Malaysian Journal of Chemistry, 2024, Vol. 26(3), 383-388
(Special Issue: *3rd Virtual International Symposium on Sciences 2023*)

Characterization of Silver Nanoparticles (AgNPs) Synthesized Using Aqueous and Methanol Extracts of *Cosmos caudatus* (*Ulam Raja*)

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Nanotechnology has been developed because of its tremendous potential for industries and silver nanoparticles (AgNPs) is known for its valuable particles. This is mainly due to its inert character as an inorganic metal element that has strong antibacterial effects and can be synthesized using plant extracts. Basically, *Cosmos caudatus* is the chosen plant but it has less exposed in the field of nanotechnology. There are a few approaches used to synthesize AgNPs including the physical method, the chemical method, and the biomimetic method. The biomimetic method is the best approach as it is environmentally-friendly and gives off non-toxic effects. This research aims for the characterization of AgNPs using the aqueous extract and methanol extract of *C. caudatus*. One of the objectives is to obtain a crude extract of *C. caudatus* by using the cold extraction method before continuing to synthesize the AgNPs from the extracted sample by treating it with $AgNO_3$. This research is aimed at determining the phytochemicals in *C. caudatus* extract that may contributes to the formations of AgNPs. In regards to the morphology characterization of the AgNPs, the synthesized AgNPs were observed for any peaks generated by UV-Vis spectroscopy and FTIR. There are reportedly five major phytochemicals in aqueous extract including tannins, alkaloids, flavonoids, phenols, and terpenoids, while there are four major phytochemicals in methanol extract such as tannins, proteins, alkaloids, and phenols. The formation of AgNPs aqueous extract and AgNPs methanol extract was confirmed by UV-Vis absorption peaks at approximately 411 nm and 410 nm, respectively. Meanwhile, the FTIR results indicates the presence of O-H, C-H, C=C bands that may contribute to the phenolics and the flavonoids compounds in the extract. In conclusion, the AgNPs have been successfully synthesized through the biomimetic process by reducing silver nitrate with several important phytochemicals of *Cosmos caudatus* aqueous and methanol extract. The potential of AgNPs may pave the way for a new range of pharmacological properties especially for antibacterial and antivirus properties.

Keywords: Silver nanoparticles (AgNPs); *Cosmos caudatus*; biomimetic synthesis; aqueous extract; methanol extract

Received: October 2023; Accepted: February 2024

Metal nanoparticles, especially silver nanoparticles (AgNPs), are the most valuable particles in nano-technology fields due to their superior physical and chemical properties and have tremendous applications. Scientists have discovered that AgNPs have anti-inflammatory effectiveness and its can be found in the antiseptic sprays, wound dressings and topical creams which are widely used in the biomedicine [1]. It must be noted that AgNPs also can be developed into catalyst agents [2], antibacterial agents [1, 3-5] or even biosensors [6]. In addition, AgNPs show magnificent antimicrobial activity especially in food and seed preservation, food packaging, cosmetics, biofertilizers and other medicinal applications [7].

Silver nanoparticles can be obtained through three synthesizing approaches which includes chemical method [8], physical method [9] and biomimetic method [10]. According to [10], biomimetic method is the best approach compared to the other two methods due to the presence of plant metabolites that can act as capping, reducing and stabilizing precursor which are safe, cheap, non-toxic and environmentally friendly. With the growing interest in the pharmacological properties of medicinal plants metabolites, it significantly proves that biomimetically synthesized AgNPs can contribute to medicinal applications as there are demands for more comprehensive scientific research in discovery AgNPs [11-12].

Several pharmacological active plants metabolites including phenolics, alkaloids, terpenoids, phenols, proteins and polyphenols plays major role in synthesizing silver nanoparticles [13]. Previous studies have revealed some medicinal plants that

†Paper presented at the 3rd Virtual International Symposium on Sciences (3rd VISoS 2023)

can be used in synthesizing AgNPs which include *Eleusine indica* extract [5, 14], *Parmotrema praesorediosum* extract [15], *Arabian Primrose* leaf extract [16], *Ramalina dumeticola* [17], *Cucumis prophetarum* [18], *Canarium patentinervium* [4], *Crocus Hausknechtii Bois* extract [2] and *Callistemon citrinus* extract [3]. In addition, studies have revealed that active plant metabolites such as phenolics and flavanoids found in Asteraceae species play a significant role in synthesizing silver nanoparticles [19].

