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Plant-mediated green synthesis of Ag nanoparticles with enhanced antibacterial efficacy

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ABSTRACT

The present study deals with the antibacterial efficacy of silver nanoparticles (AgNPs) synthesized in the aqueous leaf extracts of *Murraya koenigii* and *Moringa oleifera* on *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis* and *Staphylococcus aureus*. The results reveal that the AgNPs synthesized by this green approach have a significant impact on the antibacterial activity of the original extract itself against most of the tested microorganisms. The MIC of the original extracts, ranging from 0.150 to 0.300 mg/ml was decreased in the range of 0.075 to 0.150 mg/ml after the synthesis of AgNPs. The results obtained with the reference drug, ciprofloxacin were in the same range with those obtained with the original leaf extracts. The data suggest that the antibacterial efficacy of *M. koenigii* extract containing AgNPs was superior to that of ciprofloxacin.

Keywords: *green synthesis, silver nanoparticles, antibacterial, MIC.*

1. INTRODUCTION

Nanoparticles are of vast scientific importance because of their size related distinct properties. Study of their role in various fields is an upcoming area for invention of novel products. By restraining their size specifically at nanoscale dimensions their solubility as well as biocompatibility can be regulated and altered [1]. Thus, their application in the field of medicine cannot be no longer ignored [2]. Recently, research efforts are being focused on of the green synthesis of nanoparticles with the bio-efficacy of herbal products, which are cost-effective and safe.

Among the bioapplications of nanoparticles there are their exceptional antibacterial activity on various Gram positive and Gram negative bacteria [3]. The bactericidal effect of nanoparticles on a range of microorganisms is due to their size and the shape [4] which favor their interaction with microorganisms [5, 6, 7, 8]. Recently, gaining high interest is given in the field of nanoparticles to deal with the rising microbial resistance to various existing antimicrobial agents [9]. Hence, there is a rising need to develop new and potent antibacterial agents with a better antibacterial efficacy, lower toxicity and cost effectiveness. Therefore, the present paper deals with the unique study of plant-mediated green synthesis of AgNPs and their impact on antimicrobial activity of these plants in which they were synthesized. Among several metal nanoparticles, AgNPs have gained our attention due to their antimicrobial property associated with low toxicity [10, 11].

The plant extracts selected for the green synthesis of AgNPs were of *Murraya koenigii* and *Moringa oleifera* leaves. *M. koenigii* (L.) Spreng (Family: Rutaceae) is commonly known as 'Indian Curry Leaf' in English and 'Meethi Neem' in Hindi. It is a well-known Indian medicinal plant used as a preservative for preventing growth of pathogenic bacteria. The curry leaves have been reported to possess antibacterial, as well as antidiabetic activities (obstructing the pancreatic alpha amylase) [12, 13]. Whereas, the other selected plant *M. oleifera* Lam. (family: Moringaceae) is extensively cultivated and is native to the sub-Himalayan tracts of India and its neighboring countries [14]. It is commonly known as 'Saijan' in Hindi and 'Drumstick' in English. The plant has been the focus of significant research due to its manifold uses and reported bactericidal property [15, 16, 17, 18].

Thus, the present study deals with the synthesis of AgNPs in the aqueous leaf extracts of each, *M. koenigii* and *M. oleifera* and to assess the antibacterial activity of these extracts against five bacterial strains, viz. *Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Enterococcus faecalis*.

2. EXPERIMENTAL SECTION

2.1. Materials.

Chemicals used in the present study were of highest purity and purchased from Sigma-Aldrich (New Delhi, India); Merck and Himedia (Mumbai, India). *M. oleifera* and *M. koenigii* leaves were collected locally from the University of Allahabad, Allahabad, Uttar Pradesh, India and were authenticated by Prof. Satya Narayan, Taxonomist, Department of Botany of the same University. A voucher specimen has been submitted to the University herbarium.

2.2. Preparation of plant materials.

Aqueous leaf extracts of both *M. oleifera* and *M. koenigii* plants was prepared separately by taking 5 g of properly washed leaves. They were then cut into fine pieces and taken in a 250 mL Erlenmeyer flask with 100 mL of sterile distilled water. The mixture was boiled for 5 min and then filtered. The extract thus obtained was stored at 4 °C for further use in a week time.

2.3. Plant-mediated synthesis of AgNPs.

The aqueous solution of 1 mM silver nitrate ($AgNO_3$) was prepared to synthesize AgNPs. 190 ml of aqueous solution of 1

mM $AgNO_3$ was slowly added to 10 ml of aqueous leaf extract with constant stirring and was kept at room temperature for 6 h [10, 19]. Color changes from yellowish orange to brown suggested the reduction of Ag^+ ions into AgNPs.

