Research Article

# Assessment of Antibacterial Potency of Biosynthesized Silver Nanoparticles of *M. dioica* Leaf Extract against NDM-1 Bacteria.

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**Received:** 10-12-2020; **Revised:** 24-01-2021; **Accepted:** 02-02-2021; **Published on:** 15-02-2021.

**ABSTRACT**

The objective of the present study was to investigate the possible antimicrobial potential of *Momordica dioica* leaf extract and its bio synthesized silver nanoparticles against sensitive and resistant bacteria. $AgNO_3$ (5 mM) was allowed to react with aqueous leaf extract of *M. dioica*. The silver nanoparticles so formed were characterized using ultraviolet-visible (UV-VIS) spectroscopy, Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM) and Zeta Potential. UV-VIS spectra showed absorption peak at 416 nm. The FTIR analysis showed the alcoholic, unsaturated ester and nitro group present in the plant extract, which were responsible for the reduction in silver nanoparticles. The SEM images showed the size distribution of the nanoparticles. The negative value of zeta potential indicated the stability of bio synthesized silver nanoparticles. Phytochemical screening has shown the presence of Alkaloids, Saponins, Tannins and Terpenoids in both extracts. The study showed that the biosynthesized silver nanoparticles of aqueous leaf extract had a significant antimicrobial activity against sensitive as well as resistant NDM1 bacteria (*K. pneumoniae* and *E. coli*). This ability to inhibit even the resistant pathogens used as indicator organism holds promise for potential application in the pharmaceutical industry. Thus, the plant extracts can be further fractionated for the identification of some active compounds that could be used against NDM-1 bacteria.

**Keywords:** Antibacterial, Resistance, Nanoparticles, NDM-1, green synthesis.

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**DOI:** 10.47583/ijpsrr.2021.v66i02.018

**DOI link:** http://dx.doi.org/10.47583/ijpsrr.2021.v66i02.018

## INTRODUCTION

Antimicrobial resistance has become a global problem, constantly challenging the medical and pharmaceutical fields for last few decades. With the recent progress in medical field, surgical procedures like heart surgery and kidney transplantation are a great success; but the infections after such surgeries has become a major concern because of the growing microbial resistance. As the newer approaches are developed to treat microbial infections, microbes are also developing new ways to resist the changes by generating resistance.[1] Multi-Drug Resistance (MDR) has been a long-time concern for the government and health agencies including WHO. Some bacteria like methicillin-resistant *Staphylococcus aureus* (MRSA) and carbapenem-resistant *Enterobacteriaceae* have known to develop resistance to almost all the antibiotics leading to a serious health concern.[2] The centre for disease control and prevention therefore considers this situation as global acute health problem of this century. The recently discovered New Delhi metallo-b-lactamase 1 (NDM-1) had proved to develop extensive antibiotic resistance against almost all currently available b-lactam antibiotics. The NDM-1 was found in many species of bacteria, predominantly *K. pneumoniae* and *Escherichia coli.* In infection due to highly resistant pathogens such as NDM-positive bacteria, antimicrobial choice is limited and requires a considered analysis of risk and benefit of available agents.[3] So, it is high time to find an alternate solution to the increasing menace of MDR. Plants and metals are known for their medicinal value since ancient times. Healing power in plants and metals is a primeval belief even before any conventional drugs existed.[4] The Ayurvedic system (Materia Medica) largely includes drugs derived from plants, animals, metals and mineral sources.[5] Plants are the renewable and economical sources of antimicrobials with rich chemical diversity and least toxicity. Secondary metabolites like alkaloids, polyphenols, terpenes, glycosides, etc. in plant extracts are mainly phenolic derivatives that are known to stop the bacterial growth by binding to bacterial proteins or through reducing pH, which alters the bacterial cellular process killing the bacteria.[6] So these plant-based antimicrobials are supposed to possess certain clinical value, as the bioactivity does not impose any resistance.[7,8]