Hence, the aim of this study is to increase knowledge on biomimetic synthesis of silver nanoparticles using aqueous extract and methanolic extract of *Cosmos caudatus* (*ulam raja*).

EXPERIMENTAL

Chemicals and Materials

The fresh leaves of *Cosmos caudatus* were collected in Kuala Pilah, Negeri Sembilan. All the reagents purchased were of laboratory grade and used as they were received. Silver nitrate ($AgNO_3$) was purchased from R&M Chemicals, US. Methanol was purchased from Sigma Aldrich, Germany.

Characterization Methods

An aqueous extract of *C. caudatus* was prepared by heating a mixture of 100 g *C. caudatus* leaves in 500 mL of distilled water at a temperature of 60℃ for about 30 minutes to denature the enzyme. The solution was cooled down to room temperature and Whatman's filter paper was used to filter and remove the residue before obtaining the aqueous extract of *C. caudatus.* This method is a modification and can be referred to [15]. A methanol extract of *C. caudatus* was obtained from maceration techniques of 100 g fine powdered *C. caudatus* leaves in 1000 mL methanol at room temperature for 72 hours before filtering the extracts using the Whatman No.1 filter paper [20]. A rotary evaporator was used to get the *C. caudatus* methanol crude extract. The aqueous and methanol extract of *C. caudatus* was stored in a refrigerator at 4°C for further analysis. The present of metabolites in the *C. caudatus* extract was screened through several phytochemicals test [21, 22].

In order to synthesize silver nanoparticles, 10 mL of *Cosmos caudatus* aqueous extract was treated with 90 mL of 1 mM $AgNO_3$ solution at room temperature (24-25℃) for 48 hours. For methanol extract, about 10 mL of 10% (w/v) methanol leaves extract was added to a 90 mL of 1 mM $AgNO_3$ and left it in a dark chamber for 48 hours. After 48 hours, these samples were centrifuged for 20 min at 10,000 RPM. The supernatant was freeze dried to form a powder and kept in a vial for further analysis.

Physicochemical appearance of the synthesized AgNPs was characterized using ultra-violet visible spectra (UV-Vis) and FTIR spectroscopy analysis. The UV-Vis spectra analysis was conducted using a UV 2450 Shimadzu double-beam spectrophotometer, operated at 2 nm from 300 to 500 nm. The reduction of the silver ions to AgNPs was monitored by measuring the spectra of the solutions. The results show there was a peak in the range of 400-600 nm for the UV-Vis wavelength (λ) which confirmed the presence of AgNPs [8]. Further, Fourier-Transform Infra-Red (FTIR) Spectroscopy analysis is one of the methods to analyse the different functional group of metabolites present in *C. caudatus* extracts which may play significant roles in synthesizing AgNPs. The Fourier-Transform Infra-Red (FTIR) Spectroscopy analysis was conducted between 500 – 4000 cm^{-1} through alpha interferometer FTIR.

RESULTS AND DISCUSSION

Analyzing secondary metabolites from medicinal plants is essential to discover their medicinal properties [23]. Based on phytochemical screening tests on the *C. caudatus* extracts, there are reportedly five major phytochemicals in *C. caudatus* aqueous extract including tannins, alkaloids, flavonoids, phenols, and terpenoids, while four major phytochemicals in *C. caudatus* methanol extract such as tannins, proteins, alkaloids, and phenols (Table 1).

The present of phytochemicals in *C. caudatus* extract is similar to a previous study reported by [24] which indicates that phenolic compounds are major metabolite in primary and secondary metabolites in *C. caudatus*. This study suggests that phenolic metabolites of *C. caudatus* may act as a potential capping, reducing and stabilizing agent in the synthesis of AgNPs. According to [25], some metabolites such as flavonoids, phenolics, sugar, and proteins can serve as reducing and stabilizing agents in synthesizing AgNPs. As compared to methanol extracts, aqueous extract shows the presence of flavonoids and terpenoids which are other major metabolites contributing in the synthesizing of AgNPs [26].

