J.Mycopathol.Res. 62(1) : 93-99, 2024, ISSN : 0971-3719 (Print), 2583-6315 (Online) doi.10.57023/JMycR.62.1.2024.93

Biosynthesis of Silver Nanoparticles with Aqueous Leaf Extract of *Commiphora caudata* and their Antibacterial Activity against Human Pathogens

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Received : 27.10.2023 Accepted : 31.01.2024 Published : 25.03.2024

The biosynthesis of silver nanoparticles (AgNPs) using the aqueous leaf extract of *Commiphora caudata* has gained significant attention in recent years due to its eco-friendly and cost-effective approach. This study explores the green synthesis of AgNPs utilizing *Commiphora caudata* leaf extract as a reducing and stabilizing agent. Characterization techniques such as UV-visible spectroscopy, Fourier-transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD), and energy-dispersive X-ray spectroscopy (EDX) were employed to confirm the formation and structural properties of AgNPs. The synthesized AgNPs exhibited distinct absorption peaks in the UV-visible spectrum, indicating their nanoscale dimensions. FTIR analysis revealed the presence of bioactive compounds in the leaf extract responsible for the reduction and stabilization of AgNPs. XRD analysis confirmed the crystalline nature of AgNPs. TEM images displayed spherical and polydispersed AgNPs with an average size of approximately 20-30 nm. Furthermore, the antibacterial activity of these AgNPs was assessed against a panel of human pathogens, including *Staphylococcus aureus*, *Escherichia coli, Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*. AgNPs exhibited potent antibacterial activity, with inhibition zones observed in agar diffusion assays. Minimum inhibitory concentrations (MIC) were determined to quantify their effectiveness against the tested pathogens.In conclusion, the biosynthesis of AgNPs using *Commiphora caudata* leaf extract offers a sustainable and environmentally friendly method for the production of nanomaterials with potential applications in medicine and biotechnology. The demonstrated antibacterial activity suggests the utility of these AgNPs in combating human pathogens, paving the way for further research in the development of novel antimicrobial agents.

Keywords: Antibacterial activity, *Commiphora caudata,* Green synthesis, Human pathogens, Silver nanoparticles

INTRODUCTION

Nanotechnology, with its distinctive attributes and diverse industrial applications spanning agriculture, food, and healthcare, stands as a captivating and pivotal field of research. Nanoparticles, owing to their well-established characteristics, have emerged as promising candidates in the biomedical sector for their potential roles as antibiotics, antioxidants, and anticancer agents. These characteristics encompass their diminutive size, high surface area-to-volume ratio, and multifaceted optical, magnetic, chemical, and mechanical properties. Notably, nanoparticles of noble metals, such as silver, gold, platinum, copper, zinc, titanium, and magnesium, have garnered considerable attention for their versatile theranostic capabilities in biomedical applications . Nevertheless, conventional chemical and physical methods for nanoparticle synthesis often involve hazardous substances, posing safety concerns. In contrast, the use of plant-mediated synthesis for metal nanoparticles is gaining traction due to its advantages, including reduced time, cost-effectiveness, and efficacy . Plants offer a rich

