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Antifungal activity of Silver nanoparticles obtained by Green synthesis

Aravind Mohan V.R. & Sujamol Abraham*
Post Graduate Department of Botany, Devamatha College, Kuravilangad, 686633, Kerala, India

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Abstract

The present study presents a simple, cost-effective, and environmentally friendly method for the green synthesis of Silver nanoparticles (AgNPs) using *Piper nigrum* and *Piper longum* in an aqueous medium, without the need for synthetic chemicals. The formation of AgNPs was confirmed through UV-Vis spectroscopy and FTIR analysis. These green-synthesized nanoparticles exhibit strong antifungal activity, positioning them as a promising alternative to combat fungal infections, particularly in the context of increasing resistance to conventional antifungal treatments. This approach leverages natural plant extracts, aligning with the growing demand for sustainable, non-toxic materials in medical, agricultural, and industrial applications, offering a viable eco-friendly solution for various sectors.

1. Introduction

Silver nanoparticles (AgNPs) have gained significant attention in recent years for their broad-spectrum antimicrobial properties, including antifungal activity. *Candida albicans* is a fungal pathogen that is difficult to cure clinically due to lack of effective antifungal agents with low toxicity (Liangfu *et al*., 2021). Silver nanoparticles have attracted considerable attention due to their broad range of applications, including use in sensors, catalysts, anticancer agents, and particularly as antimicrobial agents. The unique properties of AgNPs, such as their high surface area, small size, and surface reactivity, enable them to exhibit potent antimicrobial activity against a wide range of pathogens, including bacteria, fungi, and viruses (Kashyap *et al*., 2013). In the context of fungal infections, AgNPs have demonstrated considerable efficacy, making them a promising alternative or adjunct to traditional antifungal therapies.

However, the conventional chemical methods for synthesizing AgNPs often involve the use of toxic chemicals and produce hazardous by-products, such as ammonia, which can pose risks to both human health and the environment (Panácek *et al*., 2009). The green synthesis of AgNPs has used various routes: plants, microorganisms and non-toxic substances (Iravani, 2011). The aim of this study was to synthesize AgNPs using *Piper longum* and *Piper nigrum* stem extracts and evaluate the antifungal activity of these nanoparticles against *C. albicans*.

2. Materials and Methods

2.1 Preparation of *P. longum* and *P. nigrum* stem extract

The *Piper longum* stems are freshly collected and washed with

*Corresponding author
E-mail:sujamol222@gmail.com

distilled water. The stems are finely chopped and again washed with double-distilled water for 2 times. The chopped stem is slightly dried at room temperature. About 15g of stem is weighed and boiled with 100 mL of double-distilled water at 60–80°C for 10 min. After boiling, the solution is filtered through Whatman No.1filter paper and stored at 4°C for the nanoparticle synthesis**.**

The fresh *Piper nigrum* stems are collected and washed with distilled water. The stems are finely cut into small pieces and again washed with double-distilled water. The chopped stem is slightly dried at room temperature. About 10g of sliced stem is weighed and boiled with 100 mL of double-distilled water at 60–80°C for 10 min. After boiling, the solution is filtered through Whatman No.1filter paper and stored at 4°C for the nanoparticle synthesis**.**

2.2 Synthesis of silver nanoparticles by using *P. longum* and *P. nigrum* stem extract

For the biosynthesis of silver nanoparticles from the stem extract of *Piper longum* 90ml of 1mM silver nitrate and 10 ml of extract were mixed and idle to allow reaction and development of biogenic silver nanoparticles.10 ml of fresh stem extract of *Piper nigrum* was added into the 90 ml aqueous solution of 1mM silver nitrate. The reaction mixture was kept undisturbed until the colourless solution for converted into a brown colour, which indicated the formation of AgNPs.