Momordica dioica is a perennial, dioecious, cucurbitaceous climbing creeper, commonly known as kantole or spine gourd and is known to be used as preventive and curative agent for various diseases along with a significant nutritional value over thousands of years.[9,10] The leaves, fruits and roots are being used as folk remedy for diabetes and to cure various illnesses. It is also rich in carotene, protein and carbohydrate.[11] The biosynthesised nanoparticles are extensively explored and investigated for antimicrobial.[12] One such metal nanoparticle with prominent antimicrobial activity is silver nanoparticles (AgNPs).[13] Given the importance of the green synthesis of the silver nanoparticles that have the specific biological activity and could potentially be used for the medicinal purposes, the present investigations were undertaken to find out the antibacterial potentiality of the biosynthesised silver nanoparticles of the leave extracts of *M.dioica* against some sensitive and resistant NDM1 bacteria.

## MATERIALS AND METHODS

### Materials

The reagents such as Nutrient broth & Muller Hinton Agar were obtained from Hi-Media Laboratories, Mumbai. Silver Nitrate was procured from Qualigens Fine Chemicals, Mumbai, Dimethylsulphoxide was procured from Merck, Mumbai. Antibiotic Meropenem was procured from Aristo Pharmaceuticals Pvt. Ltd. Mumbai.

### Procurement of microbial strains

Samples of *E.coli* and *K. Pneumonia* were procured from Microbiology Department of S. K. Porwal College of Sciences, Kamptee Nagpur whereas resistant NDM1 *E.coli* and *K. pneumoniae* were obtained from Hi-Media Laboratories, Mumbai. All the procured bacteria were stored at $4^0$C until required.

### Collection of plant materials

Healthy disease free, fresh plant leaves of *Momordica dioica* were collected locally from Nagpur region and was identified and authenticated from Botany Department of RTM Nagpur University, Nagpur. The leaves were rinsed thoroughly 2-3 times with running tap water and lastly in sterile water and then grounded into fine powder using an electric blender and placed in a cool place prior to its use for extraction.

### Preparation of leaf extracts

50 grams of leaf powder material was subjected to the successive Soxhlet extraction using about 500 ml of Distilled water and Ethanol separately for a period of 18 hour. The extracts thus obtained were concentrated to dryness in vacuum at 40°C and then stored at 4°C in the refrigerator until further use.[14]

### Preliminary Phytochemical Analysis

The preliminary phytochemical screening for the detection of secondary metabolites was carried out for both the extracts of *M. dioica* using standard methods.[15-17]

### Biosynthesis of Silver Nanoparticles

10 ml of aqueous plant extract was added in 50 ml of $AgNO_3$ (5 mM) solution and the mixture was stirred on the magnetic stirrer for 45 minutes. The solution obtained was then centrifuged 3 times at 9,000 rpm by washing the pallet each time with distilled water to remove the unbound ligands from the pallet. The final pallet was then dissolved in the distilled water and dried in the oven at 50°C. The dried black coloured powder obtained was used for further studies.[18]

### Characterization of biosynthesised nanoparticles

#### *UV Visible Spectral Analysis*

The reduction of silver ions into silver nanoparticles (AgNPs) during exposure to plant extracts was observed as a result of the colour change. The optical property of AgNPs was determined by using Jasco V-630 UV-Visible spectrophotometer. After the addition of Silver nitrate to the plant extract, the spectrum was taken at between 300 nm to 600 nm.

#### *Fourier Transform Infrared Spectroscopy (FTIR) Analysis*

The synthesized nanoparticles were further chemically characterized and compared using an FTIR spectroscopy. The FTIR spectrum was taken in the mid IR region of 400–4000 $cm^{-1}$. The sample was directly placed in the KBr crystal and the spectrum was recorded in the transmittance mode.

