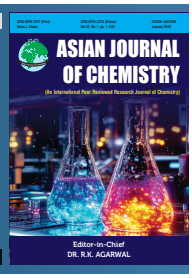
Asian Journal of Chemistry; Vol. 37, No. 5 (2025), 1044-1048

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2025.33485



Microwave Assisted *in situ* Coating of Silver Nanoparticles on Cotton Fabric using *Kalanchoe delagoensis* Leaf Extract and Screening of Biological Activity

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Received: 7 February 2025; *Accepted*: 17 March 2025; *Published online*: 30 April 2025; AJC-21968

The application of nanoparticle coatings on cotton through microwave heating is a well-established technique that has garnered considerable interest as an innovative and promising approach. Silver nanoparticles (AgNPs) get significant attention owing to their remarkable physical and chemical characteristics. The nanoparticles on cotton capping with phytochemicals has enhanced their applicability in the medicinal field. This study focuses on the green synthesis of silver nanoparticles on cotton using *Kalanchoe delagoensis* (AgNPs@KD) under microwave. UV-visible, XRD and EDX spectral reports shown the formation of AgNPs. SEM studies revealed spherical shape of nanoparticles with average particle size of 45 ± 5 nm and polydispersity index (PDI) below 0.5. AgNPs@KD and AgNPs coated cotton were meritoriously inhibited bacteria *Klebsiella pneumonia*, *Escherichia coli*, *Staphylococcus*, *Bacillus* and Fungi *Candida*, *Aspergillus*.

Keywords: Microwave, *Kalanchoe delagoensis*, Silver nanoparicles, Cotton, Antimicrobial activity.

INTRODUCTION

In recent years, metallic nanoparticles have shown much interest due to their unique properties and large applications in numerous fields [1,2]. There are different physical and chemical processes were used to generate nanoparticles [3]. Synthesis of metal nanoparticles using plant extracts and microorganisms consider as green method [4-6]. Since the physical, chemical and biological properties of silver metal are particularly remarkable, it is indispensable to the formation and applications of silver nanoparticles [7,8].

The present-day, use of plant extracts in nanoparticle synthesis is environmentally benign and sustainable approach [9]. AgNPs are employed in medicines due to their minuscule size, bioavailability, solubility and half-life of drugs [10]. Recent literature highlights the synthesis of AgNPs using various plant parts and explores their potential biological applications [11-13]. Composite materials of metal nanoparticles exhibited diverse applications, especially in enhancing properties like antimicrobial activity, conductivity and durability [14-16]. The cotton/silver/graphene, cotton/silver/carboxymethylchytosin, cotton/polypyrrole/silver composite, cotton/silver have been synthesized *in situ* so far. Recently, silver composites generated with cotton mediated by *Ocimum* leaf extract, curcumalonga, *etc.* [17]. Aloe vera known for its rich bioactive compounds has also been employed to synthesize AgNPs coated on cotton cloth, the resulted material exhibited superior wound healing properties [18]. Similarly, *Moringa oleifera* leaf extract, *Eucalyptus globulus* leaf extract, *Camellia sinensis* extract and *Azadirachta indica* leaves extract are also used and their various applications have been reported [19-21].

Microwave heating can speed up the synthesis, providing more accuracy and selectivity than the conventional heating techniques [22,23]. Microwave assisted synthesis has been increasingly applied to the development of sustainable and energy efficient approaches for synthesizing the nanomaterials [24,25]. *Kalanchoe delagoensis* (KD) or Chandelier plant is a Crassulaceae family and is well known for its unique reproductive strategy [26]. *Kalanchoe delagoensis* encompasses a variety of phenolic compounds including 4-aminobenzoic acid, *p*-anisic acid, 4-hydoxymethyl benzoic acid, aroma-dendrin, epicatechin, ellagic acid chlorogenic acid, salicylic acid and naringenin [27,28]. Remarkable, naringenin and syringic acid were also identified in significant concentrations [29]. The

unique phytochemical composition of *Kalanchoe delagoensis* leaf extract may contribute to the properties and functionality of resulting AgNPs.

This study focuses on the microwave assisted synthesis of AgNPs on cotton using leaf extract of *K. delagoensis* (AgNPs@KD), characterization and evaluation of their biological activity. The FT-IR, UV-visible, scanning electron microscopy and X-ray diffraction techniques are used to characterize the structure and morphology of the biogenic AgNPs@KD.

