



An Eco-Friendly Synthesis of Silver Nanoparticles Using Extract of *Capsicum chinense* Jacq. as A Bio-reducing Agent

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Abstract

Two different types of inductions, direct sunlight and heating, were demonstrated for the green synthesis of silver nanoparticles (AgNPs) using a natural reagent from *Capsicum chinense* Jacq. extract as a bio-reducing agent. The study was initialized by characterization of the extract using high-performance liquid chromatography-mass spectrometry to define the major compounds actively as bio-reducing agents. Both inductions techniques were studied towards the effect of extract concentrations and duration of sunlight exposure or heating temperatures to obtain optimum conditions for AgNPs synthesis. The product AgNPs were characterized using Ultraviolet-Visible and Fourier Transform Infra-Red Spectroscopy, Scanning Electron Microscopy, X-Ray Diffraction, and Particular Size Analyzer. Sunlight induction showed more effectiveness (higher product) than heating induction. The optimum conditions of biosynthesis of AgNPs were attained at 12% extract and AgNO₃ (1 mM) and a duration of sunlight exposure of 90 min, resulting in AgNPs characterized with a maximum wavelength of 422 nm and average particle sizes of 67.94 nm. From the results of LC-MS/MS analysis, it is known that capsaicin compound is a significant component that may act as a bio-reducing agent.

1. Introduction

Metal nanoparticles, such as silver nanoparticles (AgNPs), can be used as antimicrobials [1, 2, 3], biosensors [4, 5, 6], composite fibers [7], semiconductor materials [8], and other medical applications due to their unique characteristics such as surface plasmon features, morphology, and interesting physicochemical properties among various nanomaterials [9]. Nanoparticles are dispersed particulates with a size of 1-100 nm [10]. Some methods for synthesizing metal nanoparticles include chemical reduction [11], electrochemical techniques [12], and green routes [13].

Green chemistry has recently received much attention by reducing toxic chemicals and utilizing natural products. The nanoparticle synthesis method can be carried out using plant extracts as bio-reducing agents, such as *Phyllanthus acidus* extract [14], grape and tomato extracts [15], *Piper retrofractum* extract [16], *Coffea*

canephora fruit skin extract [17] and *Capsicum fluorescence* [18]. Plant extracts have heteroatomic rings and hydroxyl, carbonyl, and carboxyl groups that can act as reducing agents as well as stabilizers in the synthesis of nanoparticles. The synthesized nanoparticles using these plant extracts showed different shapes and sizes depending on the species of plants.

This study used *Capsicum chinense* Jacq., known as *cabai katokkon* or Toraja chili [19], the most popular chili widely cultivated in the highlands of the Tana Toraja district as a natural reagent for synthesizing AgNPs. This type of chili contains antioxidant compounds, including capsaicin, ascorbic acid, and secondary metabolite compounds [19, 20] which have the potential as reducing agents for synthesizing AgNPs. Besides, these compounds are also expected to act as a capping agent, a binding molecule that stabilizes the colloidal nanoparticles by inhibiting the over-growth of

nanoparticles and avoiding AgNP agglomeration. Thus, no additional capping agent is required to produce nanoparticles via green synthesis.

Therefore, the purpose of the study was to conduct the synthesis of AgNPs by utilizing an extract of *C. chinense* Jacq. as a bio-reducing agent. The effect of extract concentrations, duration of exposure to sunlight, temperatures, and pHs on AgNP formation was studied to obtain the optimum synthesis condition. Generally, the smaller sizes of nanoparticles were obtained in higher extract concentrations and higher temperatures [21, 22]. In this research, the characteristic of the biosynthesized AgNPs was analyzed using Ultraviolet-Visible Spectrophotometry (UV-Vis), Fourier Transform Infra-Red (FTIR), Scanning Electron Microscopy (SEM), X-Ray Diffraction (XRD), and Particular Size Analysis (PSA).

