JOURNAL OF TROPICAL LIFE SCIENCE
2023, Vol. 13, No. 2, 305 – 310
http://dx.doi.org/10.11594/jtls.13.02.08

Research Article

Green Synthesis of Silver Nanoparticles Using Leaves of *Chromolaena odorata* and its Antioxidant Activity

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Article history:
Submission August 2022
Revised August 2022
Accepted September 2022

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ABSTRACT

Harmful chemical waste is a serious problem being faced during the synthesis of nanoparticles due to the usage of hazardous chemicals. Synthesis of silver nanoparticles using aqueous extract of the leaves of *C. odorata* is cheap and environmentally friendly. This study reports the synthesis of silver nanoparticles (AgNPs) using *C. odorata* aqueous extract as reducing agent. The leaves of *C. odorata* was extracted by using cold maceration technique. The phytochemicals screening of leaves was done and positive results was showed for the presence of flavonoids, tannins, saponins, and phenolics in the leaves extract of *C. odorata*. The synthesized *C. odorata* extract mediated AgNPs was characterized using several techniques including UV-Visible spectroscopy and Field Emission Scanning Electron Microscopy (FESEM). The reduction of pure Ag (I) ions to Ag (0) was monitored using UV-Vis every one hour after 24 hours up to 28 hours and it showed an absorption band at 430-450 nm. Field emission scanning electron microscopy (FESEM) was utilized to determine its particle size and the average particle size obtained was 27.3 nm. The silver nanoparticles (AgNPs) produced by using *C. odorata* leaves aqueous extract was determined for its antioxidant activity by using DPPH free radical scavenging assay. The IC_{50} value obtained was 277.29 mg/ml. Based on the results obtained, it indicates that the silver nanoparticles (AgNPs) produced using *C. odorata* leaves aqueous extract possessed antioxidant activity that can scavenge free radicals.

Keywords: Antioxidant, Chromolaena odorata, Green synthesis, Silver nanoparticles

Introduction

Nanoparticles is one of the applications of nanotechnology which is adapted in the production of daily materials and processes (National Nanotechnology Coordination Office, n.d.). Silver nanoparticles (AgNPs) has made its name in the science world due to its interesting properties and wide applications as antibacterial agents, catalysts agents, or biosensor [1]. The leaves of *C. odorata* can be used as the reductants in the biosynthesis of nanoparticles since it contains a polyphenolic flavonoids molecule that is suitable to be adapted in green synthesis as a reducing and capping agent [2]. Despite the presence of a wide range of enzymatic and non-enzymatic antioxidant systems in mammalian cells and microorganisms, excessive oxidant production leads to the accumulation of novel products that damage cell function and structure, ultimately leading to cell death [3]. Based on the surge of evidence in the biological and pharmacological industries, support has been shown for the claimed of ethnomedical benefits of *C. odorata* [4].

'Daun Malaysia' (*Chromolaena odorata*) is a perennial weed that can be found in tropical shrub mostly in Africa, Asia, and some parts of Europe and is known for its healing properties in traditional medicine [5]. Primarily, the natural leaves or extract of *C. odorata* was used over the years by many tropical countries such as Malaysia for treatment of leech bite, soft tissue wounds, burn wounds, skin infection and dento-alveolitis [6]. Thus, this study was done to synthesize silver nanoparticles using the aqueous leaves extract of *C. odorata* and to determine the antioxidant

How to cite:
Hashim SE, John AP (2023) Green Synthesis of Silver Nanoparticles Using Leaves of *Chromolaena odorata* and its Antioxidant Activity. Journal of Tropical Life Science 13 (2): 305 – 310. doi: 10.11594/jtls.13.02.08.

properties of the synthesized silver nanoparticles using DPPH free radical scavenging assay.

Material and Methods

Extraction and Phytochemicals Screening

The leaves of *C. odorata* were collected from the street of Inanam, Kota Kinabalu, Sabah. Prior to the experiment, the leaves were dried under a shaded area. The dried *C. odorata* leaves sample (2 g) were grinded and added into 100 mL distilled water. The mixture was then heated at 40°C for 30 minutes while stirring. After that, the mixture was let to cool down at room temperature before being filtered using gravity filtration method. Phytochemical screening was done to screen the presence of phytochemicals that was carried out using standard qualitative methods. In this study, screening tests were done for flavonoids, saponins, tannins, and phenol [7].

