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Green Synthesis and Analysis of Antioxidant Activity of Silver Nanoparticles Derived from *Cissampelos pareira* L. Leaves

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Abstract: In nanotechnology and nano materials research, green synthesis of metal nanoparticles has emerged as a potential synthetic approach. Silver nanoparticles (AgNPs) were successfully biosynthesized in the presence of *Cissampelos pareira* aqueous extract as a reducing agent in this study. UV-Vis spectroscopy was used to evaluate the reaction's progress. X-ray Diffraction spectroscopy (XRD) and Transmission Electron Microscopy (TEM) were used to characterize silver nanoparticles (TEM). Antioxidant capacity was evaluated by radical scavenging method, IC₅₀ were found to be 45.531, 83.468 and 50.892 µg/mL for *Cissampelos pareira* of AgNPs, Aqueous extract, and Ascorbic Acid (Standard), respectively. When compared to *Cissampelos pareira* aqueous extract alone, biosynthesized AgNPs showed stronger antioxidant activity. It can be concluded that *Cissampelos pareira* AgNPs can be widely used in the pharma industries as a potential antioxidant.

Keywords: *Cissampelos pareira*, X-ray Diffraction spectroscopy, Transmission Electron Microscopy, DPPH assay

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Introduction

Nanotechnology are critical in a variety of industries and domains of science. Chemical, optical, and physical features, as well as antibacterial activity, are all unique to nanoscale materials. Antimicrobial, antioxidant, wound healing, and other biological effects of silver nanoparticles (AgNPs) have piqued the interest of researchers. Anticancer properties of Green synthesis of NPs has consistently gotten a lot of interest due to its cost-effectiveness compared to chemical and physical approaches. It is also eco-friendly because it does not necessitate the use of high pressure, energy, temperature, or hazardous

chemicals (Hembram *et al.*, 2018; Asimuddin *et al.*, 2020). Moreover, antibacterial efficacy against pathogenic microbes were demonstrated using a variety of nanomaterials such as gold, copper, zinc, magnesium, titanium, and silver (Mahadevan *et al.*, 2017; Arya *et al.*, 2018; Liao *et al.*, 2019; Ahn *et al.*, 2020).

Herbal drugs face the same clinical challenges as allopathic medicines, such as selectivity, drug delivery, solubility, safety, toxicity, efficacy, and frequent dosing (Fabricant and Farnsworth, 2011; Duan *et al.*, 2015; Mohammadalinejad *et al.*, 2019). Modern pharmaceutical research could

overcome the aforementioned challenges by developing novel herbal medicine drug distribution systems, such as micro-emulsion, nanoparticles' solid dispersion, liposomes, matrix systems, and solid lipid nanoparticles (Kumar and Rai, 2012; Lohr *et al.*, 2017; Miljkovi *et al.*, 2020).

Plant-derived bioactive compounds can act as antioxidants and reducing oxidative stress. Many plant crude extracts had a high oxidative capacity and a significant amount of total phenolic compounds (Kahkonen *et al.*, 1999; Arockia *et al.*, 2017). Natural phytochemicals derived from medicinal plants have received a great deal of attention. In the last two decades, there has been increased recognition in the treatment and management of various diseases (Dyana, 2012; Seetharaman *et al.*, 2016; Jorge *et al.*, 2019; Jalilian *et al.*, 2020). Indeed, numerous studies have shown that phytochemical constituents of plants, such as flavonoids, Polyphenols, tannins, carotenoids, and phenolic terpenes have antioxidant properties because they quench free radicals in the body production (Mathew, 2015; Arockia *et al.*, 2017; Ramya *et al.*, 2017; Seetharaman *et al.*, 2018; Jorge *et al.*, 2019; Jalilian *et al.*, 2020).

Plant extracts play a double role in the NPs synthesis process, while mediating the reduction of metal salts; they can also act as capping agents to stabilize the produced NPs. Bioreduction with plants is a complex process where a large variety of plant components like terpenoids, flavonoids, phenols, alkaloids, saponins, or proteins are involved. The antioxidant behavior of these metabolites is well known. Antioxidants are compounds capable of delaying or inhibiting the oxidation processes which occur under the influence of atmospheric oxygen or reactive oxygen species (Della Pelle *et al.*, 2018; Martínez-Cabanas *et al.*, 2021). Furthermore, some nanomaterials' strong antioxidant properties open up exciting possibilities for developing novel regimens with increased and targeted activities. Gold, silver, and selenium nanoparticles, for

example, have been found to have the ability to reduce inflammation. Due to their excellent redox-active radical-scavenging characteristics, they are effective against oxidative stress (Saad *et al.*, 2017; Arya *et al.*, 2018; Kora *et al.*, 2020).

