Green Synthesis of Silver Nanoparticles Using *Moringa Oleifera* Leaf Extract, *Tamarindus Indica* Fruit Extract and Assessment of Antibacterial Activity Against *Staphylococcus Aureus*

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Abstract

The present study mainly deals with the green synthesis, characterization and evaluation of antibacterial properties of silver nanoparticles (AgNPs) synthesized by using the leaf extract of *Moringa oleifera* and fruit extract of *Tamarindus indica*. In this study for synthesis of silver nanoparticles different ratios of 1mM silver nitrate and *Moringa oleifera* leaf extract i.e, 95:5, 90:10 and 85: 15 was taken in conical flask and kept for one 1 hr at 25 0 c on magnetic stirrer, out of which 90:10 ratio was selected for further study based on highest peak, good size and stability. *Tamarindus indica* fruit extract was added to silver nitrate solution till the colour of the solution changes from light brown to chocolate brownish colour. The synthesized silver nanoparticles were characterized by UV-Visible spectroscopy, Zeta potential, size distribution by intensity. The absorption spectrum of the silver nano solution prepared by using *Moringa oleifera* and *Tamarindus indica* fruit extract showed a surface plasmon absorption band with maximum of 420 nm and 430 nm respectively indicating the presence of silver nanoparticles. The zeta value of silver nanoparticles synthesized from *Moringa oleifera* and *Tamarindus indica* fruit extract was -12.5 mV and -15.5 mV, size of 110.2 nm and 130.2 nm respectively. The antibacterial efficacy of nanosilver was checked by agar well diffusion method, and the silver nanoparticles showed effective antibacterial activity against *Staphylococcus aureus*.

Introduction

Nanotechnology is the ability to control and restructure the matter at the atomic and molecular levels in the range of approximately 1–100 nm and exploiting the distinct properties and phenomena at that scale as compared to those associated with single atoms or molecules or bulk behaviour (1). The most important and distinct property of nanoparticles is that they exhibit larger surface area to volume ratio (2). The noble metal nanoparticles exhibit new physicochemical properties which are not observed either in the individual molecules, or in the bulk metals (3). Control of size, composition, morphology, stability and environmental friendly synthesis are the features which are highly desirable. Noble metal nanoparticles, such as Ag, Au, Pt, and Pd nanoparticles are used in physical, chemical and biological applications (4).

Silver nanoparticles represents a good candidate to carry out the nanostructured part of antibacterial and anticancer applications. AgNP's are highly in demand, because of its various applications in medicine, water treatment and catalysis. AgNP's based antimicrobial packaging is a promising form of active food packaging, which plays an important role in extending shelf-life of foods and reduce the risk of pathogens. Nanoparticles can be synthesized by physical methods, chemical methods and bio-assisted methods (5). The size and shape of AgNP's are controlled by the type of synthesis method. The chemical reduction of silver ions using sodium borohydride, hydrazine, ascorbic acid, tri sodium citrate and polyols were used in well established methods. Although chemical methods are effective, these methods may suffer from toxicity due to chemicals used and difficulty in removing them. Additionally chemical reagents are hazardous to the environment (6).

Green synthesis is the application of certain ideologies that lessens or abolishes the use or production of hazardous chemicals. Biosynthesis of metal nanoparticles has been proposed as a simple, cost effective, high yield and environment friendly way of fabricating these materials (7). Various green synthesis methods include using plants, bacteria and fungi. Plants can be described as nano factories, which provide safe and beneficial way to the synthesis of metallic nanoparticles and there are possibilities for large scale production, apart from eco-friendly, the rate of production is faster in comparison to other biological models. The reducing capacity of plant depends on the presence of various polyphenols and other heterocyclic compounds (8). AgNPs using plant extracts like *Murraya koenigii* leaf (9), Mangosteen leaf (10), *Mangifera indica* leaf (11), *Jatropha curcas* (12), *Cinnamomum zeylanicum* leaf (13), *Camellia sinensis* (14), *Aloe vera* (15) etc. The advantage of using plant and fruit extract includes the formation of stable nanoparticles without molecular aggregation even if they are stored for a longer time.