2. Methodology

2.1. Materials and Instrumentation

C. chinense Jacq. was obtained from Tana Toraja District, South Sulawesi, Indonesia. Silver nitrate (AgNO_3) was purchased from Sigma Aldrich, Singapore. Instrumentations involved in this study include a hotplate (DLAB MS-H280-Pro), UV-Vis (Shimadzu UV-Vis 1601 series), FTIR (Shimadzu 8400s), SEM (FEI Inspect-S50), XRD (Panalytical X'Pert Pro), PSA (Malvern Nano ZS ZEN35000), LC-MS/MS (UHPLC Vanquish Tandem Q Exactive Plus Orbitrap HRMS ThermoScientific), and freeze dryer.

2.2. Preparation of 20% (w/v) *C. chinense* Jacq. extract

The extract was prepared by modifying the previous method [23]. Fresh *C. Chinense* Jacq. was washed thoroughly with demineralized water, wiped with tissue to dry, and then cut into small pieces. 20 g of finely cut pieces of *C. chinense* Jacq. was transferred into a beaker glass containing 100 mL water and heated at 60°C for 15 min. The mixture was then filtered using filter paper, followed by centrifugation. The extract was collected as a natural reagent to synthesize AgNPs. This extract was characterized using UV-Vis and FT-IR spectrophotometers and LC-MS/MS.

2.3. Synthesis of AgNPs using *C. chinense* Jacq.

In this study, synthesis was done using two heating processes, electrical heating using a hot plate and sunlight exposure. The synthesis of AgNPs was done using modified previous methods [16]. The AgNPs were synthesized by mixing 0.1 mM AgNO_3 solution with *C. chinense* Jacq. extract. Then the mixture was exposed directly to sunlight radiation, and the formation of AgNPs can be visually observed by a color change from colorless to brown. The product of the AgNPs solution was characterized further by scanning using a UV-Vis spectrophotometer at a wavelength range from 200 to 800 nm. This procedure was repeated for synthesizing AgNPs using heating induction. Figure 1 illustrates the schematic steps for synthesizing the formation of AgNPs using *C. chinense* Jacq. extract using both inducing methods, sunlight, and heating inductions.

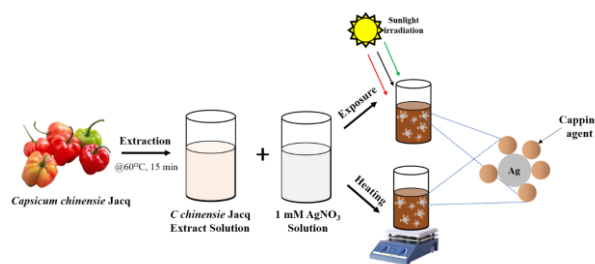


Figure 1. Illustration of the synthesis of AgNPs using sunlight and heating inductions

In this research, both induction techniques were optimized towards the % extract (4–12 %) and induction time from 15 to 120 min and processed as in Figure 1. Sunlight induction was done under bright sunlight from 10 AM to 12 PM with the intensity of sunlight ranging from 53.990 to 118.100 lux and the temperature of sample solution ranging from 30 to 36°C, while the heating induction was kept at 50°C to maintain the stability of the active compounds of extract. The product AgNPs solutions were scanned spectrophotometrically at a wavelength range from 200 to 800 nm for initial characterization.

2.4. Characterization of AgNPs

The AgNPs products were characterized further using FTIR, SEM, PSA, and XRD. The AgNPs solution was filtered and washed with distilled water and freeze-dried before being characterized using FTIR, SEM, and XRD to study the functional groups, morphological images, and crystallinity, respectively. FTIR spectrophotometer (Shimadzu 8400s) and the spectrum were recorded at 4.000–400 cm^{-1} . The XRD was set up to obtain XRD-diffractogram in the range of 2θ from 20° to 90°. The size and zeta potential of AgNPs was assessed by Particular Size Analyzer (PSA).

3. Results and Discussion

3.1. Characteristic of *C. chinense* Jacq. Aqueous Extract

The aqueous extract of *C. chinense* Jacq is colorless with a UV-Vis spectra profile showing two λ_{max} at 228 nm and 276 nm (Figure 2), representing the character of capsaicin and dihydrocapsaicin compounds [24, 25, 26]. The extract's highest content of capsaicin and dihydrocapsaicin exhibits potent antioxidant activity, which can be used as a reducing agent in the formation of nanoparticles.