Biological Synthesis of Silver Nanoparticles (AgNPs) using C. odorata Leaves Extract

Synthesis of AgNPs was performed prior to previous approach with minor modifications [8]. 2 mL of *C. odorata* aqueous extract was added to 10 mL of silver nitrate ($AgNO_3$) (1 mM) solution and was incubated at room temperature. After 1 hour, the colour change was observed from light yellow to yellowish brown which showed the formation of *C. odorata* -AgNPs and these were confirmed by UV-visible spectrophotometer. Next, the freeze-dried *C. odorata* -AgNPs was characterized using FESEM. The AgNPs mixture was centrifuged for 30 minutes at 24000 G to obtain a solid compound using aqueous solution and the extracts will be filtered by Whatman No.1 filter paper and then was dried at 40°C for 24 hours.

DPPH Free Radical-Scavenging Assay

Free radical scavenging activity of leaves extracts of *C. odorata*-AgNPs were evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) as in previous approach with minor modifications [9]. The DPPH reagent (80 µg/mL) was prepared by dissolving DPPH (8 mg) into ethanol (100 mL). A 50 µL of the sample aliquots was mixed with 100 µL of DPPH reagent. The mixture incubated under room temperature and in dark condition for 30 minutes. Using microplate reader, the absorbance of both the blank (100% methanol) and the mixture was measured at 517 nm. All measurements were triplicated using gallic acid as positive control. IC50 value was determined using extrapolation and inhibition percentage using equation below.

$$\% \text{ Inhibition} = (A_0 - A_t)/ A_0 \times 100$$

Characterization

2 mL of synthesized AgNPs was diluted with 3 mL of distilled water in a 5 mL volumetric flask and subjected to spectral analysis in the wavelength range from 200-500 nm using UV-Visible spectrometer. The analysis was observed every one hour after 24 hours up to 28 hours of reaction.

Field emission scanning electron microscopy (FESEM)

Field Emission Scanning Electron Microscopy (FESEM) is a useful technology used to frame the microstructure of materials. The dried AgNPs synthesized was used for FESEM analysis to obtain the microstructure of AgNPs with 50,000X and 120,000X of magnification.

Results and Discussion

Phytochemicals Screening of C. odorata Leaves

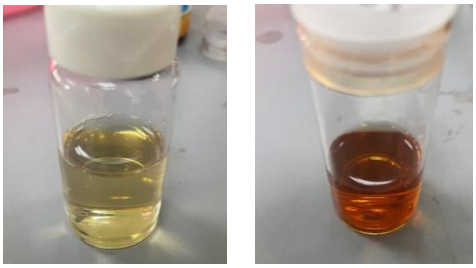
Phytochemicals screening was done for sample extracted using distilled water. The extract from leaves of *C. odorata* was screened for the presence of secondary metabolites including flavonoids, phenols, saponins, and tannins. Table 1 presents the qualitative phytochemical screening carried out on the leaves of *C. odorata*. Based on the results, flavonoids were found to be in low presence and phenolic compounds were found to be in medium presence in aqueous leaves extract. Saponins and tannins were found to be highly present in aqueous leaves extract. The qualitative analysis of phytochemicals proved that *C. odorata* leaves extract has potential to be a reducing agent since it contains secondary metabolites that possess reducing capacity.

Several studies have observed that the secondary metabolites presence in *C. odorata* extracts are used for anti-diarrheal, astringent, antispasmodic, antihypertensive, anti-inflammatory, diuretic, tonic, antipyretic and heart [10]. Overall, this study found that secondary metabolites in *C. odorata* leaves are present which played an important role in reducing Ag(I) to Ag(0) by bearing electron donor element such as oxygen.

Table 1. Phytochemicals screening tests for *C. odorata* leaves extract

Phytochemical	Leaves extract of *C. odorata*
Flavonoids	+
Phenolics	++
Saponins	+++
Tannins	+++

Note: +++: Highly Presence; ++: Medium Presence; +: Low Presence



(a) (b)

Figure 1. Visual observation of synthesis of AgNPs, (a) *C. odorata* leaves extracts after addition of $AgNO_3$ solution (b) after addition of extract lead to colour change from light yellow to reddish brown.

