ISSN: 1410-8917
e-ISSN: 2597-9914

Jurnal Kimia Sains dan Aplikasi 26 (3) (2023): 85–90

Jurnal Kimia Sains dan Aplikasi
Journal of Scientific and Applied Chemistry

Journal homepage: http://ejournal.undip.ac.id/index.php/ksa

Tyrosinase Inhibitory of Silver Nanoparticles Synthesized using *Morus Nigra* Leaves Extract

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https://doi.org/10.14710/jksa.26.3.85-90

Article Info

Article history:

Received: 21st December 2022
Revised: 26th April 2023
Accepted: 03rd May 2023
Online: 31st May 2023

Keywords:
Morus nigra L.; silver nanoparticles; tyrosinase activity; green synthesis; novel application

Abstract

A novel preparation of silver nanoparticles using *Morus nigra* leaves extract, as opposed to the physical and chemical methods, had been used in this work. Their active phytochemical compounds will reduce Ag^{+} and form AgNPs (Ag^{0}). A peak spectrum at 460 nm was formed and confirmed as the Surface Plasmon Resonance (SPR). The vibrations that appeared at 1643 cm^{-1} and 3286 cm^{-1} have the characteristics of a C=C bond and a hydroxyl group (-OH), respectively. An X- ray diffraction (XRD) examination of silver with good crystallinity revealed its distinctive pattern. According to the results of transmission electron microscopy (TEM), the produced AgNPs-*Morus nigra* leaves extract were between 10 and 20 nm in size. Using L-dihydroxyphenylalanine (L-DOPA) as the substrate, the synthesized AgNPs-*Morus nigra* were tested for their tyrosinase inhibitory activity, and the results are substantial when compared to kojic acid as a control. The percentages of inhibition from crude extract, AgNPs, and kojic acid at 100 µg/mL are found at 12.10%, 64.80%, and 59.84%, respectively. Based on the results of this work, AgNPs can be produced by utilizing a green synthesis method with leaf extract, making them a promising candidate for use in medicine and cosmetics.

1. Introduction

The multifunctional enzyme tyrosinase (EC 1.14.18.1), sometimes called polyphenol oxidase (PPO), is made of copper and has two copper ions in the catalytic center. Some plants, bacteria, fungi, and mammals contain tyrosinase [1]. Tyrosinase is an enzyme that converts the substrates L-tyrosine and L-DOPA to o-dopaquinone, assisting in the manufacture of melanin. L-DOPA is created when tyrosinase first catalyzes the hydroxylation of L-tyrosine to create dopaquinone [2]. Dopaquinone spontaneously transforms into dopachrome, which eventually transforms into melanin. The excessive formation of melanin can disturb pigmentation and may initiate hyperpigmentation [3]. These enzymes can be prevented from functioning during catalytic reactions by the inhibitor tyrosinase, which is catalyzed by tyrosinase and forms covalent bonds [4]. Tyrosinase inhibitors can be made naturally from plant tissues or by chemical synthesis [5]. The hydroxyl group on rings A and B of flavonoid compounds, which reduces the activity of the tyrosinase enzyme, has chelating and tyrosinase inhibitory properties. Anti-tyrosinase activity is also quite strong in plants with high antioxidant activity [2]. The ethanolic extract from *Asphodelus microcarpus* has shown high antioxidant and tyrosinase inhibitory activity [6].

Due to their distinctive catalytic properties, silver nanoparticles have received much interest in magnetic [7], electrical [8], oxidation resistance [9], biostabilities [10], and biocompatibility properties [11]. Physicists, chemists, biologists, doctors, and material scientists have used silver nanoparticles as functional materials

[12]. It is possible to synthesize silver nanoparticles through physical, chemical, and biological processes [13]. The most common technique is chemical, which typically involves toxicants [14]. However, expensive equipment is typically needed with physical methods [15]. Based on this problem, synthesis using biological methods has several advantages because they are inexpensive and environmentally friendly. Numerous plants, including plants [16, 17], seeds [18], leaves [19], flowers [20], fruits [21], and fruit peels [22], have been utilized to successfully create silver and gold nanoparticles as part of the ongoing study of plant-mediated synthesis.