Table 1. Phytochemical analysis on *Cosmos caudatus* crude extracts

Chemical compound	Methanol Extract	Aqueous Extract
Glycosides	-	-
Protein	+	-
Alkaloids	+	+
Tannins	+	+
Flavonoids	-	+
Phenols	+	+
Saponins	-	-
Anthraquinones	-	-
Terpenoids	-	+

(+ : present, - : absent)

Physicochemical characterization of synthesized AgNPs was done using UV-Vis spectra and FTIR spectroscopy analysis. Based on the UV-Vis spectra, the result show the surface of plasmon resonance of the synthesized AgNPs using aqueous extract and methanol extracts of *C. caudatus* were centered at 411 nm and 410 nm, respectively (Figure 1). In addition, the obvious peak at 297 nm indicates the plant metabolites peaks have significant synergistic effect on the synthesizing AgNPs [15].

The reduction of the silver ions to AgNPs was monitored by measuring the UV-Vis spectra of the solutions in the range of 400-600 nm [8]. In adition, the formation of AgNPs can also be observed physically as the reduction of Ag^{+} ions will bring a visible colour change into a brownish [27]. The results of the UV-Vis absorption spectra will have various wavelength intervals, and the peak ranging from 400 to 550 nm is based on the size, shape or amount of the synthesized AgNPs [28].

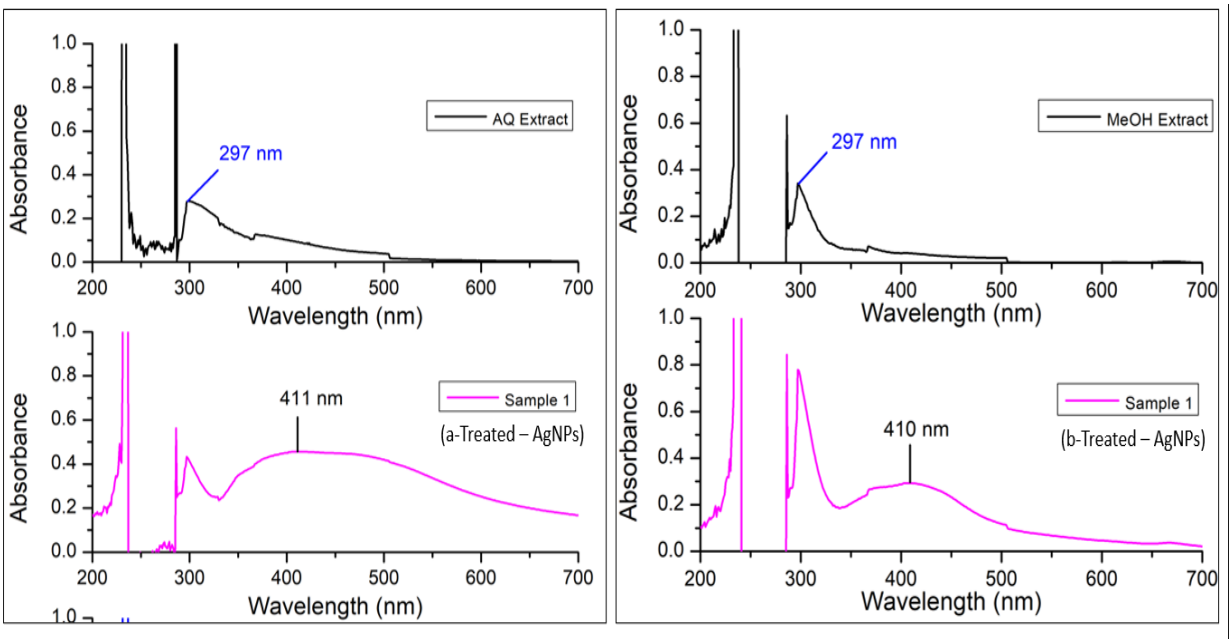

Figure 1. UV-Vis absorption spectra of *C. caudatus* aqueous extract/treated – AgNPs (a) and *C. caudatus* methanol extract/treated – AgNPs (b).