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source of bioactive secondary metabolites, including proteins, polysaccharides, flavonoids, terpenoids, tannins, alkaloids, amines, ketones, and aldehydes, which play crucial roles as reducing, stabilizing, and capping agents in the transformation of metal ions into tailored nanoparticles . Silver nanoparticles (AgNPs), owing to their distinct biological, chemical, and physical attributes, have emerged as leaders among various biosynthesized metal nanoparticles over the past two decades . Despite the potential toxicity of silver at higher concentrations, studies have indicated the superior chemical stability, catalytic activity, biocompatibility, and therapeutic potential of AgNO3 at lower concentrations. AgNPs are being explored for their antibacterial and anticancer properties, with a key advantage being their controlled and gradual release of silver ions . This research also aligns with the concept of new-age bio-Nano formulations, which integrates traditional medicine with nanotechnology. Several investigations have delved into the biosynthesis of AgNPs, utilizing indigenous plant species and employing green synthesis methods using plant leaves. One such plant of interest is *Commiphora caudata* Hillmango, an evergreen tree with aromatic leaves, found in semi-evergreen or desert forests of South India. The leaves and fruits of *Commiphora caudata* are used by local communities to enhance food flavor. The extract of *Commiphora caudata* has been found to contain various organic compounds, including sulfur compounds, enzymes, minerals, vitamins, and amino acids . E-guggulsterone, a significant biological substance found in *Commiphora caudata,* holds importance for its antioxidant properties. Additionally, *Commiphora caudata* contains bipolar compounds of steroidal and phenolic origin, known for their pharmacological activities, thermal stability, and lack of odor, with E-guggulsterone being the primary antioxidant component. Studies have demonstrated the broad-spectrum antimicrobial activity of *Commiphora caudata* against various bacteria, viruses, parasites, and fungi, including *staphylococci, Streptococcus pneumoniae, Streptococcus faecal*, *Enterobacter cloacae, Klebsiella pneumonia, and Pseudomonas aeruginosa*. E-guggulsterone, generated through enzymatic activity in *Commiphora caudata,* is considered a key contributor to its antibacterial effects. Given the rising concerns over MRSA outbreaks and multidrug-resistant bacterial strains, E-guggulsterone has gained popularity as an alternative treatment for MRSA (methicillin-resistant *Staphylococcus aureus*). Our research aims to investigate the antibacterial potential of a crude ethanol extract of *Commiphora caudata* against clinical strains of MRSA in vitro. Surprisingly, despite the plant's historical use, there is limited existing research on the phytochemical and pharmacological effects of *C. caudata* ethanol extract .

Our study seeks to shed light on the antibacterial properties associated with *C. caudata* leaves and silver nanoformulations, illustrating the potential of combining plants from the Burseraceae family with nanotechnology for a range of biomedical applications.

MATERIALS AND METHODS

Collection of Plant Material and Preparation of Extract

Fresh *Commiphora caudata* leaves were gathered in the Periyakulam, Tamil Nadu area. The Dr. V. SIVA Department of Botany at V.H.N.Senthikumara Nadar College in Virudhunagar, where a voucher specimen was deposited, taxonomically recognized, and certified the sample. The leaves were properly cleaned with distilled water before being allowed to air dry at room temperature in the shade. Using an electric lab blender, the dried leaves were ground into a fine powder. 250 mL of distilled water was added to 25 g of leaf powder, and the mixture was boiled at 80 °C for 3 hours while being constantly stirred. The resulting extract was then filtered using What Man filter paper no. 1 to produce the final product. Until usage, the extract was kept at 4 °C.

Preliminary Phytochemical Analysis

Commiphora caudata leaf extracts were freshly prepared, and preliminary phytochemical analysis was done to identify the various phytoconstituents present, including phenol, triterpenoids, tannins, flavonoids, saponins, and alkaloids, using the methods prescribed by Cyril *et al.*(2019).

Preparation of silver nitrate solution

10mm of silver nitrate solution was prepared by adding 1.68g of silver nitrate powder in 100ml of distilled water.

Synthesis of silver nanoparticles

We utilized the aqueous leaf extract of *Commiphora caudata* produced in the preceding stage for the green production of silver nanoparticles. For this, 90 mL of a 1 mM aqueous silver nitrate solution and 10 mL of leaf extract were combined, then heated at 80 °C for 3 h while stirring continuously. The transition from yellow to dark brown served as a preliminary indicator of AgNP production. Centrifugation was used to separate the green-synthesized nanoparticles at 15,000 g for 20 min. To eliminate the free silver linked to Cc-AgNPs, this procedure was carried out three times. Cc-AgNPs, the final silver nanoparticles produced via green synthesis, were freeze-dried and kept at 4 °C until needed.