Moringa oleifera (drumstick) is cultivated all most all over the country and its leaves and fruits are used as vegetables. Almost all parts of the plant have been utilized in traditional medicine properties. The leaves of plant have also been reported for its antitumor, hypotensive, cardioprotective, wound healing activities and use for eye diseases (16). First time synthesis of Ag nanoparticles were prepared by reducing the silver ions present in the solution of silver nitrate by the aqueous extract of *M. oleifera* leaf by (17). *Tamarindus indica* is a member of Fabaceae family and commonly known as 'Imli'. This is a very common fruit plant distributed throughout various regions of India. *Tamarindus indica* fruit is sweet and sour in taste and has tartaric acid, sugar and vitamins. In traditional medicine and food, it is used as the main ingredient in general. Synthesis of AgNP's using *Tamarindus indica* fruit extract without the addition of any external surfactant, capping agent or template was reported (18). The biologically synthesized nanoparticles were highly toxic against different pathogenic microorganisms at low concentrations. Silver ions have strong inhibitory and bactericidal effects as well as a broad spectrum of antimicrobial activities (19). Recent microbiological and chemical experiments implied that interaction of Ag⁺ with thiol groups played an essential role in bacterial inactivation (20). It is revealed that bulk silver in an oxygen charged aqueous media catalyzes the complete destructive oxidation of microorganisms (21).

Silver nanoparticles are the metal of choice as they hold the promise to kill microbes effectively (22). The silver nanoparticles act on a broad range of target sites both extra-cellularly as well intra-cellularly. Microbes generally have a harder time developing resistance to silver than they do to antibiotics (23). Therefore, the aim of this work was to green synthesise, characterize and evaluate the antibacterial effects of silver nanoparticles.

Materials And Methods

Materials

1 mM silver Nitrate solution was prepared and the *Moringa oleifera* leaves, *Tamarindus indica* fruits were collected from the premises of College of Veterinary Science, Rajendranagar, Hyderabad, India.

Preparation of plant extract

The collected fresh leaves of *Moringa oleifera* are as shown in Fig.1. were washed in tap water to remove adhered dust particles. Then, it is dried in ambient temperature to remove residual moisture. After removal of moisture the dried leaves of *Moringa oleifera* were powdered in grinder. 20 g of finely powdered leaves of *Moringa oleifera* added to 100 ml of double distilled water and boiled for 10 minutes. The extract cooled and filtered for further use. Similarly, 10 grams of tamarindus fruit (Fig.2.) was kept in 100 ml of double distilled water, mixed well and boiled for 20 minutes. The extract obtained was filtered through Whatman No.1 filter paper and the filtrate was collected, stored till further use.

Green Synthesis of Silver Nanoparticles using *Moringa oleifera* Leaf Extract and *Tamarindus indica* fruit extract

For synthesis of silver nanoparticles different ratios of 1mM silver nitrate and *M. oleifera* leaf extract i.e., 95:5, 90:10 and 85: 15 was taken in to a conical flask and kept for 1 hr at 25⁰C on magnetic stirrer. The bioreduction of Ag⁺ to Ag⁰ with the action of moringa leaf extract is observed with the characteristic change in color from pale yellow to brown (Fig.3.) which may be attributed due to surface Plasmon resonance (SPR) of silver nanoparticles. Similarly, 100 ml of 1mM silver nitrate solution was taken in conical flask and drop by drop *Tamarindus indica* fruit extract was added to it till the colour of the solution changes from light brown to chocolate brownish colour (Fig.4.).

Characterization of Silver nanoparticles

Characterization of silver nanoparticles will be done for the confirmation of nanoparticles formation, their size and shape.

UV-Vis Spectral Analysis

The silver nanoparticles were confirmed by measuring the wave length of reaction mixtures in the UV-vis spectrum of the Thermo fisher spectrophotometer at a resolution of 1 nm (from 300 to 600 nm) in 2 ml quartz cuvette with 1 cm path length.

Zeta Potential Analysis

The prepared sample was dispersed in deionised water followed by ultra-sonication. Afterwards the solution was filtered and centrifuged for 15 min at 25⁰c with 5000rpm and the supernatant was collected. The supernatant was diluted for 4 to 5 times and the particle distribution in liquid was studied in a computer-controlled particle size analyser (ZETA sizer Nano series, Malvern instrument Nano Zs).