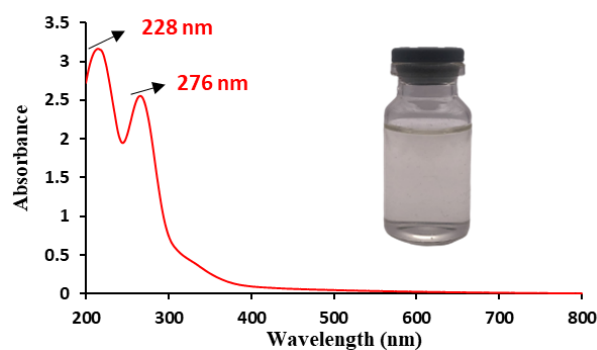


Figure 2. Profile spectra of *C. chinense* Jacq. extract

The extract was also characterized using LC-MS/MS to find the major compounds contributing to the reduction process of Ag⁺ to Ag⁰ and stabilizing agents (capping agents), which prevent agglomeration of the obtained AgNPs. The compound of the extract resulting from LC-MS/MS is shown in Table 1.

Table 1. LC-MS/MS data of *C. chinense* Jacq. extract

Compound	Formula	t _R (min)	% Area
Capsaicin	C ₁₈ H ₂₇ NO ₃	16.56	16.41
L-Phenylalanine	C ₉ H ₁₁ NO ₂	2.71	4.92
Arginine	C ₆ H ₁₄ N ₄ O ₂	1.01	4.79
Asparagine	C ₄ H ₈ N ₂ O ₃	1.05	3.17
Dihydrocapsaicin	C ₁₈ H ₂₉ NO ₃	17.67	2.57
L-Tryptophan	C ₁₁ H ₁₂ N ₂ O ₂	5.27	1.18

t_R: Retention time

Table 1 shows that six major compounds appear at retention times of 1.01, 1.05, 2.71, 5.27, 16.56, and 17.67, respectively. Based on the MS/MS fragmentation results, the six compounds can be identified as arginine, asparagine, L-phenylalanine, L-tryptophan, capsaicin, and dihydrocapsaicin. Based on the percent area, the major component in the *C. chinense* Jacq. extract is capsaicin, with 16.56 % of the area. Thus, capsaicin is one of the main compounds taking a role as a bio-reducing agent in the synthesis of AgNPs.

3.2. Synthesis of AgNPs using *C. chinense* Jacq. under sunlight exposure

The successful synthesis of AgNPs was recognized from the color change of the solution from colorless to brownish orange by irradiation under sunlight [25]. In addition, the formation of AgNPs can be characterized by the absorption peak in the 400–500 nm wavelength region, corresponding to the absorption of colloidal AgNPs [27, 28]. This absorption peak and the color change are caused by surface plasmon resonance (SPR) due to electron excitation on the surface of AgNPs.

3.2.1. Effect of Extract Concentration

The effect of extract concentration during the formation of AgNPs was observed by mixing 0.1 mM AgNO₃ solution with *C. chinense* Jacq. extract from 4 to 12%, followed by direct sunlight irradiation for 60 min. It can be seen in Figure 3 that AgNPs were successfully produced using *C. chinense* Jacq. under sunlight induction, shown by the brown color of the AgNPs solution, which gives λ_{max} at 422 nm.

As expected, higher concentrations of extracts produced higher color intensity of AgNPs solutions, with the greatest concentration obtained at 12% extract concentration (Figure 3). Under the chosen condition of extract concentration, no shift of plasmon resonance peak to a lower maximum wavelength due to the change in nanoparticle size as reported by previous researchers was observed [27]. The higher the extract concentration, the greater the amount of reducing agent and stabilizer compound, thus producing more nanoparticles with high stability [28]. Stabilizing agents also contribute to controlling the size of nanoparticles by preventing

agglomeration, i.e., clustering single nanoparticles to form a more significant size due to the high surface area of nanoparticles and the strong attractive interaction between particles [29].