#### *Scanning Electron Microscopy*

The morphological features of the silver NPs synthesized from the *Momordica dioica* leaf extract were calculated using SEM with a voltage of 10 KV.

#### *Zeta Potential*

A zeta size analyzer was used to determine the morphology based on the polydispersity index (PI) and zeta potential of the AgNPs. The value of the zeta potential was used to determine the solidity of the particles.

### Assessment of Antibacterial activity

The anti-bacterial activity of biosynthesized silver nanoparticles was performed by agar well diffusion method for which Muller Hinton Agar medium petri plates were prepared, sterilized, and solidified. After solidification, test bacterial cultures were swabbed on agar plates. A 6 mm diameter wells were punched into the Agar medium with a sterile cork borer and filled with 50µl of plant extract, $AgNO_3$NP and solvent blank. The plates were then incubated at 37 °C for 24 h. The sensitivity of bacterial strains was indicated by the zones of inhibition around the well and the diameter of clear zones of inhibitions was measured.[19]

## RESULTS AND DISCUSSION

### Phytochemical studies

The results of phytochemical studies of the leaf extract of *M.Dioica* are shown in Table no. 1 below:

**Table 1:** Phytochemical screening of Aqueous and Ethanolic leaf extract of *M.Dioica*

| Phytochemical | Test performed | Results | |
|---|---|---|---|
| | | Aqueous Extract | Ethanolic Extract |
| Alkaloids | Dragendorff's test | ++ | ++ |
| Flavonoids | NaOH test | -- | ++ |
| Phenolic compounds | Ellagic test | ++ | ++ |
| Saponin | Froth Test | ++ | ++ |
| Tannins | Ferric Chloride Test | ++ | ++ |
| Triterpenoids | Triterpenoid test | ++ | ++ |
| Glycosides | Keller Kilani Test | ++ | ++ |

++ indicates the presence and -- indicates the absence of phytoconstituent.

The phytochemical studies showed the presence of secondary metabolites like Alkaloids, flavonoids, Phenols, Saponin, Tannins, Triterpenoids and Glycosides in both the extracts accept the absence of Flavonoids in aqueous extract. The presence of alkaloids is supposed to inhibit microbial growth preferably by interfering with cell division.[20] Various tannins substances were also known to produce good antibacterial activity.[21]

### Antibacterial activity of leaf extracts

The Antibacterial activity carried out on leaf extracts (aqueous and ethanolic) obtained from Agar well diffusion method indicated that among these two extracts, aqueous extract showed more antimicrobial activity against both sensitive as well as resistant strains of bacteria. So it (aqueous extract) was selected for synthesis of nanoparticles.

### Synthesis of Nanoparticles

Synthesis was acknowledged by the colour change from green/light brown to dark brown/black due to the concept of surface Plasmon resonance that indicates the formation of silver nanoparticles. The content of sample is shown in table no. 2 and colour change as shown in figure 1.

**Table 2:** Synthesis of silver nanoparticles of leaf extract of *M.Dioica*

| Sample No | Extract $Sol^{n}$ | Silver nitrate $Sol^{n}$ | Silver nitrate Conc. | Colour change |
|---|---|---|---|---|
| 1 | 2ml | 10ml | 1mM | - |
| 2 | 2ml | 10ml | 2mM | - |
| 3 | 2ml | 10ml | 3mM | - |
| 4 | 2ml | 10ml | 4mM | - |
| 5 | 2ml | 10ml | 5mM | + |