2.4. Bacterial strains, stocks and growth *in vitro.*

The bacterial Gram negative strains of *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and Gram positive strains of *Staphylococcus aureus* and *Enterococcus faecalis* were clinical isolates obtained from the Department of Biotechnology, All India Institute of Medical Sciences (AIIMS), New Delhi. The microbiologist of the department confirmed the identity based on microscopic examination, Gram's character and biochemical test profiles. Bacterial stocks were maintained and stored as 1 ml aliquots at -80ºC in Luria Bertani (LB) broth containing 5% glycerol for all the five bacterial strains. For experimentation bacterial stocks were revived from -80ºC and grown in LB broth for each strain and all the cultures were grown overnight at 37ºC ± 0.5°C, pH 7.4 in a shaker incubator (190-220 rpm). Their sensitivity to the reference drug, Ciprofloxacin was also checked. Luria Bertani broth and standard antibiotic, Ciprofloxacin both were purchased from Himedia for antimicrobial sensitivity testing.

2.5. Determination of Minimum Inhibitory Concentration (MIC).

Minimum Inhibitory Concentration (MIC) for the freshly prepared inocula of bacterial strains were determined by the micro-dilution method using serially diluted (2-fold) plant extracts as prescribed by the National Committee for Clinical Laboratory Standards [20]. Five sets of thirty sterile test tubes each were taken for the experiment. Each set of these thirty sterile test tubes was further divided in a subset of six for six different concentrations of the five different bacterial inocula. The final working solution in each tube was of 1ml containing 50µl of bacterial strain suspension and the calculated amount of broth and various concentrations of aqueous extracts with and without AgNPs. The different concentrations of extracts ranging from 0.075 to 2.4 mg/ml were prepared from 50mg/ml stock solution. Initially, the turbidity appeared as the bacterial strain was added to the broth. The test tubes closed with cotton plugs were incubated at 37ºC for 24 h in a shaker incubator after adding extracts. The lowest concentration of the extracts at which the turbidity disappeared was considered to be the MIC for a particular bacterial strain. The effects were also compared with that of the standard antibiotic, Ciprofloxacin at the same concentration range.

3. RESULTS SECTION

The impact of the nanoparticles derived from the antibacterial activities of the aqueous extracts of *M. koenigii* and *M. oleifera* leaves was studied by the micro-dilution method and the results are shown in Tables 1 and 2, respectively. Table 1 presents the comparative MIC values of aqueous extracts of *M. koenigii* leaves with and without AgNPs and standard antibiotic, Ciprofloxacin against the five different bacterial strains. The results reveal that the synthesized *M. koenigii* mediated AgNPs exhibit a remarkable impact on the improving of the antimicrobial efficacy of the extract, higher than that of the original leaf extract as well as of the reference drug, Ciprofloxacin itself, against most of the tested strains with MICs ranging from 0.075 to 0.30 mg/ml. Comparatively, the MIC values were ranging from 0.15 - 0.30 mg/ml and from 0.075 - 0.30 mg/ml for the original leaf extract and reference drug respectively.

Table 1. MIC values of the Aq. Ext. of *M. k.* leaves with and without AgNPs & standard antibiotic, Ciprofloxacin against five bacterial strains

Microorganism	MIC(mg/mL)		
	***M. k.* leaves+ AgNPs**	***M. k.* leaves**	**Ciprofloxacin *(Standard drug)***
E. coli	0.075	0.30	0.30
K. pneumoniae	0.15	0.30	0.15
P. aeruginosa	0.075	0.30	0.075
S. aureus	0.075	0.30	0.30
E. faecalis	0.30	0.15	0.075

Similarly, Table 2 presents the comparative MIC values of the aqueous extract of *M. oleifera* leaves with and without AgNPs,with those of the standard antibiotic, Ciprofloxacin against the five different bacterial strains. The results reveal that the *M. oleifera* containing the synthesized AgNPs exhibit a noteworthy enhancement in the antimicrobial activity as compared with the original leaf extract itself and the reference drug, Ciprofloxacin against most of the tested strains at a MIC range from 0.075 to 0.30 mg/ml. Moreover, the MIC values were ranging from 0.15 to 0.60 mg/ml and from 0.075 to 0.30 mg/ml for original leaf extract and the reference drug, Ciprofloxacin, respectively.