In this article, we present and discuss the data on the tyrosinase inhibitory activity of AgNPs produced using an extract of *Morus nigra* leaves. *Morus nigra* is a member of the genus *Morus*, the tribe *Moracea*, and the family *Moraceae* [23] . Additionally, the extract of *Morus nigra* leaves includes antioxidant properties [24]. To the best of our knowledge, neither the synthesis of AgNPs by leaf extract nor the tyrosinase inhibitory activity of *Morus nigra* have been reported. According to this study, AgNPs synthesized using an extract of *Morus nigra* leaves had good tyrosinase inhibitory activity. Figure 1 depicts a schematic of the synthesis of silver nanoparticles mediated by *Morus nigra* leaf extract and their use as tyrosinase inhibitors.

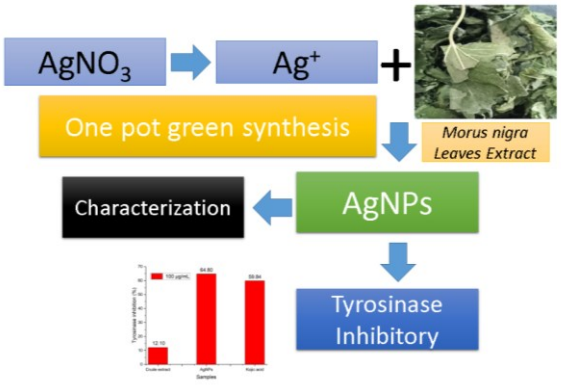

Figure 1. Schematic representation of synthesis silver nanoparticle mediated by *Morus nigra* leaves extract and its application for tyrosinase inhibitory

2. Materials and Methods

2.1. Materials

All of the chemicals were bought from Merck (Germany). At BALITTRO (*Balai Penelitian Tanaman Rempah dan Obat*), Indonesia, dried leaf powder of the *Morus nigra* was acquired.

2.2. Preparation of *Morus nigra* leaves extract

100 mL of sterile distilled water was combined with 1 g of dried *Morus nigra* leaves powder to prepare a broth solution. The filter paper was used to separate this mixture to obtain a clear yellow extract, which was then stored at 4°C.

2.3. Phytochemical analysis of *Morus nigra* leaves extract

Bioactive secondary metabolites were found via phytochemical screening. The particular tests were used to check for the presence of terpenoids, alkaloids, tannins, saponins, flavonoids, and steroids.

2.4. Synthesis of AgNPs using *Morus nigra* leaves extract

One mL of *Morus nigra* leaf extract and 5 mL of an aqueous solution containing 100 ppm silver nitrate ($AgNO_3$) were combined and agitated at 30°C for 10 minutes to synthesize AgNPs-*Morus nigra* leaves extract. After 30 minutes, there was a color change from yellow to orange-brown, which meant that AgNPs-*Morus nigra* leaves extract had formed and surface plasmon resonance excitation had occurred in metal nanoparticles. Using a Microcentrifuge KHT 410 E, AgNPs-*Morus nigra* leaves extract was centrifuged for 20 minutes at 5000 rpm for further characterization.

2.5. UV–Vis spectral analysis

The UV-Vis spectra of the $AgNO_3$ solution, leaves extract, and reduction of silver ions to produce AgNPs were observed using a PG Instrument T80+ UV-Vis spectrophotometer with a resolution of 1 nm between 350 and 550 nm.

2.6. FTIR spectral analysis

Shimadzu IRTracer-100 Fourier transformed IR spectroscopy (FTIR) spectra were used in the 500-4000 cm^{-1} range at the powdered sample. The sample was then mixed with potassium bromide (KBr) to analyze functional groups from biomolecules in AgNPs-*Morus nigra* leaves extract.