EXPERIMENTAL

Kalanchoe delagoensis leaves (Fig. 1) was collected from the Botanical Garden of Nagarjuna Government College, Nalgonda, India in the month of January 2024 (17.065500ºN, 79.263200ºE). The leaves (20 g) were thoroughly washed with tap water followed by deionized water and then were precisely crushed to obtain a fine paste like material. The resulting paste was then combined with 200 mL of deionized water and heated for 60 min at 55 ºC. Followed by cooling and double filtration using Whatman filter paper, the solution was separated and utilized for further investigations.

Fig. 1. *Kalanchoe delagoensis*

Preparation of AgNPs & AgNPs coated cotton : The leaf extract (20 mL), 0.2 N $AgNO_3$ (100 mL) and 2 cm × 2 cm cotton cloth were mixed in a Erlenmeyer flask and then kept in microwave oven for 5 min. The colour of the solution altered from light yellow to brown. The cotton silver composite was separated and the remaining mixture centrifuged at 9000 rpm for about 10 min to separate the AgNPs@KD, then washed with double distilled water and dried.

Antibacterial activity: Active cultures of Gram-positive (*Staphylococcus* and *Bacillus*) and Gram-negative (*Escherichia coli* and *Klebsiella*) was used in antibacterial activity and the minimum inhibitory concentration (MIC) analysis [30]. The nutrient agar media, dextrose agar and yeast extract peptone media were used for the bacterial growth. A pure culture was incubated for 8-12 h at 37 ºC to prepare the bacterial cultures. The swab strip method was applied for the antibacterial test, with bacterial cultures distributed evenly on the sterilized and solidified nutrient agar plates for antibacterial tests. Each well was prepared in the agar using a sterile borehole drill and 100 µL sample added to each well. Additionally, 5 mm × 5 mm cotton and AgNPs@KD coated cotton were introduced onto the bacterial plate for evaluating the antibacterial activity of AgNPs@KD coated cotton composite. The antibacterial activity was evaluated *via* plates incubated at 37 ºC for 18-24 h and zones of inhibition (ZOI) were measured. The study was performed by preparing a sample at 10 mg/mL successive dilution of samples to achieve varying concentrations. The MICs were measured using samples of 25 µL,50 µL, 75 µL and data was collected.

Antifungal activity: *Candida* and *Aspergillus* species were used in the antifungal assay by well-diffusion method [31-33]. Autoclave was used to sterilize two media, yeast extract peptone agar and dextrose agar respectively. Antibiotic streptomycin was added to the media to prevent the bacterial contaminations. Plates were then allowed to solidify and 5 mm wells were created using sterile well borer in a 5 mm wide. Sample were inoculated into 100 µL of sample (AgNPs@KD) into every well. Afterwards plates are incubated at 25 ºC about 96 h and the results were noted.

RESULTS AND DISCUSSION

The synthesized AgNPs@KD coated on cotton and AgNPs formation was detected by colour change of reaction mixture from light yellow to dark brown. The silver ions were reduced, capped and stabilized by the secondary metabolites of *K. delagoensis* leaf extract.

UV-visible studies: The UV-Vis spectral analysis validated the presence of silver nanoparticles in an aqueous solution, the absorption peak obtained at 255 nm and 380 nm in the spectrum (Fig. 2). The maximum absorption at 255 nm for polyphenols and 380 nm is evidenced of the formation of silver nanoparticles.

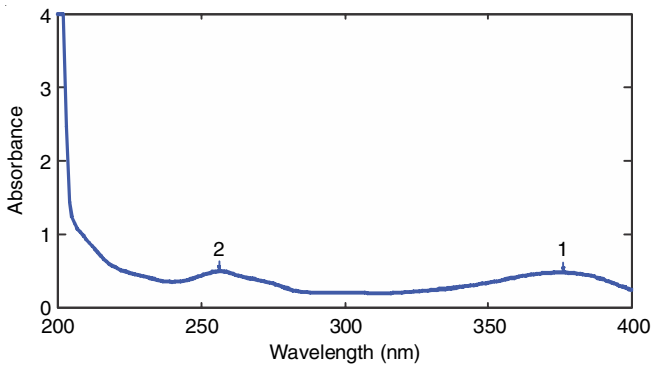

Fig. 2. UV-Vis spectrum of AgNPs@KD

XRD studies: The distinct peaks of XRD of the synthesized AgNPs@KD appeared at 2θ 28.89º, 34.58º, 48.90º and 73.31º are associated with the (111), (200), (222) and (311) crystal planes, respectively as shown in Fig. 3.