2.3 Characterization studies

UV–Visible spectroscopy is an effective technique that can help characterize synthesized AgNPs. The absorbance spectra can confirm the formation of synthesized AgNPs in a solution, as previously reported (Ahmad *et al*., 2003). *The analysis measures the intensity of light transmitted through the sample and compares it with a reference measurement of the incident light source***.** *Wavelengths* ranging from 400 to 800 nm *are commonly used to* indicate the presence of nanoparticles. AgNPs are established to induce surface plasmon resonance (SPR) at a certain range of wavelengths. Lower and higher maximum wavelength (λmax) values are associated with a smaller average size and higher concentration of AgNPs, respectively (Prathna*et al*., 2011). Moreover, broad and narrow peaks at higher and shorter wavelengths, respectively, indicate an increase and decrease in AgNPs size, respectively (Khan *et al*., 2013). The quality of the synthesized nanoparticles can be illustrated by the intensity and position of the SPR peak, which occurs at wavelengths between 380 and 450 nm. A narrow and low wavelength absorption peak implies a small size of the nanoparticles, while a broad peak at a high wavelength implies a large size or aggregated AgNPs, as previously reported by Njud*et al*., (2022).

Fourier Transform Infrared Spectroscopy (FTIR) analysis can assist in identifying biomolecules responsible for reducing Ag^+ ions and stabilizing the AgNPs synthesized (Anandalakshmi *et al.,* 2016). This qualitative analysis is based on using infrared light scanning to observe the chemical bonds of samples. The silver nanoparticles were also characterized by FTIR spectroscopy which measures the prominent peak in ranges between 400-4400cm^{-1}

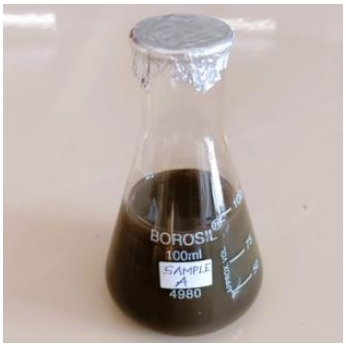
2.4 Antifungicidal activity of synthesized AgNPs

Agar well diffusion method is carried out here, pure strain of *C. albicans* was streaked from stock onto a petri plate with potato dextrose agar and incubated aerobically for 72 h at 25°C. Briefly, the fungal spores were prepared by adding 5% Tween 80 to the petri plate, containing the fungus culture. The concentration of the collected spore suspension was then adjusted to an OD of 0.1. Afterwards, fungal suspension (100 µL) was aseptically spreading on the surface of the sterile PDA plates. Wells of around 6 mm diameter were cut on the agar and then test solution (60 µL) was added to the well. Then it was incubated at 37 °C for 24 h for the appearance of zone of inhibition. The formation of zone of inhibition or zone of clearance around the test film was observed and the zone diameter or diameter of inhibition (DI) in mm was measured. The assays were performed in triplicates.

3.Results and Discussion

Silver nitrate solution is colourless and adding *Piper longum* and *Piper nigrum* plant extract to silver nitrate solution, the colourless silver nitrate solution became dark brown in colour (**Fig.1 a&b**). It confirms that the silver nitrate was reduced and transformed into silver nanoparticles.

(a)

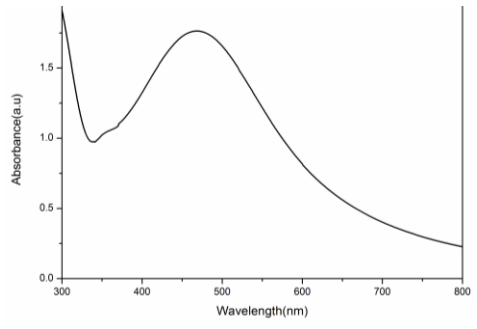


(b)

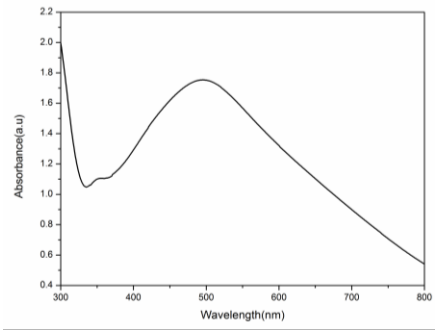
Fig. 1 Biosynthesis of AgNPs from stem extracts (a). *P. longum* (b) *P. nigrum*