2.7. TEM and XRD analysis of AgNPs

Tecnai G2 20S-Twin Function Transmission Electron Microscope (TEM) was used to conduct electron microscopy investigations. The crystallinity and the particle sizes of AgNPs-*Morus nigra* leaves extracts were evaluated using Shimadzu X-ray Diffractometer 7000 X- ray Diffraction (XRD) at 30 kV, 40 mA, and Cu Kα radians at a 2theta angle.

2.8. Tyrosinase inhibitory (%) activity

The tyrosinase inhibitory activity was assessed by spectrophotometric analysis using L-DOPA as the substrate. A 12.428 U of mushroom tyrosinase, 3.0 mL of reaction mixture containing 1.5 mM L-DOPA, 0.1 mM sodium phosphate buffer (pH 6.5), and the test sample were incubated at 30°C for 20 minutes. At 475 nm, the development of dopachrome was seen. The proportion of inhibited tyrosinase activity was calculated using the formula (1).

$$\text{Tyrosinase inhibitory activity (\%)} = \frac{(A-B)-(C-D)}{(A-B)} \text{ x } 100\ \% \quad (1)$$

where, A and B are the absorbances of the blank solution after and before incubation, respectively. C and D are the absorbances of the sample solution after and before incubation, respectively.

3. Results and Discussion

Using the following chemicals, the extracts were qualitatively screened for the presence of phytochemical

components such as alkaloids, tannins, saponins, flavonoids, steroids, and terpenoids (Table 1).

Table 1. List of the chemicals used to identify secondary metabolites in extracts

Phytochemicals	Method	Results for the presence
Alkaloids	Extract + H_2SO_4 + Dragendorff reagent	Reddish-orange precipitation
Tannins	Extract + $FeCl_3$	Dark green color
Saponins	Extract + H_2O (boiled)	Frothing happened
Flavonoids	Extract + NaOH + HCl	The addition of HCl caused the yellowish tint to fade
Steroids	Extract + acetic acid + H_2SO_4	The color changed to green
Terpenoids	Extract + chloroform + H_2SO_4	Reddish brown color

Table 2 lists the qualitative screening for evaluating bioactive secondary metabolites, such as alkaloids, tannins, saponins, flavonoids, steroids, and terpenoids in *Morus nigra* leaves extract. From the previous study, these compounds have a significant role in reducing silver nanoparticle synthesis [25, 26, 27, 28, 29, 30].

Table 2. Phytochemical evaluation of *Morus nigra* leaves extract

Constituent	Results
Alkaloids	+
Tannins	+
Saponins	+
Flavonoids	+
Steroids	+
Terpenoids	+

The compound is indicated by "+" when it is present and "-" when it is absent

UV-visible spectroscopy is a technique that is regarded as an extension of colorimetry. It operates on the idea of light absorption from the test sample. It uses a variety of colorimeter-like components, but spectroscopy has the advantage of improved accuracy over a broad range of wavelengths between 200 and 800 nm [31]. The addition of *Morus nigra* leaves extract to $AgNO_3$ solution results in a color change from light pale yellow to dark brown during the first 30 minutes of the reaction. The formation of silver nanoparticles in the reaction medium enables to produce color change due to their specific optical properties. Surface plasmon resonance (SPR) revealed that AgNPs-*Morus nigra* leaves extract were successfully synthesized when there was no peak seen in the spectra of the $AgNO_3$ solution between 350 and 500 nm by using a UV-Vis spectrophotometer (Figure 2). A peak was seen at 460 nm when the solution mixture ($AgNO_3$ + *Morus nigra* leaves extract) was examined at the same wavelengths. This peak indicates the reduction of silver ions to AgNPs [32].