Bacterial culture and antibacterial activity

The *Staphylococcus aureus* organism was isolated from 100 mastitis milk samples collected from different dairy farms in and around Hyderabad and confirmed by various biochemical tests (Table 1), catalase test, Gram staining. The pure culture was obtained from MTCC Chandigarh. The antibacterial sensitivity test was carried out against *Staphylococcus aureus* pure culture and isolates to check the

efficacy of green synthesized AgNPs, by agar well diffusion method. Antimicrobial susceptibility of the isolates was done by the disc diffusion assay with Muller-Hinton (MH) agar and in accordance with CLSI recommendations. MH broth was inoculated with five colonies of the isolate and tubes were incubated at 37°C for 2-8 h until achieving a turbidity equivalent to 0.5 on the Mac Farland scale. After turbidity adjustment, a sterile swab was introduced, pressed against the tube well in order to remove any excess liquid and then seeded on the surface of a petri dish containing MH agar, for *S. aureus* rotating at least twice. Using sterile forceps 4 discs (Table 2) impregnated with antimicrobials and 50 µl of AgNP's of *M. oleifera*, *Tamarindus indica*, AgNO₃, extracts and distilled water were placed at equal distances from each other on the surface of inoculated agar plate. Subsequently the plate was inverted and incubated at 37°C for 24 h. Disc readings were performed after incubation and the diameter of inhibition halos was measured with the aid of a ruler. The interpretation was made as per the zone size interpretation chart provided by manufacturer of discs. The antibacterial activity against *Staphylococcus aureus* pure culture, isolates from mastitis milk was compared.

Table 1 Biochemical tests

S. No	Tests	Typical reactions of <i>S. aureus</i>
1.	Indole	Negative
2.	Methyl Red	Positive
3.	Voges Proskauer	Positive
4.	Citrate (Simmons)	Positive
5.	Catalase	Positive
6.	Coagulase	Positive

Table 2 Antibiotics used in the antibiotic resistance/susceptible test

S.No:	Antibiotics	Abbreviations	Concentration(µg/unit)
1.	Tetracycline	TE	30
2.	Ampicillin	AMP	10
3.	Ciprofloxacin	CIP	5
4.	Ceftriaxone	CTR	30

Results

CHARACTERIZATION OF SILVER NANOPARTICLES

UV-Visible Spectroscopy

The silver nanoparticles were characterized by UV-Visible spectroscopy, for confirmation of silver nanoparticles formation and the absorption spectrum of the brown silver nano solution prepared by using *Moringa oleifera* and *Tamarindus indica* showed a surface plasmon absorption band with maximum of 420 nm (Fig.5.) and 430 nm (Fig.6.) respectively, indicating the presence of silver nanoparticles.

Zeta Potential and Size distribution by intensity

Zeta potential measures the potential stability of the nanoparticles in the colloidal suspension. The zeta potential values of silver nanoparticles synthesized from *Moringa oleifera* and *Tamarindus indica* was -12.3 mV and -15.5 mV (Fig.7, 8.). These zeta potential values indicate good stability of silver nanoparticles synthesized. The size of the silver nanoparticles was 110.2 nm and 130.2 nm for AgNP's with *Moringa oleifera* and *Tamarindus indica* respectively (Fig.9,10.).

Isolation and Identification of *Staphylococcus aureus* by cultural method

Out of 100 total milk samples collected from different sources, 27 (27 %) milk samples were positive by traditional cultural methods. The isolation of *Staphylococcus aureus* was carried out by using BHI broth for enrichment and Mannitol salt agar media. The colonies were small size with yellow colour (Fig.11.). The presumptive colonies were subjected to gram staining (Fig.12.), biochemical tests (Fig.13.) and catalase test (Fig.14.) for confirmation.

Antibacterial activity

The antibacterial activity was recorded as the mean of zone of inhibition (mm). The antibacterial activity of leaf extract of moringa and fruit extract of tamarind, pure silver nitrate solution, Ag nanoparticles with Moringa leaf and Tamarind fruit and antibiotics on *Staphylococcus aureus* pure culture and isolates was presented in the (Table 3 and Fig.15-18.).

Table 3 The antibacterial activity of silver nitrate solution, extracts, silver nanoparticles and antibiotics on pure cultures and isolates of *Staphylococcus aureus* from milk samples

S.No	Antibiotic/ material	Zone of inhibition (mm)	
		On pure cultures	On isolates (Mean± SE)
1	AgNO ₃ Solution	14	13.00±0.25
2	<i>Moringa oleifera</i> leaf extract	2	1.02±0.11
3	<i>Tamarindus indica</i> fruit extract	3	2.00±0.14
4	<i>Moringa oleifera</i> AgNP's	26	24.00±0.29
5	<i>Tamarindus indica</i> AgNP's	18	16.08±0.34
6	tetracycline	26	24.65±0.29
7	ciprofloxacin	10	8.15±0.28
8	ampicillin	6	4.29±0.27
9	ceftriaxone	32	28.29±0.43