FTIR studies: The FTIR spectral analysis of the aqueous *K. delagoensis* plant extract (Fig. 4a) reveals the prominent

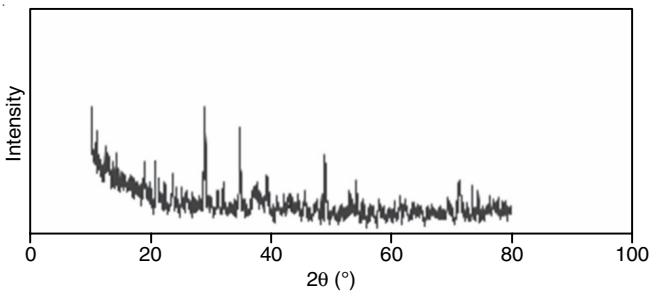

Fig. 3. XRD of AgNPs

functional groups typically associated with phytochemicals. A broad peak at 3273 cm^{-1} suggests the occurrence of alcoholic or phenolic groups, which are the common constituents of plant metabolites such as flavonoids and tannins. The presence of a medium to high-intensity peak at 1634 cm^{-1} indicating the presence of the carbonyl groups (C=O) of organic acids, esters, ketones, aldehydes. Moreover, the spectrum displays the low-frequency bands at 569-461 cm^{-1} signifying the presence of the secondary metabolites.

The FTIR spectrum of AgNPs@KD (Fig. 4b) has one prominent peak at 3163 cm^{-1}, which is attributed to the O-H group. The other prominent groups like the C-H stretch and carbonyl (C=O) groups were identified at 2821 and 1757 cm^{-1}, respectively. The comparative IR studies of fresh leaf extracts with that of AgNPs@KD reveals a decrease in the peak intensity of the -OH (alcohol) group, while the -C=O (carbonyl) spectral intensity becomes more prominent. This indicates that the leaf extracts (alcohols) underwent oxidation to form carbonyls and the silver ions were reduced to silver metal.

SEM-EDX studies: The SEM investigation (JOEL SEM instrument at 5.0 kv, 3000 and 30,000 magnification) revealed morphology and size of the nanoparticles. The AgNPs@KD displayed regular distribution and less aggregation. The size of biogenic AgNPs@KD was calculated as 45-50 nm having a spherical shape as shown in Fig. 5. The EDX spectrum (Fig. 6) also confirmed the elemental composition of AgNPs @KD.

Antibacterial activity: The silver nanoparticles coated on cotton and AgNPs@KD were screened against two Gram-positive and two Gram-negative bacteria. Fig. 7 confirmed the good inhibition of bacterial growth in the presence of AgNPs @KD & AgNPs coated cotton. The zone of inhibition values for the studied bacterial strains showed that the treated fabric was successful in preventing the growth of bacteria (Table-1) and thus can be considered to develop promising antibacterial cotton fabrics.

Antifungal activity: The green synthesized capped AgNPs @KD exhibited strong inhibition against *Candida* but no inhibitory activity against *Aspergillus* is observed (Table-2).

Conclusion

The microwave assisted synthesis is a clean energy utilizing, fast resulted method for the production of AgNPs@KD which is a biodegradable, easy and inexpensive method. The