3.1 Ultraviolet -visible spectroscopic analysis of AgNPs

The formation of the green synthesized AgNPs from the *Piper longum* and *Piper nigrum* stem extracts was visually confirmed via color change after 5 minutes. Analysis with UV–visible spectrophotometer at wavelengths of 400–500 nm showed the formation of AgNPs as shown in **Fig.2 a&b**. The absorption peak at 480 nm in the spectrum of AgNPssynthesised using *Piper longum* extract and peak at 495 nm in the *Piper nigrum* stem extracts confirmed their synthesis. The appearance of this peak could be attributed to the SPR of AgNPs.



(a)



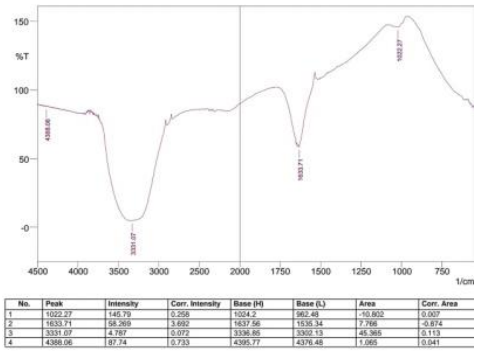
(b)

Fig.2 UV-vis spectroscopic analysis of AgNPs from stem extracts (a). *P. longum*(b) *P. nigrum*

3.2 FTIR Spectroscopic analysis of synthesized AgNPs

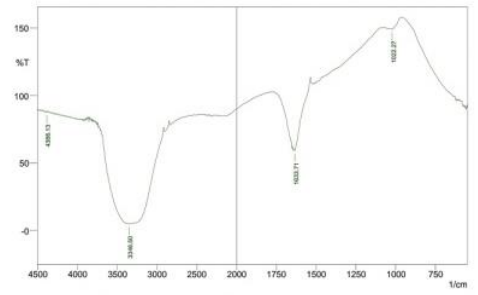
In FTIR spectroscopy analysis, the form of the absorption spectrum profile exhibits different peaks representing the high concentration of specific types of chemical bonds, and various functional groups, such as alkanes, ketones, and amines, absorb infrared radiation of different wavelengths, thus allowing the identification of biomolecules (Palithya*et al.*, 2022). A comparison between the FTIR spectrum of a medicinal plant extract and that of the biosynthesized AgNPs can reveal the functional groups that are involved in the surface coating and effective stabilization of the produced nanoparticles (Akintelu*et al.*, 2020). Therefore, FTIR spectroscopy is a valuable and economical technique to determine the role of biological molecules in the synthesis and stabilization of green synthesized AgNPs.Mehata (2021a,2021b) demonstrated the FTIR spectra illustrating plant extracts and biosynthesizedAgNPs that contain some biomolecules

The **Fig.3 (a&b)** depicts the FTIR absorption spectrum of the *P. longum* and *P. nigrum* synthesized AgNPs.



No.	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	1022.27	140.79	0.258	1024.2	962.48	-10.802	0.007
2	1633.71	58.269	3.692	1637.56	1535.34	7.766	-0.874
3	3331.07	4.787	0.072	3336.85	3302.13	45.365	0.113
4	4386.06	87.34	0.733	4395.77	4376.48	1.065	0.041

(a)



No.	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	1022.27	148.919	1.804	1035.77	962.48	-13.448	0.167
2	1633.71	58.872	3.504	1637.56	1535.34	7.366	-1.056
3	3346.5	4.876	0.025	3348.42	3336.85	15.152	0.023
4	4386.13	87.497	0.712	4395.77	4378.41	0.976	0.03