Discussion

Physical examination of silver nano solution

In the present study when the *Moringa oleifera* leaf extract was added to the colorless silver nitrate solution, the colour changed from yellow to light brown and/or thick brown colour. The colour of silver nano solution prepared with *Moringa oleifera* leaf extract was dark brown colour (24). The change of colour from watery to yellowish brown and finally to green-black due to reduction of silver ion which indicated formation of silver nanoparticles using *Moringa oleifera* leaf extract (25). Change of colour from pale colour to yellowish brown (26), whereas observed change in colour from pale yellow to reddish brown (27) which confirmed the reduction of silver ions into nanoparticles using *Moringa oleifera* leaf extract. The colour of *Tamarindus indica* fruit extract was light brown and when it was added to the silver nitrate solution the color changed to dark brown color. The colour change of silver nitrate solution into brown to dark brown color indicates that silver nanoparticles were formed (28). The reason for the brown colour is due to the extraction of surface Plasmon vibrations in the silver nanoparticles prepared by using *Tamarindus indica* fruit shell (29). Yellowish brown color, reported for the Ag nano solution prepared by *Tamarindus indica* fruit extract (30).

Characterization of silver nanoparticles

UV-Visible Spectroscopy

The structural characterization of silver nanoparticles was characterized by UV-Visible spectroscopy. The formation of AgNP's will be confirmed by UV-Vis spectral study, which is an authentic technique to monitor the progress of the reaction during the reduction of silver ions. The absorption spectrum of the brown silver nano solution prepared by using *Moringa oleifera* and *Tamarindus indica* showed a surface plasmon absorption band with maximum of 420 nm, 430 nm indicating the presence of silver nanoparticles. The occurrence of peak at 420 nm, 430 nm is due to the phenomenon of surface plasmon resonance, which occurs due to the excitation of the surface plasmon present on the outer surface of the silver nanoparticles which gets excited to the applied electro-magnetic field (31). The UV absorption peak of silver nanoparticles range from 400 nm to 450 nm (32). The peaks obtained in this study for nanoparticles were within the range, clearly indicating the formation of silver nanoparticles. The UV-Vis spectral peak of 420 nm (33), 430 nm (34), 460.8 nm (35), 450 nm (24) was reported for the AgNP's prepared by using *Moringa oleifera* leaf extract, where some was lower, higher and similar to the peak observed in the present study.

Higher UV-Vis spectral peak at 432 nm for the AgNP's prepared by using *Tamarindus indica* leaf extract were reported (28), UV-Vis spectral peak at 450 nm for the AgNP's prepared by using *Tamarindus indica* fruit shell extract, which was higher than the present study (430 nm) was reported (29). Very high UV-Visible spectra peak (491 nm) was observed for the AgNP's prepared by using *T. indica* leaf extract (36). Very low UV-Vis spectral peak at 348 nm using tamarind fruit extract compared to the peak (430 nm) observed in the present study (37).

Zeta Potential

Zeta potential measures the potential stability of the nanoparticles in the colloidal suspension. The zeta potential of silver nanoparticles synthesized from *Moringa oleifera* leaf extract and *Tamarindus indica* fruit extract was -12.3 mV and -15.5 mV respectively. This zeta potential value indicates good stability of silver nanoparticles synthesized. The zeta potential to know the stability of AgNP's synthesized by using *Ocimum sanctum* leaf extract was - 55.0 mv (38), whereas, the zeta potential value is -47.1 mV and -43 mV at pH 5.5 for the silver nanoparticles synthesized by using extract of callus cultures of pumpkin (*Cucurbita maxima*) (39). The zeta potential value in the range of -10 to -30 mV for the gold nanoparticles synthesized by using different plant extracts was reported (40).