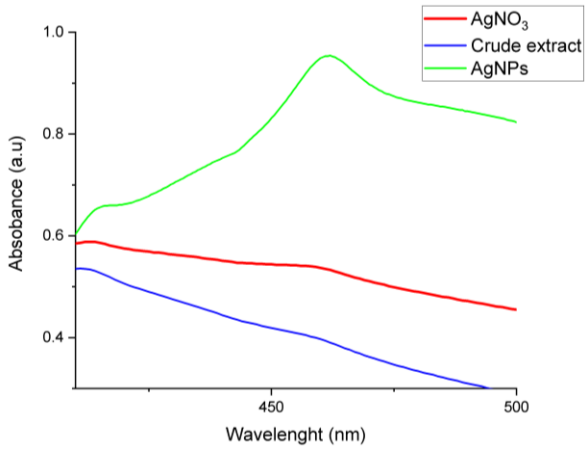

Figure 2. UV-Vis spectra of AgNPs, $AgNO_3$, and *Morus nigra* leaves crude extract

Using a Fourier transform infrared spectrometer (FTIR), the identification of functional groups on AgNPs-*Morus nigra* leaves extract was assessed in the range 4000-500 cm^{-1}. Figure 3 shows the spectrum acquired by analyzing the AgNPs-*Morus nigra* leaves extract. FTIR spectra showed strong bands at 3286 cm^{-1} assigned to hydroxyl groups and the band at 1643 cm^{-1}, characteristic of C=C bonds. From this spectrum, flavonoid and phenolic groups from leaves extract could be responsible for the reduction of metal ions into nanoparticles.

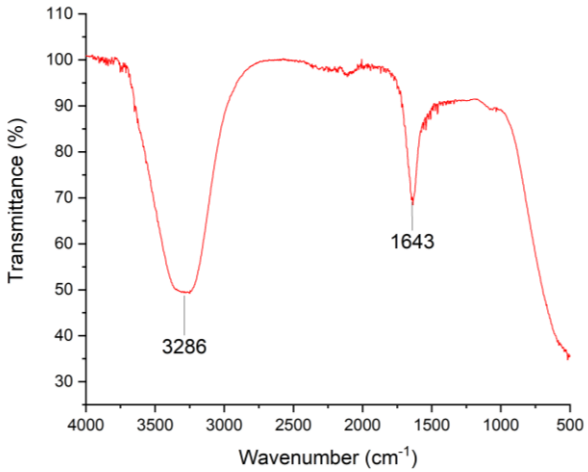

Figure 3. FTIR spectra of AgNPs-*Morus nigra* leaves extract

TEM analysis determined the size and form of the AgNPs-*Morus nigra* leaves extract with different magnifications. The nanoparticle with a mean size of 16.69 ± 4.69 nm is depicted in Figure 4 as dispersed and spherical. Distribution and variations in particle sizes were due to the formation of silver nanoparticles at different time intervals [33].

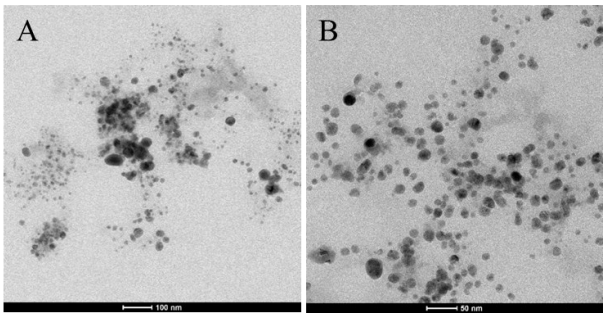

Figure 4. TEM micrographs of AgNPs under different scale bar corresponds to (A) 100 nm, (B) 50 nm

AgNPs exhibit four diffraction peaks at 2theta values of 38.17°, 45.50°, 64.43°, and 76.10° in their XRD patterns, which, respectively, correspond to the (111), (200), (220), and (311) planes (Figure 5). The obtained data were consistent with the database (JCPDS No.01-087-0717). The subsequent XRD spectrum showed that the generated AgNPs were crystallized, and the patterns observed were consistent with prior studies [34]. The bio-organic phase crystallization that took place on the surface of the nanoparticles is what caused the unassigned peaks (*) to be seen [4]. Similar outcomes in silver nanoparticles synthesized by *Skimmia laureola* leaf extract have also been reported [35].

