



**BIOSYNTHESIS OF SILVER NANOPARTICLES FROM *MUKIA
MADERASPATANA* LEAF EXTRACT AND EVALUATION OF THEIR
ANTIMICROBIAL ACTIVITY**

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ABSTRACT

Biosynthesized silver nanoparticles are gaining attention because of biologically active plant secondary metabolites that help in green synthesis and also due their unique biological applications. This study reports the reliable, ecofriendly and cost-effective synthesis of silver nanoparticles using the aqueous leaf extract of *Mukia maderaspatana*. The biosynthesized *Mukia maderaspatana* silver nanoparticles were characterized using UV-visible spectroscopy. Phytochemical analysis was performed to determine the phytochemicals responsible for the reduction and capping of the biosynthesized silver nanoparticles. The antibacterial activity of biosynthesized silver nanoparticles was tested against human bacterial pathogens. The green synthesized silver nanoparticles showed strong antibacterial activity against *Klebsiella pneumoniae*, *Proteus mirabilis* and *Pseudomonas aeruginosa*. The observations in this study show that the biosynthesized *Mukia maderaspatana* silver nanoparticles can be developed as a promising nanomaterial in pharmaceutical and health care sector.

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INTRODUCTION

Nanotechnology has emerged as one of the most attractive areas of research, offering unique features and extensive applications in various sectors such as biomedicine, food and agriculture. Properties associated with nanoparticles such as their small size (1-100 nm), large surface area to surface volume ratio, magnetic, optical and chemical properties have made them candidates for novel applications in the biomedical field as antimicrobial, antioxidant, and anticancer agents (Altammar, 2023). Metal nanoparticles, such as silver, copper, platinum, gold, titanium, zinc and magnesium, have gained considerable attention for biomedical applications due to their multifunctional abilities (Behboodi *et al.*, 2019). Nanoparticles are synthesized using various approaches, such as physical, chemical or biological methods with the conditions to control the size/shape and stability of nanoparticles (Irvani *et al.*, 2014)

Physical and chemical methods are employed in the synthesis of nanoparticles. Chemically prepared nanoparticles are not appropriate for medical use due to toxic chemicals binding on

their surface. On the other hand, plant-mediated synthesis of metal nanoparticles is gaining intensive importance because of the low toxicity, eco-friendliness, low time consumption and cost effectiveness (Gengan *et al.*, 2013). Furthermore, bioactive phytochemicals found in plants, such as proteins, polysaccharides, amines, alkaloids, flavonoids, polyphenols, terpenoids, tannins, and ketones, are readily available and serve as reducing, stabilizing, and capping agents in the process of converting metal ions into metal nanoparticles, which in turn produces desired nanoparticles (Rajan *et al.*, 2015).

Among metal nanoparticles, silver nanoparticles have emerged as the champion in the last two decades due to their unique physical, chemical and biological properties. Even though silver is toxic at higher concentrations, many studies have established that a lower concentration of silver nitrate has higher biocompatibility, catalytic activity, chemical stability, and therapeutic potential. Silver nanoparticles are reported to have potential antimicrobial and anticancer activity. Silver nanoparticles have wide applications in medicines including skin creams and ointments containing silver to inhibit infection

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of burns and wounds and medical devices. (Haggag *et al.*, 2019).

Mukia maderaspatana belongs to *Cucurbitaceae* family and is distributed in equatorial regions of Asian countries. The plant has numerous medicinal values in ayurveda, siddha and naturopathy. The plant is used in the treatment of fever, cold, asthma, bronchitis and flu. Its leaves are prescribed for jaundice. It is found to play roles in protecting subjects from rheumatoid arthritis and hypertension. The fruits of the plant have been used as a brain tonic and to allow free flow of urine in case of difficulty in passing urine (Sankaranarayan *et al.*, 2010; Petrus *et al.*, 2011). The present study is aimed to synthesize silver nanoparticles from *Mukia maderaspatana* leaf extract and evaluation of their antibacterial activities.

