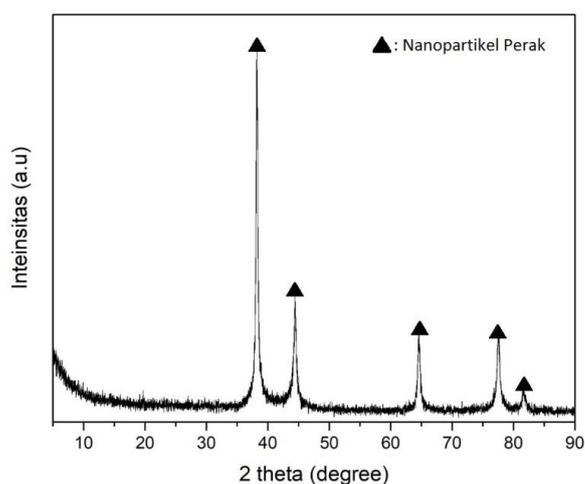


Figure 4. XRD Characterization of Silver Nanoparticle

The bacterial test in this study aimed to determine the antibacterial activity of silver nanoparticles that were successfully synthesized. In this study, an antibacterial test was carried out on *S. aureus* using the paper disc method. The testing process used disc paper soaked in a solution of silver nanoparticles then attached to the bacterial growth medium and incubated for 24 hours. The results of the antibacterial test of silver nanoparticles can be seen in Figure 4. The formation of a clear zone on the agar medium, called the inhibition zone, of 12.091 mm indicates that the silver nanoparticles synthesized in this study have strong antibacterial properties.

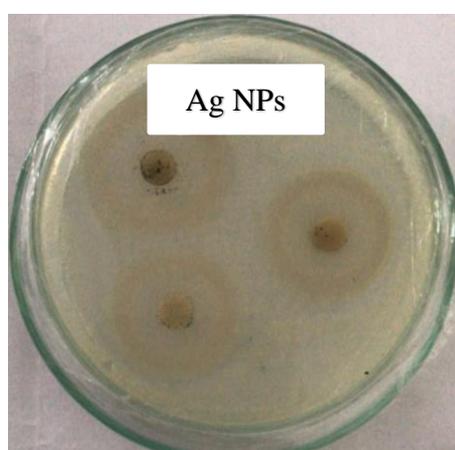


Figure 5. Activity of Silver Nanoparticles Against *S.aureus*

CONCLUSION

Based on this research, it is known that silver nanoparticles can be synthesized using the green-synthesis from a natural bioreductant of key orange peel. Through XRD analysis, it is known that the crystal structure of the synthesized silver nanoparticles has a cubic shape with a crystallite size of 39.84 nm. XRF analysis also showed that the main component of the synthesized silver nanoparticles was Ag at 97.66%. Through the antibacterial activity test of synthesized silver nanoparticles against *S. aureus*, it was found that silver nanoparticles had a strong category of inhibition. Based on these parameters, it can be stated that this research can provide an alternative method of synthesizing silver nanoparticles that are environmentally friendly.

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REFERENCES

- [1] Prasetiowati, A. L., Prasetya, A. T., & Wardani, S. (2018). Sintesis Nanopartikel Perak dengan Bioreduktor Ekstrak Daun Belimbing Wuluh (*Averrhoa Bilimbi* L.) sebagai Antibakteri. *Indonesian Journal of Chemical Science*, 7 (2), 160-166.
- [2] Prasad, S. B. (2013). Current Understanding of Synthesis and Pharmacological Aspects of Silver Nanoparticles. *American Journal of Photomedicine and Clinical Therapeutics*, 1 (7), 536-547.
- [3] Khayadrov, R. R., Khayadrov, R. A., Estrin, Y., Evgrafova, S., Scheper, T., Endres, C., et al. (2009). Silver Nanoparticles. *Nanomaterials : Risks and Benefits*, 4, 287-297.
- [4] Lalena, J. N., Cleary, D. A., Carpenter, E. E., & Dean, N. F. (2008). *Inorganic Materials Synthesis and Fabrication*. Hoboken: Jhon Wiley & sons, Inc.
- [5] Raghunandan, D., Mahesh, B. D., Basavaraja, S., Balaji, S. D., Manjunath, S. Y., & Venkataranam, A. (2011). Microwave-Assisted Rapid Extracellular Synthesis of Stable Bio-functionalized Silver Nanoparticles from Guava (*Psidium guajava*) Leaf Extract. *Journal Nanopart Res*, 3, 2021-2028.
- [6] Sileikaite, A. P., Igoris, P., Judita, J., & Algimantas, G. A. (2006). Analysis of Silver Nanoparticles Produced by Chemical Reduction of Silver Salt Solution. *Journal Material Science*, 12 (4), 1392-1320.
- [7] Shankar, S. S. (2004). Rapid Synthesis of Au, Ag and Bi, metallic Au Core-Ag Shell Nanoparticles Using Neem (*Azadirachta indica*) Leaf Broth. *Journal Colloid Interface Science*, 275 (4), 496-502.
- [8] Roanisca, O., Rani, & Mahardika, R. G. (2021). PHYTOCHEMICALN SCREENING AND ANTIBAKTERIAL POTENCY OF JERUK KUNCI FRUIT WASTE (*Citrus x microcarpa bunge*) EXTRACT AGAINST *Propionibacterium acnes*. *Journal Pijar MIPA*, 16 (3), 387-392.
- [9] Philip, D. (2010). Green Syntesis of Gold and Silver Nanoparticles Using *Hibiscus rosasinesis*. *Physica E*, 42(5), 1417-1424.
- [10] Wahyudi, T., & Sugiyana, D. d. (2011). Sintesis Nanopartikel Perak dan Uji Aktivitasnya terhadap bakteri *E.coli* dan *S.aureus*. *Arena Tekstil*, 20 (1), 55-60.
- [11] Skoog, D. A. (2007). *Principles of Instrumental Analysis*. USA: Thomson Higher Education.

- [12] Gurunathan, S., Kalimuthu, K., Ramanathan, V., Venkataraman, D., Sureshbabu, R., Jeyaraj, M., et al. (2009). Biosynthesis, purification, and characterization of silver nanoparticles using *E. coli*. *Colloids and surface B: Biointerfaces*, 74, 328-335.
- [13] Sankar, N. S., Dipak, P., Nilu, H., Dipta, S., & Samir, K. P. (2014). Green Synthesis of Silver Nanoparticles Using Fresh Water Green Alga *Pithopora Oedogonia* (Mont). Wittrock and Evaluation of Their Antibacterial Activity. *Appl Nanosci*, 5, 703-709.
- [14] Thakkar, K., & Mathre, S. d. (2010). Biological synthesis of metallic nanoparticle. *Nanomedicine: nanotechnology, biology and medicine*, 6, 257-262.
- [15] Yugandhar, P., & Savitramma, N. (2015). Biosynthesis, Characterization and Antimicrobial Studies Green synthesis Silver Nanoparticles From Fruit Extract of *Syzygium Alternifolium* (Wt.) Walp. an Endemic, Endangered Medicinal tree Taxon. *Appl Nanosci*, 6, 223-233.
- [16] Notirawan, H., Ernisa Gustria., et al (2015). Aktivitas Antibakteri Nanopartikel Perak Hasil *Green Synthesis* menggunakan ekstrak kulit buah kemuning (*murraya paniculata*). Jurnal pendidikan Biologi dan Sains (BIOEDUSAINS), Vol.3 No.2, 140-144.