Biosynthesis of C. odorata – AgNPs

The addition of leaves extract of C. odorata to the 10 mL (1 mM) of silver nitrate solution showed the colour changed from light yellow to reddish brown within 5 hours (Figure 1). These changes were due to the excitation of surface plasmon vibration of metal nanoparticles, and it delivers a suitable spectroscopic signature to designate the formation of AgNPs [11, 12]. When the silver nanoparticle interacts with the electromagnetic wave, its surface free electrons produce the Surface Plasmon Resonance (SPR) band [13].

Ultraviolet Visible (UV-Vis) of C. odorata – AgNPs

Figure 2 shows the UV absorption spectrum of the synthesized AgNPs by using *C. odorata* leaves extract. Broad peak at a higher wavelength shows bigger in particle size while a narrow line at shorter wavelength indicates a smaller particle size [14]. Absorption band in the range of 350-750 nm was measured. Maximum absorption of the synthesized AgNPs for aqueous leaves extract of *C. odorata* was 464.5 nm. It was observed that the increasing of incubation time influenced the absorption spectra which was also gradually increasing. The increasing intensity as the time increased could be related to the increasing amount of *C. odorata* -AgNPs formed. However, the SPR peak position also showed no changes indicating that the nanoparticles formed were similar in shape at different incubation time [15, 16]. Furthermore, size of nanoparticles could affect the UV absorption of nanoparticles. In general, smaller the wavelength of absorption, smaller the size of particles and vice versa [17].

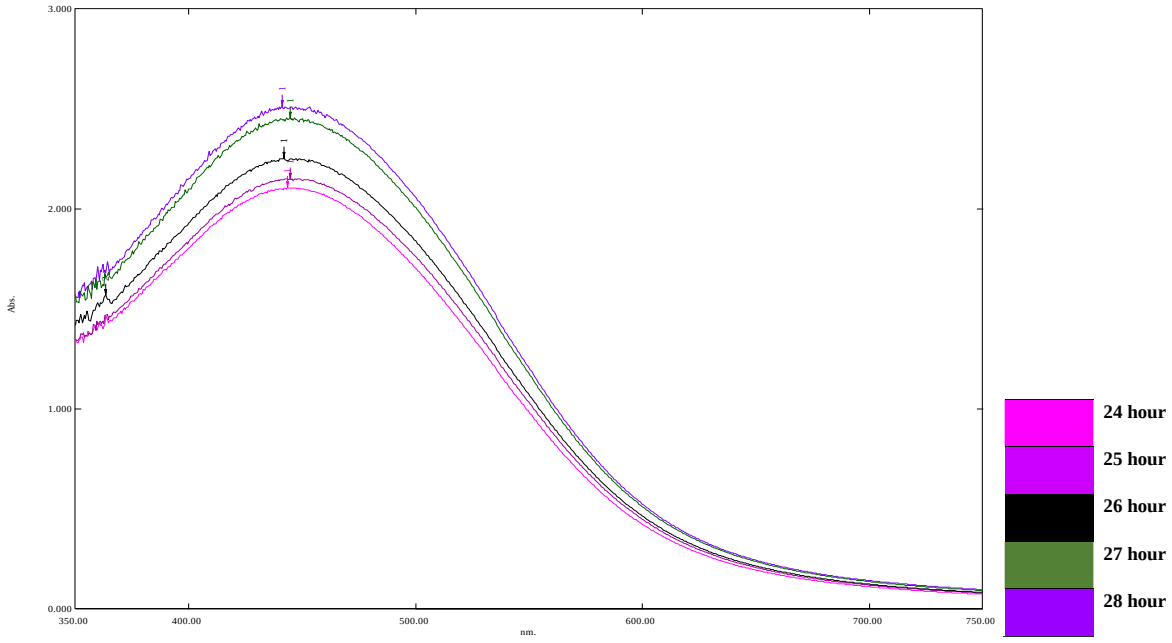
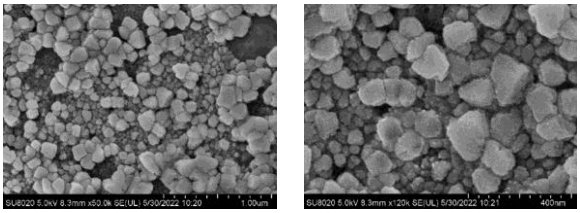