MATERIALS AND METHODS

Collection and Processing of *Mukia maderaspatana* Leaves

Healthy and fresh leaves of *Mukia maderaspatana* were collected from Varadharajanpettai, Ariyalur district, Tamil Nadu. The leaves were washed thoroughly under tap water to remove dirt and impurities. The leaves were shade dried at room temperature for two weeks. After complete drying, the leaves were powdered and stored in an air-tight bottle until further use.

Preparation of Aqueous Crude Leaf Extract

Aqueous crude leaf extract of *Mukia maderaspatana* was prepared by adding 10 g of leaf powder to 100 ml distilled water in a 250 ml conical flask and boiled for 10 minutes. The obtained extract was then filtered twice through Whatman No.1 filter paper. The filtrate was allowed to evaporate and the dried extract was used for phytochemical analysis and synthesis of silver nanoparticles (Khandel *et al.*, 2018).

Phytochemical Screening of Aqueous Leaf Extract

Qualitative phytochemical analysis of aqueous crude leaf extract of *Mukia maderaspatana* was performed using standard phytochemical methods (Harborne, 1973; Swaminathan, 2017).

Biosynthesis of Silver Nanoparticles

Green synthesis of silver nanoparticles was carried out by adding 10 ml of aqueous crude leaf extract of *Mukia maderaspatana* to 90 ml of 1mM aqueous silver nitrate solution. The mixture was incubated in dark condition at room temperature for 24 hours. The visual colour changes in the reaction mixture from yellow to reddish brown indicated the presence of silver nanoparticles. The bio-reduced aqueous component was subjected for characterization of silver nanoparticles and determination of antimicrobial activity (Khandel *et al.*, 2018).

Characterization of Silver Nanoparticles

Characterization of the silver nanoparticles was carried out using UV-visible spectroscopy. The spectra were recorded at room temperature regulated at a resolution of 1 nm between 300 and 700 nm ranges and plotted the values on a graph (Kalpana *et al.*, 2019).

Determination of Antimicrobial Activity of Silver Nanoparticles

The silver nanoparticles synthesized by using leaf extract of *Mukia maderaspatana* was tested for antibacterial activities by agar well diffusion method against *Klebsiella pneumoniae*, *Proteus mirabilis* and *Pseudomonas aeruginosa*. Levofloxacin and silver nitrate were chosen as the control group for the study of antimicrobial activity. The test bacterial strain was inoculated in to Mueller-Hinton broth and incubated at room temperature for 6 hours. After incubation, Mueller-Hinton agar plates were seeded with a lawn of bacteria and allowed to dry for about 3 minutes. By using a sterile cork borer, three wells measuring the diameter of 8 mm were made in the agar medium. 'A' well was loaded with 50 µl of levofloxacin (100 µg/ml), 'B' well was loaded with 50 µl of biosynthesized silver nanoparticles and 'C' well was loaded with 50 µl of 1mM silver nitrate solution. The plates were incubated in an upright position at room temperature for 24 hours. Antibacterial activities were evaluated by measuring the diameters of zone of inhibition in mm against the test organism (Khandel *et al.*, 2018).

RESULTS AND DISCUSSION

Phytochemical Screening of Aqueous Leaf Extract of *Mukia maderaspatana*

Preliminary phytochemical analysis of aqueous leaf extract of *Mukia maderaspatana* revealed the presence of carbohydrates, reducing sugars, phytosterols, saponins, flavonoids, proteins and free aminoacids, whereas alkaloids and tannins were not found during the analysis (Table 1). Devi and Sathishkumar (2017) reported the presence of carbohydrates, alkaloids, flavonoids, tannins, phenols, saponins, glycosides and triterpenes in the aqueous extract of *Mukia maderaspatana*.

Table 1 Phytochemical Screening of *Mukia maderaspatana* Aqueous Leaf Extract

S.No.	Phytochemicals	Test	Aqueous Leaf Extract
1	Carbohydrates	Molisch's test	+
		Barfoed's test	+
2	Reducing sugars	Fehling's test	+
		Benedict's test	+
3	Alkaloids	Mayer's test	-
		Dragendorff's test	-
4	Phytosterols	Salkowski test	+
5	Saponins	Froth test	+
6	Proteins and free aminoacids	Biuret test	-
		Ninhydrin test	-
		Millon's test	+
7	Flavonoids	Shinoda's test	+
		Zinc chloride reduction test	-
8	Tannins	Bromine water test	-