Based on the literature survey it was understood that there was not much work on biomedical application of *C.pareira* AgNPs. Therefore this present study has been biosynthesis of AgNPs by reduction of aqueous silver nitrate (AgNO₃) using *C. pareira* aqueous leaves extract for their potential antioxidant effects.

Materials and Methods

Biosynthesis of Silver Nanoparticles:

Silver nitrate was obtained from Himedia Laboratories Pvt. Ltd., Mumbai, India. 3 g of the leaves powder of *C. pareira* was boiled in 100 ml of de-ionized water. 80 ml solution of 1 mM AgNO₃ was taken which is followed by addition of 5 ml plants extract and the final volume was adjusted to 100 ml by adding the appropriate amount of de-ionized water. Color change of solution confirms the synthesis of AgNPs from yellowish to bright yellow and to dark brown. The conical flasks were incubated at room temperature under 24 h (Kasthuri *et al.*, 2009; Duan *et al.*, 2015) for further observation.

UV-Vis analysis:

The absorption spectrum of the reaction mixture was recorded at room temperature by using UV-vis spectrophotometer (Rayleigh, UV-2100).

XRD analysis:

The structure of silver nanoparticles was determined by X-ray diffraction using the Bruker – D8 Advanced (Germany) instrument.

HR-TEM:

A transparent electron microscope, the Tecnai G2 F20 X-TWIN (FEI, USA), was used to examine the size of the synthesized nanoparticles (TEM). The

diluted solution was used in the TEM experiment. Drops of samples were deposited onto carbon-coated copper TEM grids. Schottky's the emission electron source was used, and the accelerating voltage ranged from 20 to 200 kV. The microscope's resolution ranged from 10 to 30 nm.

DPPH radical scavenging assay:

By following the standard method of Mensor *et al.* (2001), the DPPH scavenging capability of silver nanoparticles from *Cissampelos pareira* leaf extract was evaluated. Various concentrations of silver nanoparticles (12.5, 25, 50, 100 and 200 µg/mL) and the standard (ascorbic acid) were used in separate test tubes. After that, each test tube was filled with 1 ml of newly produced DPPH (0.3 mM) and vortexed vigorously. Finally, the solution was incubated for 30 min in a dark environment. In addition of silver nanoparticles, 2 ml of methanol was introduced and the test was conducted simultaneously. The formula was used to calculate the silver nanoparticles' % DPPH radical scavenging activity.

The per cent inhibition of activity was calculated as $[(A_o - A_e) / A_o] * 100$

Where A_o = absorbance without extract; and A_e = absorbance with extract.

The results were expressed as IC_{50} which is the concentration of the sample required to inhibit 50 % of DPPH concentration.

Results and Discussion

The AgNPs synthesized by aqueous extract alone showed dark brown colour within 24 h and the peak was recorded at 430 nm (Fig. 1). According to Kim *et al.* (2016) the reduction of Ag^+ could be due to the presence of Polyphenol hydroxyl group oxidation. Complexation of polyphenol with metallic silver, binds with the biomolecules which are responsible for the stabilization of the nanoparticles according to Nunes *et al.* (2018) and Sganzerla *et al.* (2020).

The synthesized Silver nanoparticles were further confirmed by XRD images in the characteristic peaks (Fig. 2). Silver nanoparticles of nanocrystals in nature as evidenced by the peak at 2θ values in 28.2° , 32.45° , 46.3° and 55.34° and other observed peaks suggested that the crystallization of bio-organic phase occurs on the surface of the silver nanoparticles (Sathyavathi *et al.*, 2010; Saravanakumar *et al.*, 2015 Oliver *et al.*, 2018; Saratale *et al.*, 2020). It was also noticed that the diffraction angles of Ag-NPs were to bulk silver crystal ($2\theta = 33^\circ$). The definite line broadening of the peaks suggests that the particles are in the nanometer range. Based on the Debye-Scherrer's equation (Krishna *et al.*, 2013; Jorge *et al.*, 2019; Bhuiyan *et al.*, 2020) average crystallite size is calculated and was found to be 10-30 nm in size (diameter).