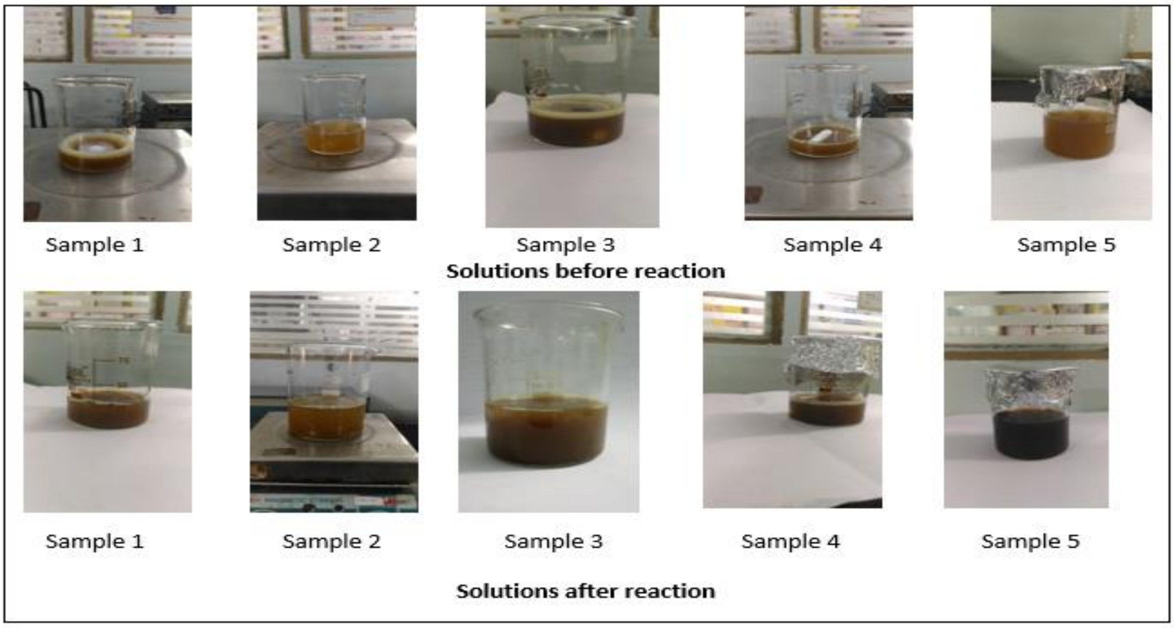



**Figure 1:** Biosynthesis of silver nanoparticles through green synthesis

**UV Visible Spectral Analysis**

The spectral study of synthesized Silver nanoparticles was done using U.V. Visible-spectrophotometer in range of 300-600nm. Strong absorption peak was observed at 416 nm as shown in figure 2, which indicated the formation of Silver Nanoparticles (MDL-AgNPs).

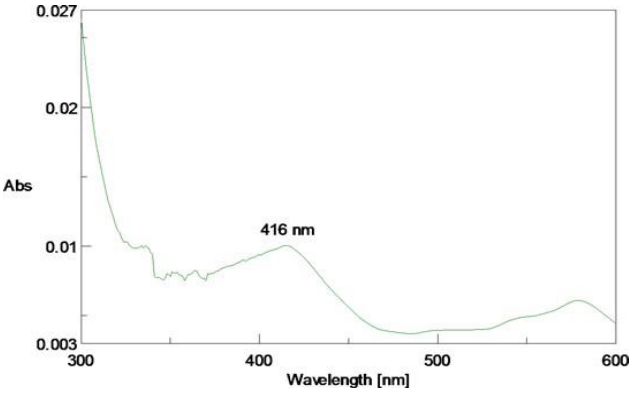



**Figure 2:** UV Vis-Spectral Analysis of Nanoparticles of Silver Nanoparticles (MDL-AgNPs)

**Fourier Transform Infrared Spectroscopy (FTIR)**

The FTIR determination was performed to identify the possible biomolecules responsible for the reduction of the silver ions into the silver nanoparticles. The IR spectrum of MDL-AgNPs Silver nanoparticles showed band at 3600-4000 $cm^{-1}$ corresponding to O-H stretching (Figure 3). The peak around 1716 $cm^{-1}$ corresponds to C=O stretching (α, β-unsaturated ester), peak around 1653 $cm^{-1}$ for C=N stretching (imine /oxime), and peak around 1558 $cm^{-1}$ for C=C stretching (cyclic alkene).