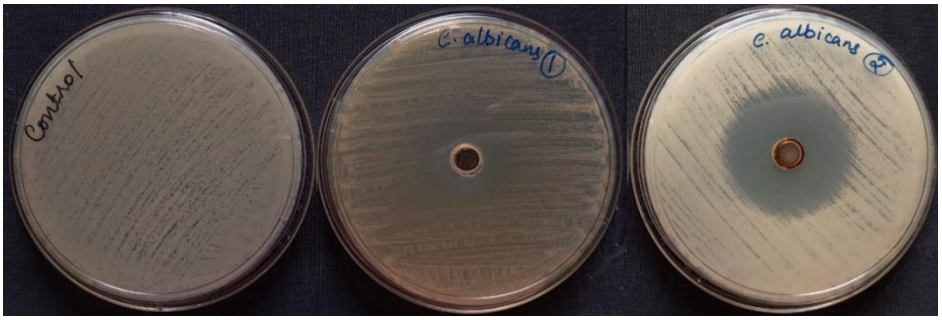
(b)

Fig. 3 FTIR analysis of AgNPs from stem extracts (a). *P. longum* (b) *P. nigrum*

3.3 Antifungicidal activity

The antifungicidal activity of AgNPs of *Piper longum* and *Piper nigrum* stem extracts and stem extracts alone were studied and the results were recorded. The result conveys that all the experimented extracts have antifungicidal properties. It was observed that the synthesized AgNPs from *Piper longum* stem extract possess more antifungicidal activity than that of *Piper nigrum*AgNPs.

Antifungicidal activity of *Piper longum* (**Fig.4**) and *Piper nigrum* (**Fig.5**) stem extract and AgNPs showed the zone of inhibition against *C. albicans* **Table-1)**



(a) (b) (c)

Fig. 4 The antifungicidal activity of *P. longum* against *C. albicans*. (a) control (b) stem extract (c) AgNPs synthesized from *P. longum*

Fig. 5 The antifungicidal activity of *P. nigrum* against *C. albicans*. (a) control (b) stem extract (c) AgNPs synthesized from *P. nigrum*

Table-1 Antifungicidal activity of various extract

Extracts	***C. albicans* zone of inhibition (diameter in mm)**
P. longum stem extract	16 mm
P. longum AgNPs	25 mm
P. nigrum stem extract	13 mm
P. nigrum AgNPs	19 mm

C. albicans was employed in the present study to investigate the antifungicidal properties of the synthesized AgNPs. The augmented fungicidal activity may be attributed due to the effects of silver nanoparticless. The activity of these solutions was mainly due to the presence of AgNPs formed upon the addition of different concentrations of extracts. The synthesized AgNPs from *Piper longum* stem extract possess more antifungicidal activity than that of AgNPs synthesized from *Piper nigrum* stem extract. The antifungal activity of AgNPs is primarily attributed to their ability to disrupt the integrity of fungal cell membranes, penetrate fungal cells, and interfere with vital cellular processes. The mechanisms underlying this activity include the generation of reactive oxygen species, which lead to oxidative stress and damage to cellular components such as proteins, lipids, and DNA. Additionally, AgNPs can bind to

fungal cell walls and membranes, causing structural damage and leakage of intracellular contents. This disruption not only impairs fungal growth but can also lead to fungal cell death.

4.Conclusion

The present study reported a simple, facile, inexpensive, eco-friendly and green synthesis of silver nanoparticles from the *Piper nigrum* and *Piper longum* in aqueous medium without employing manmade chemicals. The UV-Vis spectroscopy and FTIR analysis is confirmed the preliminary confirmation of the formation of silver nanoparticles.

Green-synthesized AgNPs retain their potent antifungal properties, making them a promising solution for combating fungal infections, especially in light of the increasing resistance to conventional antifungal drugs. The use of plants, microorganisms, and non-toxic substances for nanoparticle synthesis provides an eco-friendly alternative that aligns with the growing demand for greener and safer materials in medical, agricultural, and industrial applications.

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