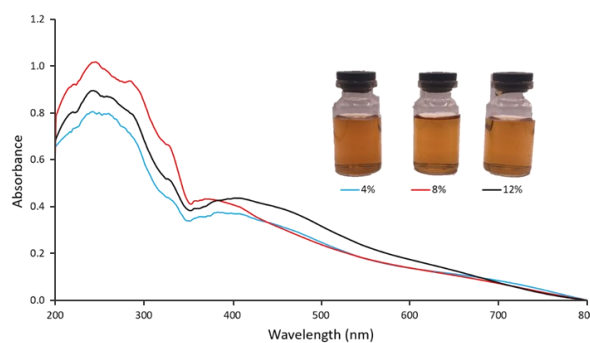


Figure 3. UV-Vis spectra profile of extract and AgNPs at various ratios

3.2.2. Effect of Duration of Sunlight Exposure

It is common to involve heat in the synthesis of nanoparticles to control the magnitude of the nanoparticles' size with uniform distribution and high crystallinity. Sunlight induction was studied under various duration of exposure up to 120 min. The absorbance of AgNPs increases with the increase of the duration of sunlight exposure up to 90 min and remains constant for a longer time (Figure 4).

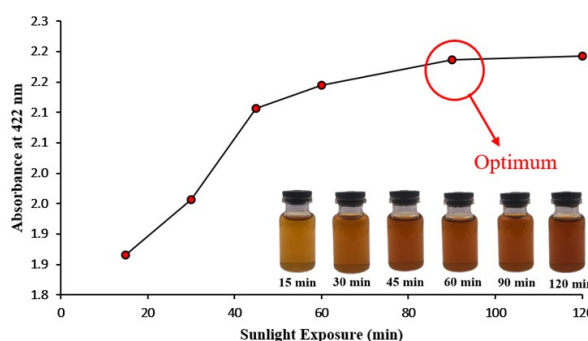


Figure 4. Effect of duration of exposure to sunlight

It can be seen from Figure 4 that the longer the exposure time, the more significant nanoparticles are formed and reach the maximum at 90 min. No significant increase of nanoparticle product was observed when the exposure was increased up to 120 min. The 90 min exposure was then used as the optimum time for the AgNPs synthesis process.

3.3. Synthesis of AgNPs Using *C. chinense* Jacq. with Electrical Heating

3.3.1. Effect of Extract Concentrations

Similar to that of sunlight induction, the effect of extract concentration using heating induction during the formation of AgNPs was conducted by mixing 0.1 mM AgNO₃ solution with *C. chinense* Jacq. extract from 4 to 12% followed by heating at 50°C for 90 min. The product of AgNPs under heating induction showed less brown color with λ_{max} at a lower wavelength (410 nm) than that of sunlight irradiation. As expected, higher concentrations of extracts produced greater AgNPs

concentration, shown by higher absorbance, with the greatest concentration obtained at 12% extract concentration (Figure 5).

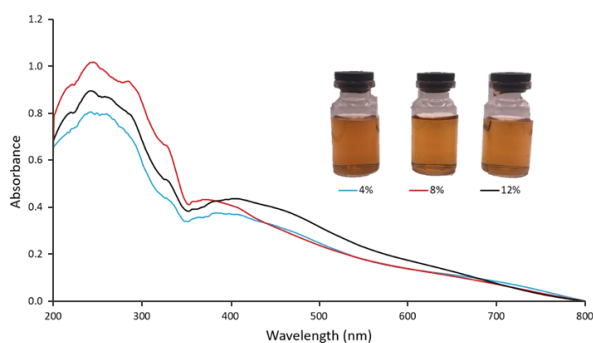


Figure 5. UV-Vis spectra profile of AgNPs using electrical heating at various ratios

3.3.2. Effect of Duration of Heating

Heating induction was studied under various durations of heating up to 210 min and maintained the temperature constant at 50°C. No color change was observed under heating up to 45 min, indicating no formation of AgNPs. The AgNPs started to form at a heating time of 60 min, and the color intensity of AgNPs (and absorbance) increased dramatically by increasing the duration of heating up to 120 min. However, more prolonged heating (above 120 min) shows a less significant increase and lower reaction rate towards equilibrium.