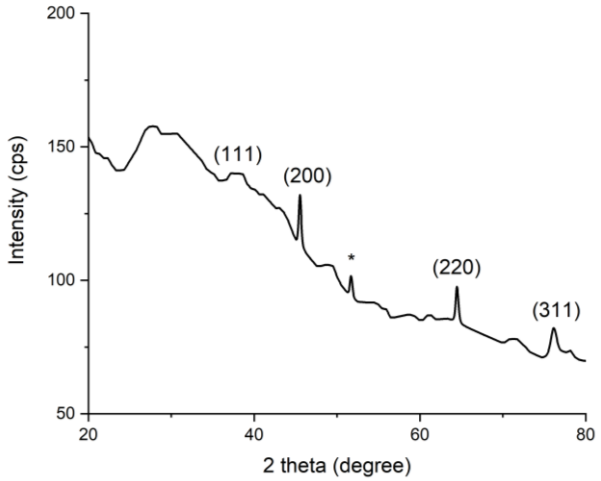

Figure 5. XRD patterns of AgNPs

Using the representative enzyme mushroom tyrosinase in a preliminary screening, we found that AgNPs significantly inhibited tyrosinase L-DOPA oxidation (64.80% at 100 µg/mL) compared to crude extract and kojic acid at the same concentration. Figure 6 displays the percentage of inhibition. Tyrosine is converted to dihydroxy-phenylalanine (DOPA) and then to DOPA quinone by the tyrosinase enzyme involved in melanin production. Tyrosinase is a metalloenzyme containing copper at an active site. It catalyzed to change the oxidative site of copper atoms [5]. The proposed mechanism of tyrosinase inhibitory by using AgNPs can be seen in Figure 7. In this case, silver nanoparticles (AgNPs) may compete with copper atoms as a cofactor and deactivate tyrosinase.

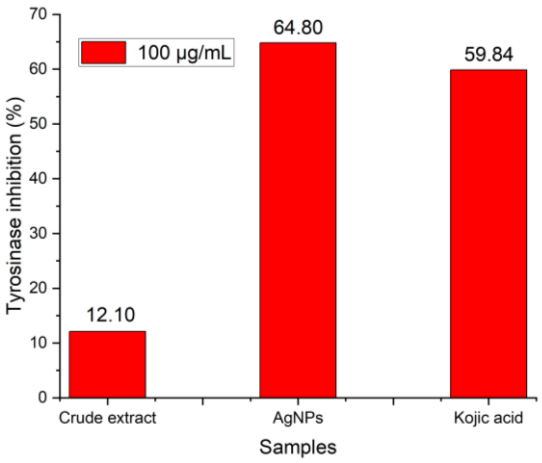

Figure 6. Tyrosinase inhibitory activity of AgNPs

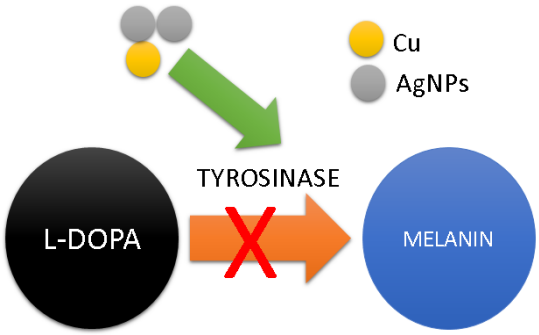

Figure 7. Proposed mechanism of tyrosinase inhibitory using AgNPs

4. Conclusion

In conclusion, we synthesized and demonstrated how to make spherically shaped and dispersed AgNPs by using an extract of *Morus nigra* leaves as a reducing agent. According to UV-Vis and FTIR data, biomolecules found in leaves were crucial in the synthesis of AgNPs-*Morus nigra* leaves extract. TEM and XRD measurements showed that AgNPs-*Morus nigra* leaves extract are highly crystalline and have an average size of 16.69 ± 4.69 nm. From this work, AgNPs-*Morus nigra* leaves extract is thought to have a high tyrosinase inhibitory activity that can be used in the pharmaceutical industry.

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