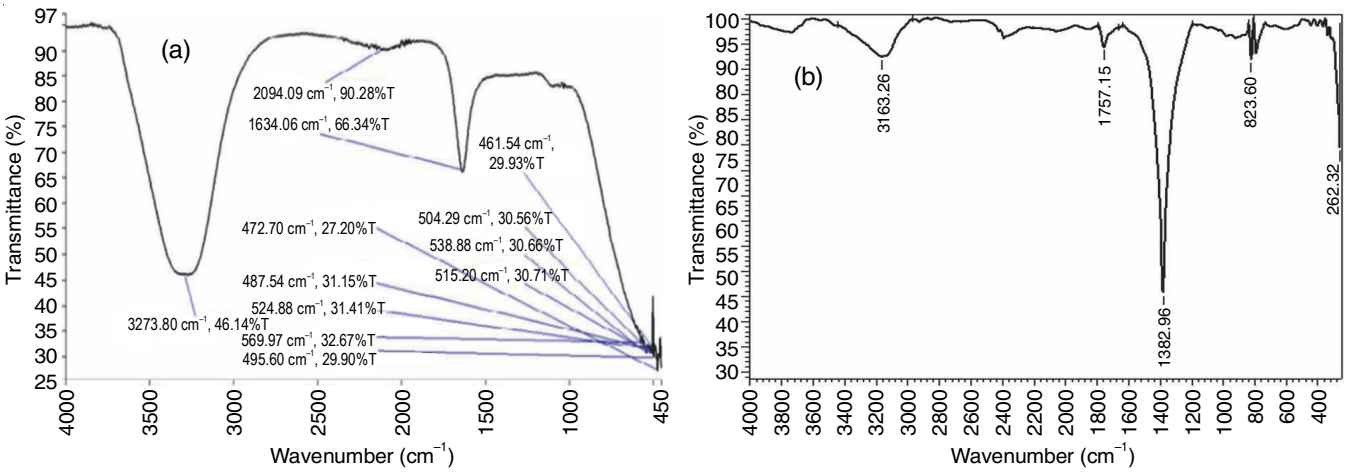

Fig. 4. FTIR spectrum of (a) aqueous extract of KD and (b) AgNPs@KD

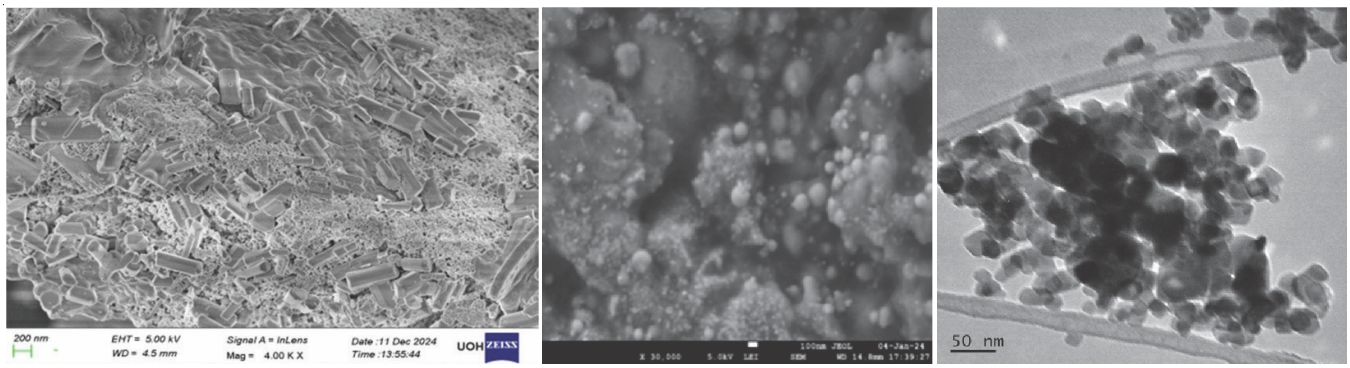

Fig. 5. SEM images of AgNPs@KD

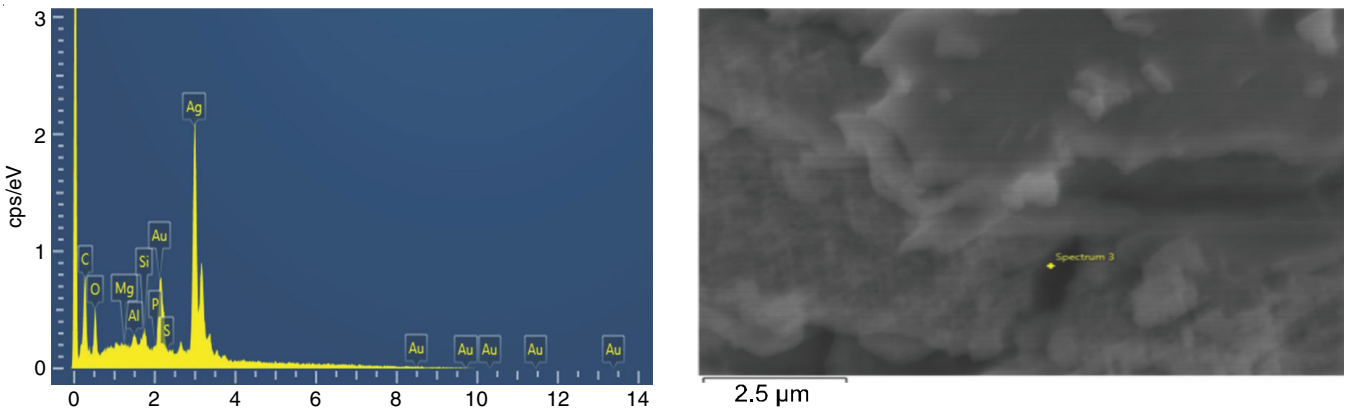

Fig. 6. EDX images of AgNPs@KD

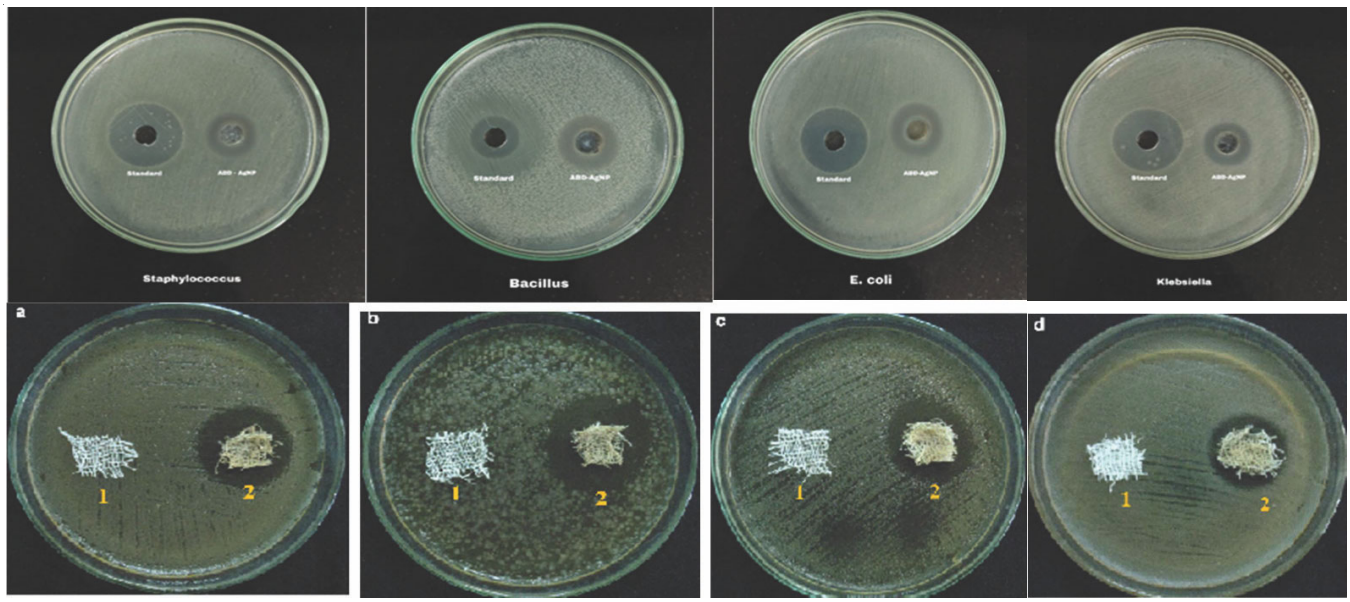

Fig. 7. Antibacterial activity of AgNPs@KD & AgNPs coated cotton

TABLE-1
ZONE OF INHIBITION OF AgNPs@KD

Compound	Gram-positive bacteria		Gram-negative bacteria	
	Staphylococcus	*Bacillus*	*E. coli*	*Klebsiella*
AgNPs@KD	12 ± 1.34 mm	13 ± 1.9 mm	12.5 ± 1.25 mm	11 ± 1.4 mm
AgNPs@KD/Cotton	13	14	13	12

TABLE-2
ANTIFUNGAL ACTIVITY OF AgNPs@KD

Sample	Fungal pathogens	
	Candida	*Aspergillus*
AgNPs@KD	12 mm	No activity
Fluconazole (standard)	14 mm	10 mm

Kalanchoe delagoensis plant extract used for novel production of nanoparticles and coating the nanoparticles on cotton fabric and characterized meticulously using advanced XRD, SEM, EDX, UV & IR techniques. Silver nanoparticles coated cotton fabric meritoriously given the promising results of the antibacterial and antifungal activity. Thus, the AgNPs@KD coated cotton fabric paving the way for use as a bandage and further exploration in medicinal research is required.

ACKNOWLEDGEMENTS

The authors express their gratitude to the Department of Chemistry, Osmania University, Hyderabad, for providing the UV, SEM and FTIR analysis facilities. Thanks are also due to Nishka Scientific Solutions for conducting the XRD and EDX analysis.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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