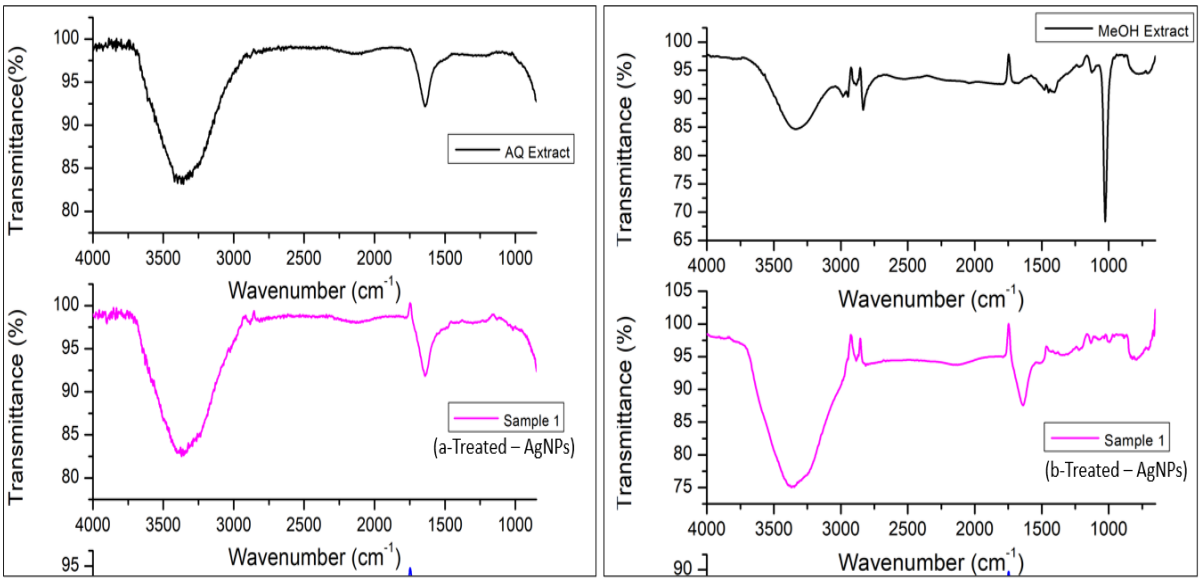

Figure 2. FTIR absorption spectra of *C. caudatus* aqueous extract/treated – AgNPs (a) and *C. caudatus* methanol extract/treated – AgNPs (b).

The biomimetically synthesized silver nanoparticles morphology was further characterized using FTIR analysis. The FTIR absorption spectra are shown in Figure 2.

Figure 2 reveals there are functional groups such as hydroxyl group and vinyl carbon group in both extracts. The intense and broad absorption peak at 3500-3200 cm^{-1} indicates the O-H stretching. The absorption peak at 1680-1600 cm^{-1} indicates the presence of C=C alkene. The result is supported by the finding of a study by [29] which found that important absorption peaks at 3200-2800 cm^{-1} (hydroxyl) and 1680-1600 cm^{-1} (C=C) in *C. caudatus* extract may be attributed to flavonoid and phenolic compounds. According to [25] and [30], some metabolites such as flavonoids, phenolics, sugar and proteins play a significant role in synthesizing AgNPs.

CONCLUSION

In conclusion, the AgNPs have been successfully synthesized through a biomimetic process by reducing silver nitrate with *Cosmos caudatus* aqueous and methanol extract. The phytochemical analysis of *C. caudatus* aqueous extract indicates the presence of tannins, alkaloids, flavonoids, phenols and terpenoids. Meanwhile, the methanol extract has shown four positive results including the presence of tannins, proteins, alkaloids and phenols. The physicochemical appearance of AgNPs shows strong UV-Vis absorption peaks approximately at 411 nm and 410 nm for both aqueous and methanol extracts, respectively. The results of FTIR spectra analysis indicate that both extracts have an absorption peak of hydroxyl (O-H) and vinyl functional groups which contribute to the presence of phenolic and flavonoid compounds. This study shows that *C. caudatus* extract contains several important phytochemicals that can aid in the potential of AgNPs which may pave the way for a new range of pharmacological properties. The synthesized AgNPs can be further investigated on the biological properties, especially for antibacterial and antivirus properties.

ACKNOWLEDGEMENTS

The authors would like to acknowledge Universiti Teknologi MARA (UiTM), Negeri Sembilan Branch, Kuala Pilah Campus, Negeri Sembilan for providing the research facilities. This research received no external funding.

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