Characterization of silver nanoparticles

A UV-visible spectrophotometer with a wavelength range of 400–600 nm was used to monitor the early characterizations of silver nanoparticles. The blank was made up of an aqueous extract of *Commiphora caudata* leaves. The pellet of silver nanoparticles was centrifuged at 2000 rpm for 30 minutes, washed three times with distilled water, dried at 50°C grinding, and stored in a dark environment. X-ray diffraction (XRD) of BRUKER-binary V4 (.RAW) using Cu K1 radiation (= 1.540562 A^o) at 30 mA current and 40 kV voltages was used to characterize the silver nanoparticle powder further. Using a spectrophotometer, Fourier Transform Infrared (FTIR) Spectra were captured. The recorded scans were in the 4000-400 cm^{-1} range.

Antimicrobial activity

Cultured bacteria for single colony isolation, Methicillin-resistant *Staphylococcus aureus, Lactobacillus sp., E. coli, Staphylococcus aureus, Streptococcus mutans,* were kept on nutrient agar medium. A new colony of each strain was taken from the Petri plate and suspended equally in separate tubes with 10 ml Luria broth in order to examine the antibacterial activity of the silver nanoparticle suspension. After the tubes were incubated for an hour, 1 ml of each culture was added to a separate flask containing 10 ml of LB broth. The flask was then kept at 37°C with constant shaking at 150 rpm for 20 hrs.

Agar well diffusion method

The well diffusion technique was used to assess the antibacterial activity of the aqueous leaf extract of *Commiphora caudata* and biosynthesized Cc-AgNPs. A sterile swab was used to disperse a bacterial inoculum suspension on Muller-Hinton Agar (MHA) that had hardened. Methicillin-resistant *Staphylococcus aureus, Lactobacillus sp., E. coli, Streptococcus mutans,* were the bacterial strains employed in this investigation. Different wells on Petri plates were filled with a constant volume of about 25 L containing various concentrations of the aqueous leaf extract (50, 75, 100, and 150 g/mL) and green-synthesized Cc-AgNPs (20, 50, and 75 g/mL) for incubation at 37 °C for 24 hours. The zones of inhibition that were produced were measured in millimeters. A loaded disc with 10 ìg of gentamycin was used as the positive control and DMSO was used as the negative control.

Statistical Analysis

GraphPad Prism was used to do a grouped analysis of variance (ANOVA) on the data. Between the treatment and control sets, a P-value of 0.05 was regarded as significant. The findings of three experiments were expressed as mean standard deviation.

RESULTS AND DISCUSSION

Biosynthesis of silver nanoparticles (AgNPs)

The biosynthesis of silver nanoparticles (AgNPs) using the aqueous leaf extract of *Commiphoracaudata* was successful, as evidenced by changes in color and the emergence of characteristic features. The yellowish-brown appearance of AgNPs in the aqueous solution, as described by surface plasmon resonance, was observed (Fig. 1a). The colorless extract

turned yellowish-brown upon interaction with the Ag ion complex, indicating the reduction of Ag+ ions and the formation of AgNPs (Fig. 1b, c). The powdered form of silver nanoparticles was evident in Fig. 2.

UV-Visible Spectroscopy

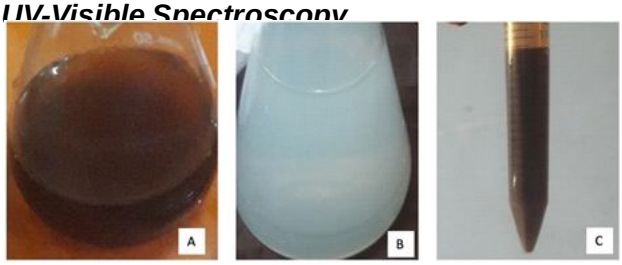

Fig. 1: *Commiphora caudata* leaf extract (a); 10mM of silver nitrate solution (b); 10mM of AgNO3 with leaf extract after 36 hrs (c).

Fig 2: Silver nanoparticles

The UV-Visible spectroscopy results revealed a notable change in the color of the solution from light yellow to reddish-brown within 3 hours of adding the aqueous leaf extract to the silver nitrate solution (Fig. 3A). The strong peak at 420 nm is characteristic of the surface plasmon resonance (SPR) of AgNPs, indicating their successful biosynthesis (Bilal *et al*. 2017). The increases in SPR band intensity over time supports the continuous synthesis of AgNPs.The UV-Visible spectroscopy results showing the characteristic SPR peak at 420 nm are consistent with similar studies.