Table 2. MIC values of the Aq. Ext. of *M. o.* leaves with and without AgNPs & standard antibiotic, Ciprofloxacin against five bacterial strains

Microorganism	MIC(mg/mL)		
	***M. o.* leaves + AgNPs**	***M. o.* leaves**	**Ciprofloxacin *(Standard drug)***
E. coli	0.075	0.15	0.30
K. pneumoniae	0.30	0.30	0.15
P. aeruginosa	0.075	0.15	0.075
S. aureus	0.15	0.30	0.30
E. faecalis	0.30	0.60	0.075

Thus, it is evident from Table 1 that *M. koenigii* leaf extract with AgNPs possessed the greatest antibacterial activity against most of the bacterial strains, viz. *E. coli*, *S. aureus* and *P. aeruginosa* (MIC 0.075 mg/ml for all three strains) as compared to the original extract without nanoparticles (MIC 0.30 mg/ml for all three strains). Moreover, the extract containing AgNPs

demonstrated the higher antibacterial activity against *E. coli* and *S. aureus* (MIC 0.075 mg/ml for both the strains) in comparison with the standard drug, Ciprofloxacin (MIC 0.30 mg/ml for both the strains).

Similarly, Table 2 apparently confirms that *M. oleifera* leaf extract with AgNPs possessed a greater antibacterial activity against most of the bacterial strains, viz. *S. aureus*, *P. aeruginosa, E. coli* and *E. faecalis* (MIC 0.15, 0.075, 0.075 and 0.30 mg/ml, respectively) as compared to the original extract without nanoparticles (MIC 0.30, 0.15, 0.15, 0.60 mg/ml respectively). Furthermore, the nanoparticles containing extracts exhibited higher antibacterial activity against *E. coli* and *S. aureus* (MIC 0.075 and 0.15 mg/ml respectively) in comparison to the standard drug, Ciprofloxacin (MIC 0.30 for both the strains).

The results summarized in Table 1 clearly reveal that the *M. koenigii* extract containing AgNPs has added value to the antibacterial potential of the original leaf extract towards the tested bacterial strains excepting *E. faecalis* in comparison to the original leaf extract. In the present study, the growth of *K. pneumoniae* and *P. aeruginosa* strains was remarkably inhibited by the synthesized AgNPs in the extract exhibiting an efficiency similar with that of the second generation antibiotic drug, Ciprofloxacin *viz*., 0.15 and 0.075 mg/ml, respectively. Whereas, a much better antimicrobial profile was observed with the extract containing AgNPs in comparison to the reference drug, Ciprofloxacin (MIC 0.30 mg/ml in both the strains) against *E. coli* and *S. aureus* strains (MIC 0.075 mg/ml in both the strains).

The impact of plant-derived Ag nanoparticles on the antimicrobial activity of the aqueous extract of *M. koenigii* leaves was compared with the reference drug, Ciprofloxacin as well as with the activity of original extract without these nanoparticles can be ordered as follows based on the obtained MIC values.

Antimicrobial efficacy in terms of MIC

***M. koenigii* aqueous leaf extract with AgNPs**:
E. coli = P. aeruginosa = S. aureus >K. pneumoniae>E. faecalis.
0.075 = 0.075 = 0.075 > 0.15 > 0.30**(mg/ml)**

***M. koenigii* aqueous leaf extract without NP**:
E. faecalis> E. coli = K. pneumoniae = P. aeruginosa = S. aureus.
0.15 > 0.30 = 0.30 = 0.30 =0.30 **(mg/ml)**

Ciprofloxacin:
P. aeruginosa = E. faecalis>K. pneumoniae > E. coli = S. aureus.
0.075 = 0.075 > 0.15 >0.30 = 0.30 **(mg/ml)**

Similarly, data given in Table 2 illustrate that the *M. oleifera* extract containing AgNPs has proved enhancement in the antibacterial activity of the original leaf extract without AgNPs towards four bacterial strains, i.e. *E. coli*, *P. aeruginosa*, *S. aureus* and *E. faecalis*. The aqueous extract of *M. oleifera* with AgNPs exhibited antibacterial efficacy similar with the original extract without AgNPs only in case of *K. pneumoniae*.