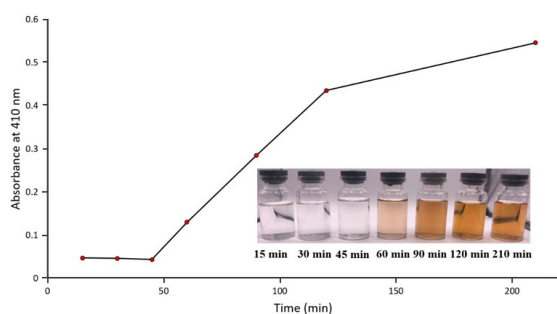


Figure 6. Effect of duration of heating

3.4. Comparative Analysis Sunlight vs. Heating Induction

Based on the results, using both inductions, sunlight and heating can produce AgNPs as discussed above. However, as can be seen from the overlay of UV-Vis spectra (Figure 7a), sunlight irradiation showed superior shown by greater absorbance compared to that heating induction. The results were strengthened by Figure 7b, in which under various extract concentrations, the product AgNPs under sunlight have greater concentrations indicated by higher absorbances.

The stability of AgNPs using the sunlight induction based on the absorbance measurement showed constant for up to 11 days. A significant decrease in absorbance was observed after 11 days. The decrease in absorbance could be due to agglomeration of the resulting AgNPs as a result of the decomposition of capping agents from the extract by the bacteria. Surprisingly, although the AgNPs under

heating induction produce lesser AgNPs at the beginning (day-1), the absorbance increased significantly during prolonged storing up to day-13. Based on this observation, the reaction rate under heating induction is lower than sunlight induction, and the reaction continued up to day-13. (Figure 8a).

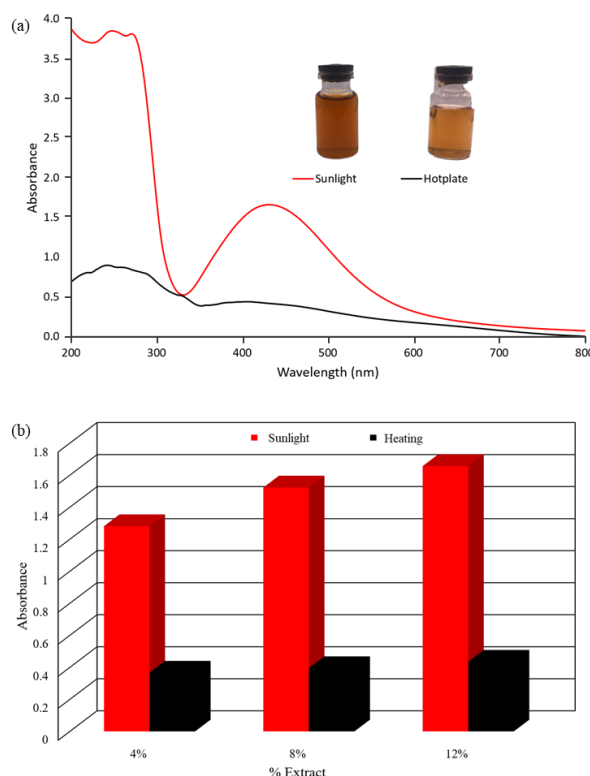


Figure 7. AgNPs sunlight vs. AgNPs heating: (a) UV-Vis profile and color changes, (b) composition of the extracts with AgNO₃

3.5. Characterizations

UV-Vis spectrophotometer was the first to identify AgNPs through the maximum wavelength and absorbance formed. Characterization technique using UV-VIS spectroscopy can provide information about the size and shape of silver nanoparticles, in which small spheres of silver nanoparticle (10–50 nm) commonly have an absorbance peak near a λ_{max} of 400 nm, while larger spheres (100–200 nm) appear as a broader peak with a λ_{max} near 500 nm. The maximum λ obtained from the UV-Vis spectra of synthesized AgNPs using sunlight induction and heating induction shows a specific band that appeared at 422 nm and at 410 nm, which corresponds to the size of AgNPs at 50–100 nm.

The role of the active compound of extract in the formation and stabilization of AgNPs was evaluated by FT-IR analysis. The spectra were recorded in the region 400–4000 cm^{-1} to determine the specific absorption of AgNPs and the functional groups of extract compounds involved in forming AgNPs. The spectra of AgNPs are shown in (Figure 8b), which shows the new absorption band in the fingerprint region around 400–600 cm^{-1} , which is expected to be the characteristic band of Ag-OH [30].