Figure 2. UV-Vis absorption spectrum of synthesized leave *C. odorata* - AgNPs at different hours



(a) (b)

Figure 3. FESEM image of *C. odorata* -AgNPs with (a) 50,000X magnification (b) 120,000X of magnification

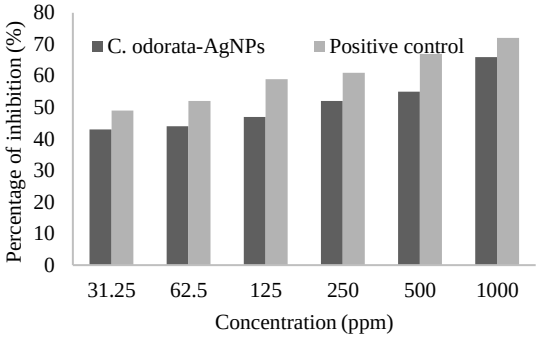


Figure 4. Percentage of inhibition for synthesized silver nanoparticles

FESEM Analysis for Leaves Extract of C. odorata–AgNPs

Figure 3 shows the FESEM image for *C. odorata*-AgNPs with the 50,000X magnification and 120,000X of magnification. The assumption that has been made for theoretical prediction of particle size is to be spherical [18, 19]. In this study, the shape that has been observed is varies in shape as much as spherical, triangular, and decahedral. There are many factors affecting the shape of the synthesized nanoparticles including extract concentration, contact time, pH and silver salt concentration [20]. A good separation was observed between the AgNPs in the FESEM image could be due to capping effect of *C. odorata* extract. Thus, this explains the narrow SPR bands shape which is related to characteristic of well-dispersed AgNPs (Figure 2). Besides, the presence of hydroxyl groups from the extract, which can act as a good reducing agent but weak capping potential, could explain agglomeration of the particles [21]. It was noticed that the size ranges from 2 to 29 nm and the average particle size obtained was 27.3 nm.

Antioxidant Activities of Synthesized Silver Nanoparticles

The antioxidant activity for the obtained biological silver nanoparticles using the leaves extract of *C. odorata* was assessed using DPPH radical scavenging assay (Figure 4). The IC_{50} value represents the concentration of sample that is required to scavenge 50% of the free radicals. The percentage of inhibition obtained were 65.65, 54.80, 51.66, 47.29, 44.27, and 42.75 % respectively for 1000, 500, 250, 125, 62.5 ppm with IC_{50} value 277.29 mg/mL. The DPPH radical scavenging activity of AgNPs using the leaves extract of *C. odorata* displayed a rapid rise when the concentration is less than 600 ppm. As the concentration exceeds 600ppm, the free radicals scavenging activity increases slowly. Phytochemicals present in the extract with high scavenging activity causes the rapid production of small AgNPs seeds, which develop into larger nanoparticles [3].

Conclusion

Aqueous extract of *C. odorata* mediated silver nanoparticle was successfully synthesized in this study. Phytochemicals screening analysis of the extract has been determined and found that flavonoids, tannins, saponins, and phenols were present in leaves extract of *C. odorata.* It has been found that the surface plasmon resonance (SPR) band for AgNPs is at 464.5 nm. Other than that, the establishment of *C. odorata* -AgNPs was characterized by FESEM. FESEM micrograph displayed that *C. odorata* -AgNPs was varies in shape as much as spherical, triangular, and decahedral shape with few agglomerated structures. Furthermore, in the antioxidant activity of *C. odorata* -AgNPs, the value of inhibition percentage represents the relationship between concentration of extract and the antioxidant activity, and the percentage of inhibition obtained were 65.65, 54.80, 51.66, 47.29, 44.27, and 42.75 % respectively for 1000, 500, 250, 125, 62.5 ppm with IC_{50} value 277.29 mg/mL. Thus, this study has shown that the synthesized *C. odorata* -AgNPs can be used as antioxidant agents.

Acknowledgment

The authors acknowledge Universiti Teknologi Malaysia for the financial support under Research University Grant (RUG) with vote number QJ130000.2654.15J91.

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