Moreover, the growth of *E. coli* and *S. aureus* strains was exceptionally inhibited (MIC 0.075 and 0.15 mg/ml respectively) by the plant-derived AgNPs, their activity being similar to that of Ciprofloxacin (MIC 0.075 mg/ml) against the bacterial strain *P. aeruginosa* (MIC 0.075 mg/ml). However, *E. faecalis* was found to be more resistant towards the aqueous extract of *M. oleifera* leaves with AgNPs (MIC 0.30 mg/ml) than to Ciprofloxacin (MIC 0.075 mg/ml)

The impact of the plant-derived Ag nanoparticles on the antimicrobial activity of the aqueous extract of *M. oleifera* leaves was compared with that of the original extract, without these nanoparticles as well as with the activity of Ciprofloxacin drug taken as standard and was found to be of the following order based on the MIC values.

Antimicrobial efficacy in terms of MIC

M. oleifera* aqueous leaf extract with AgNPs:*
P. aeruginosa =E. coli>S. aureus >K. pneumoniae = E. faecalis.
0.075 = 0.075 > 0.15 > 0.30 =0.30 **(mg/ml)**

M. oleifera* aqueous leaf extract without NP:*
E. coli = P. aeroginosa> K. pneumoniae = S. aureus > E. faecalis.
0.15 = 0.15 > 0.30 = 0.30 >0.60 **(mg/ml)**

Ciprofloxacin:
P. aeruginosa = E. faecalis>K. pneumoniae > E. coli = S. aureus.
0.075 = 0.075 > 0.15 > 0.30 = 0.30 **(mg/ml)**

Thus, it may be concluded that the *E. coli* and *S. aureus* bacterial strains were inhibited mostly by the *M. koenigii* extract with AgNPs than by the standard drug Ciprofloxacin and by the original extract. On the other hand, the extract containing AgNPs showed an enhanced antibacterial efficacy as compared to the original extract against four out of five tested bacterial strains, i.e. *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *S. aureus.*

An interesting observation was that the bacterial strains, *E. coli* and *S. aureus* were also inhibited mostly by the *M. oleifera* extract with AgNPs than by the standard drug Ciprofloxacin. Whereas, the AgNPs showed a more improved antibacterial activity than the original extract against four out of five the bacterial strains viz. *E. coli*, *P. aeruginosa, S. aureus* and *E. faecalis*.

Another important finding was that *E. faecalis* was resistant towards the aqueous leaf extracts with Ag nanoparticles of both plants, *M. koenigii* and *M. oleifera* in comparison to the standard drug, Ciprofloxacin. However, *M. oleifera* extract with AgNPs was found to be more active against *E. faecalis* than the original extract without nanoparticles.

Hence, the AgNPs derived from *M. koenigii* leaf extract enhanced the antibacterial activity of the original extract against the tested bacterial strains, superior to the standard drug, Ciprofloxacin against *E. coli* and *S. aureus* strains. AgNPs also rendered the extract equally effective against *P. aeruginosa* and *K. pneumoniae* in comparison to the reference drug.

Similarly, AgNPs derived from the *M. oleifera* leaf extract not only improved the antibacterial efficacy of the original extract against almost all the tested bacterial strains, i.e. *E. coli*, *P. aeruginosa*, *S. aureus* and *E. faecalis* but also proved to be superior to that of the drug against *E. coli* and *S. aureus*. The interesting achievement is that AgNPs were found to be more effective against *E. coli* and *S. aureus* in comparison to both the original extract as well as the standard drug.

Even though the exact mechanism for the growth inhibition by AgNPs is not known, different plausible mechanisms have been suggested. Generally, silver ions from nanoparticles are assumed to be attached to the negatively charged bacterial cell wall and rupture it, which results in protein denaturation and finally cell death [21]. Alternatively, silver ions or nanoparticles attached to the cell wall lead to accumulation of protein precursors, which gives rise to dissipation of the proton motive force. AgNPs also revealed weakening of the outer membrane and falling-out of the plasma membrane, thus resulting in intracellular ATP decline [22]. Another suggested mechanism is the association of silver with oxygen and its reaction with sulfhydryl (–S–H) groups on the cell wall to form R–S–S–R bonds, thus obstructing respiration leading to cell death [23]. Related methods of action have been stated for silver ions and silver nanoparticles, while Cho et al. reported that the nanoparticles at lower concentration were considerably effective [24]. Also, AgNPs exhibited better antibacterial efficacy in comparison to penicillin, in case of *E. coli* and *S. aureus* strains [25].

4. CONCLUSIONS

The present investigation justifies not only the traditional use of the two medicinal plants as potent antimicrobial agents but also the role of AgNPs in enhancing the antimicrobial activities of these plant extracts. Hence, these nanoparticles derived from herbal sources can be employed effectively in enhancing the growth inhibition of microorganisms including those strains which have already developed resistance towards the existing antibacterial agents.

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