FTIR Analysis

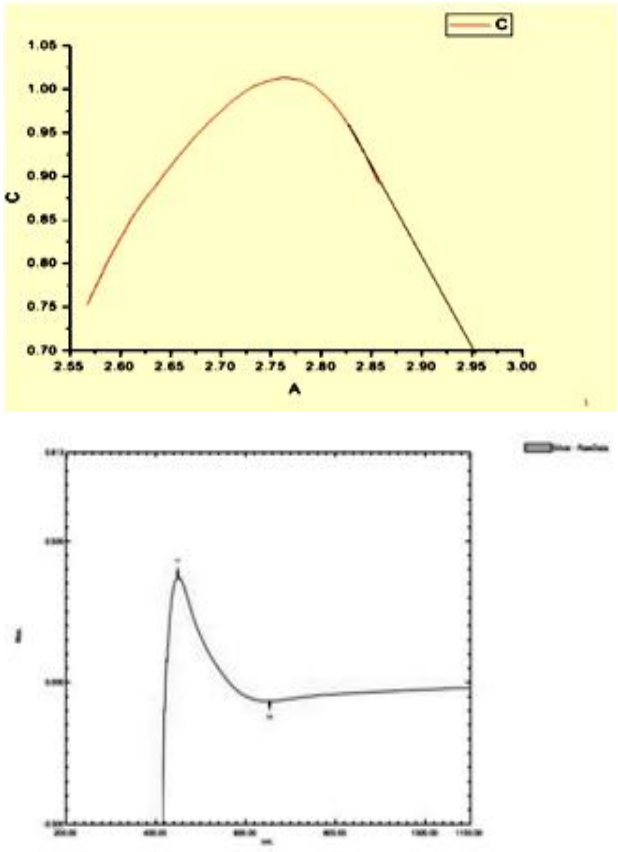

Fig 3.UV-Vis spectrum of silver nanoparticles &Band gap of silver nanoparticles

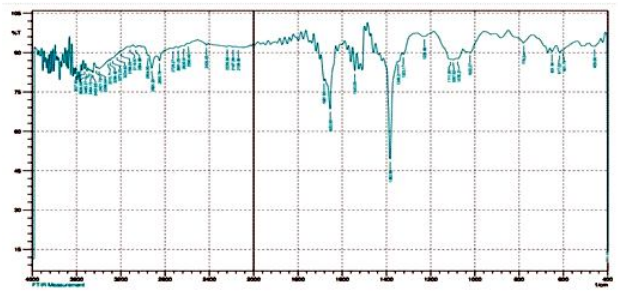

Fig. 4: (FTIR) spectroscopy Image of nanoparticles

FTIR analysis provided insights into the biomolecules responsible for the reduction and capping of Cc-AgNPs. Major absorption peaks in the leaf extract at specific wavenumbers (3450, 2920.3, 1653, 1543.18, 1382.27, 1228.05, 1097.14, 617.4, and 459 cm^{-1}- Table 1) were associated with functional groups (Fig. 3A, red). Similarly, Cc-AgNPs displayed absorption peaks at different wavenumbers (3309.15, 2927, 1602, 1075, and 538 cm-1), indicating the presence of

capping phytoconstituents (Fig. 4, blue).). FTIR analysis supports the involvement of functional groups from the plant extract in the reduction and stabilization of AgNPs, as reported in previous works (Raut *et al*. 2010).

XRD Analysis

XRD analysis demonstrated that the synthesized Cc-AgNPs exhibited sharp diffraction peaks, confirming their crystalline nature. The calculated crystalline size of 18.29 nm and mixed-phase formation (cubic and hexagonal) was revealed in the XRD pattern (Fig. 5, Table 2). The basic cubic structure of the silver nanoparticles was confirmed by the orientation of diffraction peaks.The XRD pattern revealing crystalline structures and the mixed-phase formation of AgNPs is in agreement with expectations based on the literature (Sondiet al. 2004). Additionally, the phytochemical analysis aligns with the known composition of *Commiphora caudata* leaf extract, providing a foundation for the observed reduction and capping processes.