The morphological structure and size of the synthesized Ag particles were determined by using Transmission Electron microscopy. TEM revealed that the synthesized AgNPs were spherical in shape with average size from 10 to 30 nm (Fig. 3), same trend of observation were made by Hussein *et al.* (2015); Arya *et al.* (2018) and Kamath *et al.* (2020) also reported average size of 10-20 for Ag-NPs.

DPPH radical scavenging activity:

The DPPH radical scavenging activity of *C. pareira* green synthetic and aqueous extract was investigated and the results are given in Table 1 and Figure 4. When purple DPPH, a stable free radical, is reduced by the sample, it decolorizes to a yellow DPPH molecule with a distinctive absorbance at 517 nm. The AgNPs produced from the aqueous extract of *C. pareira* are potential free radical scavengers with dose-dependent inhibitory action. When compared to aqueous extract, SNPs has the highest per cent of inhibition, with an IC_{50} of 45.531 µg/ml, aqueous extract has an IC_{50} of 83.468 µg/ml. As demonstrated in Figure 4, both SNPs and aqueous extract had substantial antioxidant capacity when compared

Fig. 1: UV-vis absorption spectra of biosynthesized silver nanoparticles from *C. pareira* L.

Fig. 2: XRD pattern of AgNPs using *C. pareira* L.

Fig. 3: TEM images for nanoparticles size of *C. pareira*.

Table 1: DPPH radical scavenging activity of *C. pareira* aqueous extract and Synthesized Silver Nanoparticles

Concentration	Aqueous extract	Silver nanoparticles	Standard (Ascorbic Acid)
Control	100 ± 0	100 ± 0	100 ± 0
12.5 µg/ml	77.37±0.346 (-22.62)	72.94±0.235 (-27.05)	75.54±0.22 (-24.54)
25 µg/ml	71.14±0.355 (-25.654)	59.50±0.371 (-40.49)	67.62±0.275 (-32.37)
50 µg/ml	60.21±0.273 (-39.788)	47.92±0.216 (-52.07)	50.17±0.239 (-49.828)
100 µg/ml	43.47±0.280 (-56.52)	33.13±0.33 (-66.86)	40.53±0.25 (-59.46)
200 µg/ml	35.20±0.364 (-64.79)	19.08±0.421 (-80.91)	23.05±0.31 (-76.94)
IC ₅₀	83.468	45.531	50.892

Values are mean ± S.E. of six individual observations; - % reduction in comparison to the control

Fig. 4: Antioxidant activity of *C. pareira* L. leaf extract and biosynthesized silver nanoparticles.

to standard L-ascorbic acid, which had an IC₅₀ value of 50.892/µg/ml. The AgNPs produced from the aqueous extract of *C. pareira* are potential free radical scavengers with dose-dependent inhibitory action (Xing *et al.*, 2019; Akintola *et al.*, 2020). Bioactive compounds such as terpenoids, alkaloids, polyphenols, and phenolic acids have been found in algae, fungi, lichens, and plants. These bioactive compounds have been shown to have antioxidant properties and to decrease and stabilise metallic ions. The various types of nanoparticles with antioxidant fictionalization

were generated from diverse biological extracts (Seetharaman *et al.*, 2018; Harsh Kumar *et al.*, 2020). These research results may refer to the impact of nanoparticle synthesis in various plant extracts. Other researchers obtained comparable results with grapefruit AgNPs from *Thymus citriodorus* and *Thymus vulgaris* (Arya *et al.*, 2018; Xing *et al.*, 2019; Saratale *et al.*, 2020; Taghout *et al.*, 2020).

Conclusion

This study presents green method for the

synthesis of Ag-NPs using *C. pareira* L. leaves extracts and reveals that efficient biological reducing agent that can be easily scaled up as its economic and environmentally benign features. Active biological constituents of leaves extract act as effective stabilizing and reducing agents that were investigated by UV-Vis studies. XRD and HR-TEM provided information regarding the crystallite size and phase-purity, average crystalline size of the AgNPs was recorded in the range of 10–30 nm. In this study, Ag-nanoparticles were synthesized from *C. pareira* leaves and characterized and their antioxidant activity was analyzed. The recorded activity is remarkable, despite the problem about the synthesis of uniform products in this study. This rapid synthesis of Silver nanoparticles by Menispermaceae family provides a good alternate for chemical reduction methods owing to its non-toxic nature. It is clear that green synthesized Ag-nanoparticles should be investigated with advanced studies to be potential candidates in various biomedical applications.

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