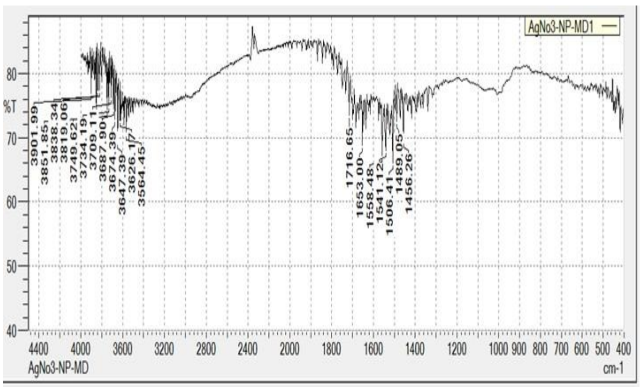



**Figure 3:** Fourier Transform Infrared (FTIR) spectrum of MDL-AgNPs

**Scanning Electron Microscopy**

The morphological features of biosynthesized silver nanoparticles of *M. dioica* extract were studied by Scanning Electron Microscope. The figure 4 shows the high density that confirms the presence of silver nanoparticles. The Synthesized nanoparticles were relatively spherical and crystal shape. *M. dioica* silver nanoparticles (MDL-AgNPs) were in the average range of 150nm. The size was more than the desired size probably due to the proteins which might have bound in the surface of the nanoparticles.

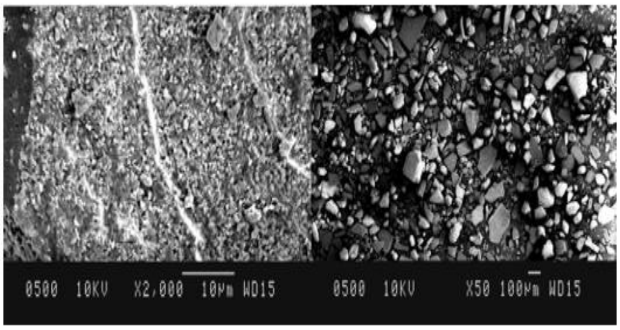



**Figure 4:** SEM images of MDL-AgNPs

**Zeta Potential**

The firmness of the silver-based NPs was detected using the zeta potential and found to be −16.6 mV. In the present study, the negative value of zeta potential confirms the repulsion among the particles and thereby increases the stability of formulation (Figure 5). Based on the above results, it was observed that the synthesized nanoparticles were very stable.