Table 1: FTIR peaks and Functional groups

Observed peak cm-1	Functional group	Vibrational mode
3450	O-H	Stretching mode
2920	C-H	Stretching mode
1653	C=O	Stretching mode
1543	C-H	Stretching mode
1382	C-N	Stretching mode
1228	C-O	Stretching mode
1097	C-O	Stretching mode
617	C-Br	Stretching mode
459	S-S	Stretching mode

Phytochemical Analysis

The phytochemical analysis of *Commiphora caudata* leaf extract identified the presence of various bioactive compounds, including steroids, flavonoids, glycosides, phenolics, tannins, terpenoids, and sugars. These compounds likely played a role in the reduction and stabilization of silver ions during the synthesis process.

Antimicrobial Activity

The synthesized AgNPs exhibited significant antimicrobial activity against various human pathogens, as demonstrated by the agar well diffusion technique (Fig. 6). The zones of inhibition against Methicillin-resistant *Staphylococcus aureus, Lactobacillus* sp., *E. coli*, and

Table 2: XRD spectral analysis details

2θ (degrees)	FWHM (Degrees)	Size D (10-? m)	Dislocation density (m-1)	Strain (no unit)	h k l planes
27.9267	0.4723	18.102	0.003	0.002	(001)
32.2821	0.3936	21.945	0.002	0.0016	(110)
38.2369	0.2362	37.179	0.0007	0.0009	(111)
44.2689	0.6298	14.227	0.004	-0.0027	(200)
46.3138	0.4723	19.946	0.002	-0.0008	(201)
56.5304	3.7786	2.493	0.1608	-0.0164	(300)
64.5618	0.6298	15.583	0.004	0.0017	(310)
77.4842	0.6298	16.891	0.003	0.0013	(311)

Streptococcus mutans highlight the potential of Cc-AgNPs as effective antibacterial agents.The observed antibacterial activity against Methicillin-resistant *Staphylococcus aureus*, *Lactobacillus sp., E. coli, and Streptococcus mutans* confirms the potential of Cc-AgNPs as effective antibacterial agents, consistent with similar studies (Arokiyaraj *et al.* 2014). The suggested mechanisms of action, including membrane disruption and interference with DNA replication and protein synthesis, are in line with the broader understanding of silver nanoparticles' antimicrobial properties (Jeyaraj *et al*. 2013).