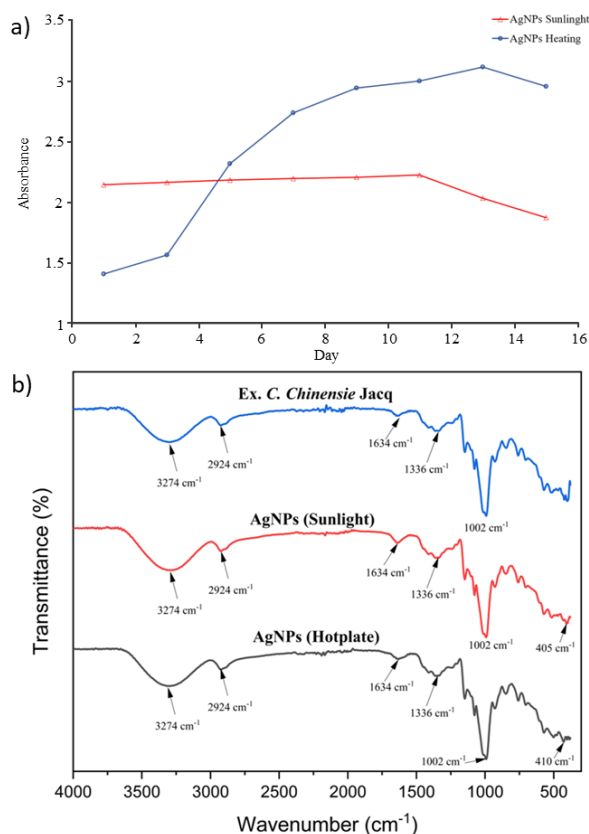


Figure 8. Characterizations of *C. Chinense Jacq.* extract synthesized AgNPs using sunlight and synthesized AgNPs using hotplate: a) UV-Vis spectrum of stability compound, b) FTIR spectra

Figure 8b displayed the FTIR spectrum of 3 samples: extract, AgNPs/extract/sunlight, and AgNPs/extract/heating with a difference in intensity and slight shift, confirming the coordination of phytochemicals with the AgNPs during the synthesis. The spectrum likely represents the character of capsaicin as the major compound in the extract of *C. Chinense Jacq.* The peaks in the region of 3200–3600 cm⁻¹ (broad, medium) can be assigned to the presence of N-H and O-H stretching vibration characteristics for amine and alcohol. Wavenumbers in 2926–2866 cm⁻¹ range show an aliphatic C-H stretching vibration, and the presence of C=O stretching vibration is recognized at 1634 cm⁻¹. AgNPs/extract for both techniques showed slightly stronger C=O stretching vibrations, which were likely assigned to the other C=O group resulting from the oxidation of the O-H group of capsaicin as a reducing agent of Ag⁺ to Ag⁰. All samples showed peaks in the range of 1400–1556 cm⁻¹ due to the presence of C–C vibration at stretching vibration in the aromatic ring and C–H bending at a range of 804–775 cm⁻¹. A range of 1000–1300 cm⁻¹ was also observed in all samples due to the stretching vibration in C–O.

Characterization using SEM aimed to show the morphology of the AgNPs. The result of SEM images can be seen in Figure 9. Figure 9b of SEM images at 10,000× magnification gives a more apparent morphology of AgNPs than Figure 9a at 5000× magnification. The size range varied from 80 to 120 nm in diameter. The shapes of AgNPs was dominated irregular, and some fraction

resulted in spherical. The previous study reported the irregular shape using another reducing agent, such as *Artocarpus heterophyllus lam* [29]. Meanwhile, the spherical shape also has been reported using other plant extracts, including *Azadirachta indica* [31]. The irregular and roughly spherical shape of AgNPs may be caused by the bulky size of tannin and smaller molecules, such as chlorogenic acid, as stabilizers during the interaction for forming AgNPs [32].

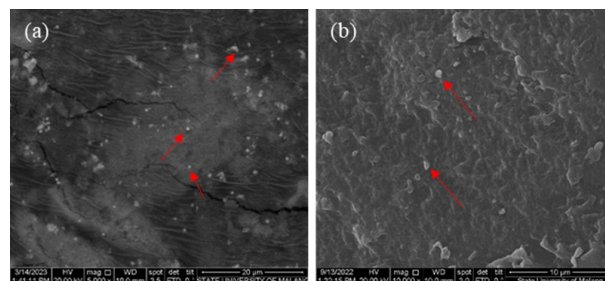


Figure 9. SEM analysis of (a) AgNPs with 5000× magnification and (b) AgNPs with 10,000× magnification

The size distribution, stability, and net surface of the synthesized AgNPs were determined using the particle size analyzer (PSA) (Malvern instrument). The products of AgNPs prepared by both inductions (sunlight and electrical heating) are shown in Table 2.