+: Positive

-: Negative

Biosynthesis and Characterization of Silver Nanoparticles

Silver nanoparticles can be synthesized using plant extracts as reductants and stabilizers. Mixing plant extracts with silver nitrate solution causes the formation of silver nanoparticles

(Ritu Verma *et al.*, 2023). In the present study, the reaction mixture, *Mukia maderaspatana* aqueous leaf extract with aqueous solution of silver nitrate, started to change its colour from yellowish brown to reddish brown and thus marked the formation of silver nanoparticles with the reduction of silver ion (Figure 1). The change in colour of green synthesized silver nanoparticles is due to the excitation of surface plasmon resonance. Several phytochemicals contribute to the formation of metal nanoparticles, including flavonoids, protein, alkaloids and secondary metabolites (Bawazeer, 2021). Alkaloids can act as reducing agents, flavonoids and terpenoids can act as stabilizing and capping agents, while carbohydrates and proteins can act as reducing and stabilizing agents during the conversion of metallic salts to metallic nanoparticles (Ritu Verma *et al.*, 2023). It has been reported that spherical shaped silver nanoparticles absorbed surface plasmon resonance band around 400 – 420 nm (Shameli *et al.*, 2012). In the present study, the absorption spectrum of the incubated solution at different wavelengths ranging within 300–700 nm revealed a peak at 425 nm which is similar to the work reported by Arunachalam *et al.*, (2013) and Devi and Sathishkumar (2017).



Fig. 1 Color change after the process of bio-reduction of silver-to-silver nanoparticles

Determination of Antimicrobial Activity of Silver Nanoparticles

The antibacterial activity of *Mukia maderaspatana* leaf extract mediated biosynthesized silver nanoparticles were tested against *Klebsiella pneumoniae*, *Proteus mirabilis* and *Pseudomonas aeruginosa* (Table 2). The inhibitory activities of silver nitrate and biosynthesized silver nanoparticles were compared with levofloxacin. Levofloxacin showed highest antibacterial activity against all the tested bacterial strains (Figure 2). Biosynthesized silver nanoparticles showed antibacterial activity against *Klebsiella pneumoniae* (11 mm), *Proteus mirabilis* (17 mm) and *Pseudomonas aeruginosa* (15 mm). On the other hand, silver nitrate did not show any antibacterial activity. The silver nanoparticles showed efficient antibacterial activity due to their extremely large surface area providing better contact with cell wall of bacteria (Ibrahim, 2015).

Table 2 Antimicrobial Activity of Biosynthesized Silver Nanoparticles

S.No.	Organism	Diameter of Zone of Inhibition (mm)		
		Levofloxacin	Silver Nitrate	Silver Nanoparticles
1	<i>Klebsiella pneumoniae</i>	20	-	11
2	<i>Proteus mirabilis</i>	24	-	17
3	<i>Pseudomonas aeruginosa</i>	26	-	15

-: No zone of inhibition

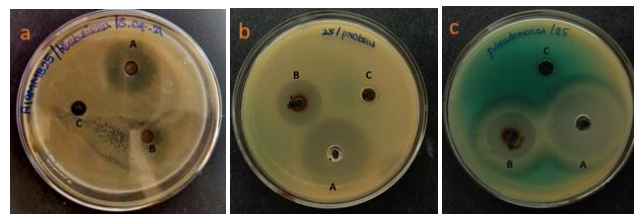


Fig. 2 Antimicrobial activity of biosynthesized silver nanoparticles. (a) *Klebsiella pneumoniae*; (b) *Proteus mirabilis*; (c) *Pseudomonas aeruginosa*

CONCLUSION

Silver nanoparticles were successfully obtained from bio-reduction of silver nitrate solution using *Mukia maderaspatana* leaf extract. The biosynthesized silver nanoparticles were confirmed by UV visible spectroscopy. The biosynthesized silver nanoparticles showed efficient antibacterial activities against *Klebsiella pneumoniae*, *Proteus mirabilis* and *Pseudomonas aeruginosa*. The study revealed that the phytochemicals found in leaf extracts of *Mukia maderaspatana* play a vital role in the formation of silver nanoparticles.

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