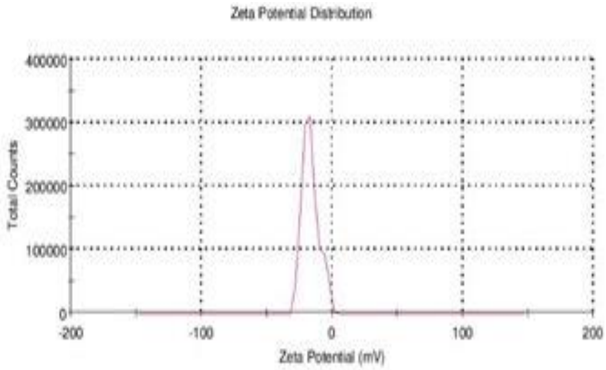



**Figure 5:** Zeta Potential of biosynthesised MDL-AgNPs

**Antimicrobial Susceptibility of Resistant Strains to biosynthesised silver Nanoparticles**

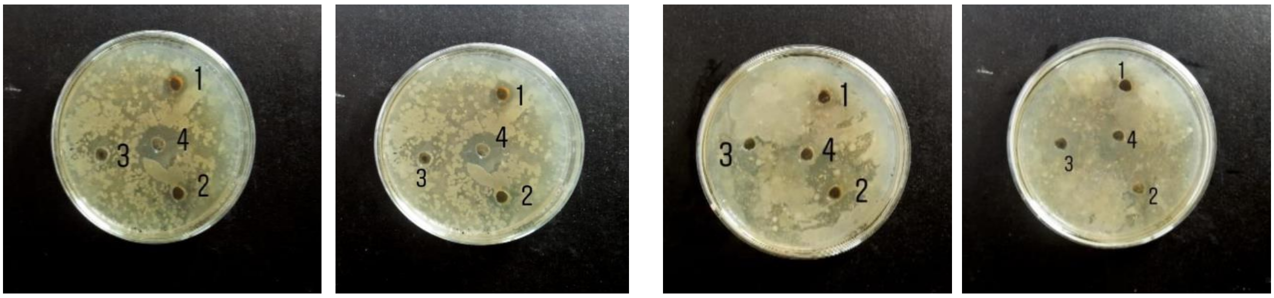
**Zone of Inhibition**

The antibacterial effectiveness of the biosynthesized silver nanoparticles of leaf extract of *M. dioica* were investigated against normal as well as resistant bacterial strains, *E. coli* and *K. pneumoniae* using the agar well diffusion method (Table 3 and figure 6).

The biosynthesized MDL-AgNPs had shown good activity against sensitive strains of *E. coli* and *K. Pneumoniae.* Similarly, it also showed a significant activity against resistant strains (NDM1 strains of *E. coli and K. Pneumoniae*), which indicates that the synthesized nanoparticles showed the combined antibacterial effect of extract and metals against test organisms' i.e. sensitive as well as resistant strains of *E. coli* and *K. Pneumoniae*. Thus, silver nanoparticles synthesized using leaf extract of *M. dioica* showed a significant antibacterial activity against NDM1 *E. coli and NDM1 K. Pneumoniae*.

**Table 3:** Zone of Inhibition of MDL-AgNPs against Test Organisms

| Sr. No | Microorganism | Bacterial strain | Diameter of Zone of Inhibition | | | |
|---|---|---|---|---|---|---|
| | | | MDL-AgNPs Nanoparticles (1) | $AgNO_3$ (2) | DMSO (3) | Meropenem (4) |
| 1. | *E. coli* | *Normal* | 11mm | 9mm | 8mm | 16mm |
| 2. | *K. pneumoniae* | *Normal* | 10mm | 5mm | 8mm | 15mm |
| 3. | *E. coli* | *NDM1* | 8mm | 5mm | -- | -- |
| 4. | *K. pneumoniae* | *NDM1* | 9mm | 6mm | -- | -- |




*E.c oli* *K. pneumoniae* *NDM1 E.coli* *NDM1 K. pneumoniae*

**Figure 6:** Zone of Inhibition of MDL-AgNPs for normal as well as resistant Bacterial Strain

(1) MDL-AgNPs; (2) AgNO3; (3) DMSO (4) Meropenem

## CONCLUSION

In the present study, a simple methodology was used to attain a green eco-friendly approach for the biosynthesis of metallic nanoparticles using *M. dioica* leaf extracts. The synthesized nanoparticles showed significant antibacterial activity. The study showed that the biosynthesized silver nanoparticles of aqueous plant extract showed significant antimicrobial activity against the resistant bacterial species especially against *K. pneumoniae* and *E. coli* compared to the standard antibiotic.

So it could be concluded that the silver nanoparticles of selected plant extract can serve as a promising natural alternate source against the growing concern of multi drug resistant strains like NDM-1 bacteria. This biosynthesised silver nanoparticle holds a promise for potential application in the pharmaceutical industrial applications like filters, masks etc. Therefore, these plants can be used to discover activities suggesting their use as potential antimicrobial agent to cure bacterial infection and can be further fractionated for the detection of some active compounds that could be used against resistant bacteria.

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**Source of Support:** None declared.

**Conflict of Interest:** None declared.