Table 2. The effect of different inductions on the particle size of AgNPs

Parameters (Ratio)	λ_{max} (nm)	Size of AgNPs (d.nm)	PdI	Zeta Potential (mV)
AgNPs Sunlight	422	67.94	0.419	-20.3
AgNPs Heating	410	38.13	0.339	-19.5

The synthesized AgNPs using sunlight induction resulted in a larger particle size (67.94 nm) than heating induction (38.13 nm). This is supported by the surface plasmon resonance peak (UV-Vis spectra), in which the AgNPs Sunlight induction absorbed a longer wavelength (422 nm) than the AgNPs–heating induction (410 nm). The size distribution of metallic nanoparticles is presented by the PdI index from “0” to “1”, indicating monodisperse distribution to polydisperse distribution of silver nanoparticles. The PdI values of AgNPs from both induction techniques exist at 0.419 and 0.339, indicating the formation of a relatively monodisperse distribution of nanoparticles [33]. Furthermore, the negative charge indicates a high electrical charge on the particle surface due to their strong repulsion forces and to prevent the possibility of aggregation of AgNPs. The negative value confirms the particle’s repulsion and ensures stable synthesized AgNPs [34].

The XRD pattern of crystalline silver nanoparticles crystalline should be supported by the appearance of four distinct diffraction peaks at 2θ values of 38.05°, 44.23°, 64.41°, and 76.66° which correspond to crystallin plane (111), (200), (220), and (311) of the face-centered cubic structure of silver. A diffractogram of synthesized AgNPs is shown in Figure 10. X-ray diffraction (XRD) patterns

were conducted by PAN Analytical using Cu K radiation ($K = 1.5406 \text{ \AA}$) to confirm the crystal of AgNPs. However, the results only showed a 2θ value of 38.05 , corresponding to crystalline plane 111 . This is perhaps the resulting AgNPs powder contained matrix extract with sticky properties; thus, a refinery process must be truly free from extract to obtain the actual structure of AgNPs.

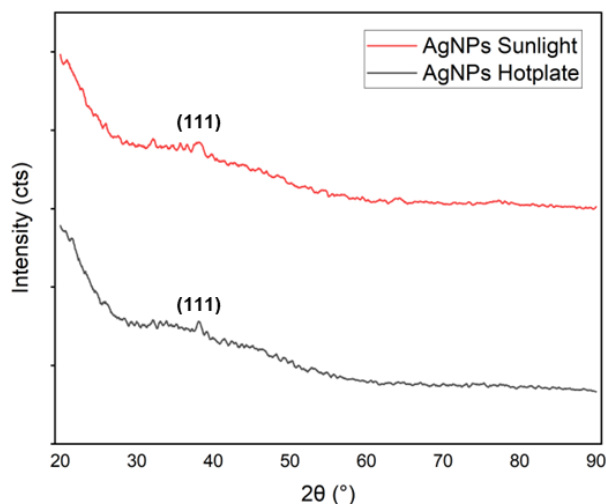


Figure 10. Diffraction pattern of synthesized silver nanoparticles (AgNPs) using sunlight and hotplate

4. Conclusion

The new eco-friendly method for synthesis of silver nanoparticles using a *C. chinense* Jacq. extract as a bio-reducing agent was successfully developed. UV-Vis spectra of *C. chinense* Jacq. provided λ_{\max} at 228 and 276 nm correspond to the character of capsaicin and dihydrocapsaicin compounds. AgNPs under sunlight exposure showed better results than the heating procedure with the optimum synthesis condition: 12% extract and AgNO_3 (0,1 mM) and 90 min sunlight exposure with λ_{\max} 422 nm. The character of the AgNPs under sunlight exposure got satisfactory results with 67.94 nm particle size and storage stability for up to 11 days.

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