CONCLUSION

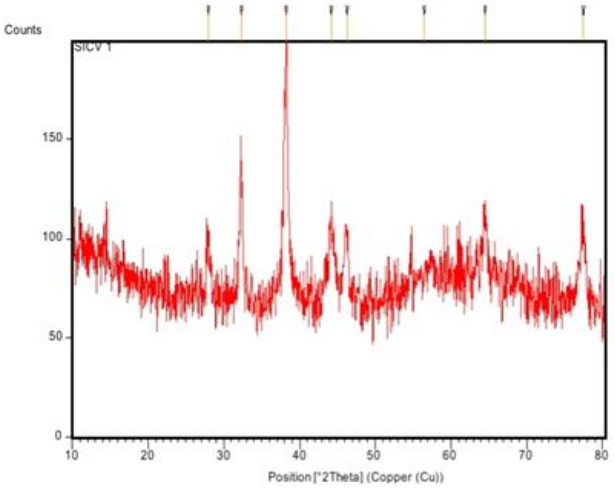

Fig. 5 XRD spectra of silver nanoparticles

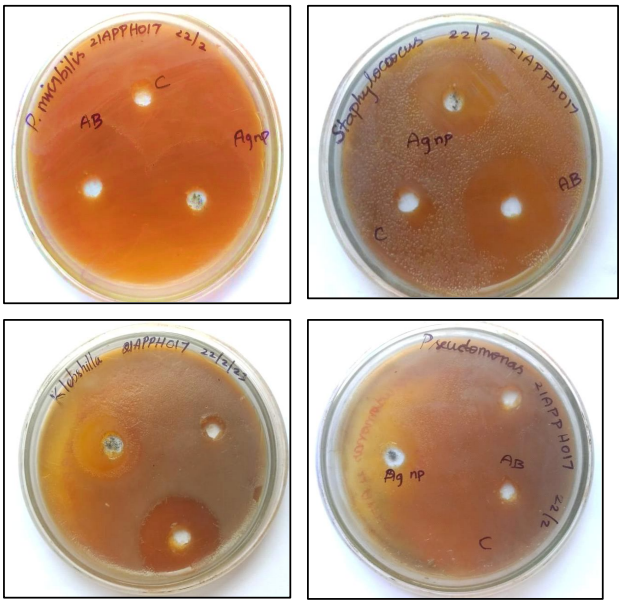

Fig.6: Antimicrobial activity of silver nanoparticles –AgNps against 4 selected Human pathogens

In summary, the biosynthesis of silver nanoparticles (AgNPs) using *Commiphora caudata* leaf extract holds significant promise for various applications. The study successfully achieved green synthesis, characterizing AgNPs with distinct properties suitable for diverse uses. The demonstrated antibacterial activity against human pathogens highlights their potential as effective antimicrobial agents, supported by agar diffusion assays and minimum inhibitory concentration (MIC) determinations. The eco-friendly and cost-effective nature of this biosynthesis process offers sustainable nanomaterial production, suggesting applications in biomedicine and beyond. The integration of traditional knowledge and modern nanotechnology showcased in this study underscores the potential for addressing contemporary challenges, particularly in combating human pathogens. Overall, the biosynthesis of AgNPs using *Commiphora caudata* leaf extract opens avenues for further exploration in developing novel antimicrobial agents and eco-friendly nanomaterial production techniques, showcasing exciting possibilities at the intersection of biology, chemistry, and nanotechnology for public health and environmental benefit.

DECLARATIONS

Conflict of interest: Authors declare no conflict of interest.

REFERENCES

Ahn, E. Y., Jin, H., Park, Y. 2019. Assessing the antioxidant, cytotoxic, apoptotic and wound healing properties of silver nanoparticles green-synthesized by plant extracts. *Materials Sci. Engineer. C* **101**: 204-216.

Arokiyaraj, S., Arasu, M. V.,Vincent, S. 2014. Rapid green synthesis of silver nanoparticles from Chrysanthemum indicum L and its antibacterial and cytotoxic effects: An *in vitro* study. *Inter. J.Nanomed*.**9**: 379.

Behboodi, S., Baghbani-Arani, F., Abdalan, S.,Sadat Shandiz, S. A. 2019. Green engineered biomolecule-capped silver nanoparticles fabricated from Cichorium intybus extract: in vitro assessment on apoptosis properties toward human breast cancer (MCF-7) cells. *Biologic.Trace Elem.Res.* **187**:392-402.

Bilal, M., Rasheed, T., Iqbal, H. M., Li, C., Hu, H., Zhang, X. 2017. Development of silver nanoparticles loaded chitosan-alginate constructs with biomedical potentialities. *Int. J. Biol. Macromol.* **105**: 393– 400. DOI: 10.1016/ j.ijbiomac.2017.07.047.

Cyril, N., George, J. B., Joseph, L., Raghavamenon, A. C. 2019. Assessment of antioxidant, antibacterial and anti-proliferative (lung cancer cell line A549) activities of

green synthesized silver nanoparticles from *Derris trifoliata.Toxicol. Res*. **8**: 297- 308. DOI: 10.1039/ c8tx00323h.

Deepa, V. S., Kumar, P. S., Latha, S., Selvamani, P., Srinivasan, S.2009. Antioxidant studies on the ethanolic extract of Commiphora spp. *Afr. J.Biotechnol.* **8**: 1630-1636.

Erci, F., Cakir-Koc, R.,Isildak, I.2018. Green synthesis of silver nanoparticles using Thymbra spicata L. var. spicata (zahter) aqueous leaf extract and evaluation of their morphology-dependent antibacterial and cytotoxic activity. *Artificial Cells, Nanomed.Biotechnol.* **46**(sup1): 150-158.

Fahimirad, S., Ajalloueian, F.,Ghorbanpour, M. 2019. Synthesis and therapeutic potential of silver nanomaterials derived from plant extracts. *Ecotoxicol.Environ. Safety*, **168**: 260-278.

Gengan, R. M., Anand, K., Phulukdaree, A.,Chuturgoon, A. 2013. A549 lung cell line activity of biosynthesized silver nanoparticles using Albizia adianthifolia leaf. *Colloids and Surfaces B: Biointerfaces* **105**: 87-91.

Haggag, E. G., Elshamy, A. M., Rabeh, M. A., Gabr, N. M., Salem, M., Youssif, K. A., Abdelmohsen, U. R. 2019. Antiviral potential of green synthesized silver nanoparticles of *Lampranthuscoccineus* and *Malephora lutea. Inter.J.Nanomed.***14**: 6217-6229.

Jeyaraj, M., Sathishkumar, G., Sivanandhan, G., MubarakAli, D., Rajesh, M., & Arun, R. (2013). Biogenic silver nanoparticles for cancer treatment: an experimental report. *Colloids and Surfaces B: Biointerfaces***106**: 86-92.

Manikandan, S., Balasubramanian, A., Lalitha, A. V., Siva, V., Palpperumal, S., Harinathan, B., Lakshmi, B. M. Characterization of Commiphoracaudata and its ability to combat Methicillin-resistant Staphylococcus aureus (MRSA). *J.Mycopathol.Res.* **61**: 245-251.

Oyawoye, O. M., Olotu, T. M., Nzekwe, S. C., Idowu, J. A., Abdullahi, T. A., Babatunde, S. O., ... Faidah, H. (2022). Antioxidant potential and antibacterial activities of Allium cepa (onion) and Allium sativum (garlic) against the multidrug resistance bacteria. *Bull. of the Nat. Res. Centre*, **46**:1-7.

Rai, M., Yadav, A., Gade, A. 2009. Silver nanoparticles as a new generation of antimicrobials. *Biotechnol. Adv.***27**: 76-83.

Rajan, R., Chandran, K., Harper, S. L., Yun, S. I., Kalaichelvan, P. T. 2015. Plant extract synthesized silver nanoparticles: an ongoing source of novel biocompatible materials. *Industr. Crops Prod.* **70**: 356-373.

Rasheed, T., Bilal, M., Iqbal, H. M.,Li, C. 2017. Green biosynthesis of silver nanoparticles using leaves extract of Artemisia vulgaris and their potential biomedical applications. *Colloids and Surfaces B: Biointerfaces* **158**: 408-415.

Raut, R., S., Rai, A., Ahmad, A.,Sastry, M. 2004. Rapid synthesis of Au, Ag, and bimetallic Au core-Ag shell nanoparticles using neem (*Azadirachta indica*) leaf broth. *J. Colloid and Interface Sci.* **275**: 496-502.

Raut, R. W., Kolekar, N. S., Lakkakula, J. R., Mendhulkar, V. D., Kashid, S. B. 2010. Extracellular synthesis of silver nanoparticles using dried leaves of *Pongamia pinnata* (L) Pierre. *Nano-Micro Lett.***2**: 106-113.

Sondi, I., Salopek-Sondi, B. 2004. Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. *J. Colloid and Interface Sci*. **275**: 177-182.

Tanase, C., Berta, L., Coman, N. A., Ro'ca, I., Man, A., Toma, F., ... Mare, A. 2019. Investigation of *in vitro* antioxidant and antibacterial potential of silver nanoparticles obtained by biosynthesis using beech bark extract. *Antioxidants* **8**: 459.

Totaro, P., Rambaldini, M. 2009. Efficacy of antimicrobial activity of slow release silver nanoparticles dressing in post-cardiac surgery mediastinitis. *Interactive Cardiovascular and Thoracic Surgery* **8**:153-154.