Antibacterial activity and comparison against *Staphylococcus aureus*

The antibacterial activity was recorded as the mean of zone of inhibition (mm). Distilled water has been taken as control which showed no zone of inhibition against *Staphylococcus aureus*. The mean of ZOI of tetracycline, ciprofloxacin, ampicillin, ceftriaxone, AgNP's with *Moringa* and AgNP'S with tamarind were 26 mm, 10 mm, 6 mm, 32 mm, 26 mm and 18 mm respectively against *Staphylococcus aureus* pure cultures, whereas mean of ZOI against *Staphylococcus aureus* isolates from milk samples was 24.65 ± 0.29 mm, 8.15 ± 0.28 mm, 4.29 ± 0.27 mm, 28.29 ± 0.43 mm, 24.00 ± 0.29 mm and 16.08 ± 0.34 mm respectively. The mean of ZOI with *Moringa* leaf extract was 2 and 1.02 ± 0.11 mm respectively for pure culture and isolates from milk samples of *Staphylococcus aureus*. On contrary to the present findings (34,27) reported no zone of inhibition against *Staphylococcus aureus* with *Moringa* leaf extract. The ZOI as 5.67 ± 1.52 mm, 13.00 ± 2.64 mm for *Moringa oleifera* crude leaf extract at concentration of 50 and 100 µg/disc respectively which was higher than the present study was reported (41). The mean of ZOI of pure AgNO₃ solution was 14 and 13.00 ± 0.25 mm respectively for pure culture and isolates of *Staphylococcus aureus* from milk samples. Very low antibacterial activity was reported for AgNO₃ solution against *Staphylococcus aureus* with zone of inhibition of 2 mm (27). Slightly higher ZOI (15 mm) than the present study was reported for AgNO₃ solution (18). The mean of ZOI of Ag nanoparticles prepared using *Moringa oleifera* leaf extract was 26 and 24.00 ± 0.29 mm respectively for pure culture and isolates from milk samples in the present study. This zone of inhibition for silver nanoparticles was higher compared to AgNO₃ solution, whereas similar zone of inhibition (2 mm) against *Staphylococcus aureus* for both silver nitrate solution as well as silver nanoparticles was reported (38).) The ZOI against *Staphylococcus aureus* as 15 mm by using AgNP's synthesised by using *Moringa oleifera* leaf extract, which was less than the ZOI (24.00 ± 0.29 mm) observed in the present study was reported (17,34). The ZOI against *Staphylococcus aureus* as 21.67 mm and 12.60 mm for AgNP's prepared by using *Moringa oleifera* leaf extract, which was less than mean of ZOI observed in the present study was reported (41-42). The ZOI against *Staphylococcus aureus* increased as the concentration of AgNP's increased and reported the ZOI of 15, 18, 20 mm at 25, 50, 100 microliters AgNP's concentration respectively (33). The mean of inhibition zone with *Tamarindus indica* fruit extract was 3 mm and 2.00 ± 0.14 mm respectively for pure culture and isolates from milk samples against *Staphylococcus aureus*. The antibacterial activity of tamarindus fruit extract might be due to Tartaric acid present in the fruit, which increases the acidity that effects the bacterial growth (28). No ZOI for *Staphylococcus aureus* (18), whereas (28) observed minor ZOI with

tamarind fruit extract. The mean of zone of inhibition of AgNP's prepared using tamarind fruit extract against *Staphylococcus aureus* was 18 mm and 16.08 ± 0.34 mm respectively for pure cultures and isolates from milk samples, which was similar to the value reported by (18), whereas slightly less ZOI (14 mm) was reported by (28). The ZOI of tetracycline and ampicillin as 18 mm and 25 mm respectively on *Staphylococcus aureus*, which was lower than the value for tetracycline and higher than the value for ampicillin in the present study was reported (43). The ZOI of ciprofloxacin as 21 ± 1.41 mm on *Staphylococcus aureus*, which was higher than the value in the present study was reported (44). The mean of ZOI against *Staphylococcus aureus* with antibiotics used in this study was high with ceftriaxone (28.29 ± 0.43 mm) followed by tetracycline (24.65 ± 0.29 mm) and less with ciprofloxacin (8.15 ± 0.28 mm) and ampicillin (4.29 ± 0.27 mm). ZOI of ampicillin as 15.03 ± 0.54 mm against *Staphylococcus aureus* was reported (45) which was slightly near to the ZOI value of AgNO_3 solution. The study on AgNP's prepared by using *Moringa oleifera*, *Tamarindus indica* and comparing their antibacterial efficacy with antibiotics was less, but there is lot of scope for future study.

Conclusion

The conclusion of the present study indicates that the AgNP's prepared with *Moringa oleifera* and *Tamarindus indica* was almost equally efficient against *Staphylococcus aureus* and the AgNP's antibacterial activity is almost comparable with most of the antibiotics studied in this work.

Declarations

Conflict of interest

The authors declare that there are no conflicts of interests.

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Figures

Figure 1

Moringa oleifera fresh leaves

Figure 2

Tamarindus indica fruit

Figure 3

Silver nano solution with Moringa before and after addition of AgNO₃

Figure 4

Silver nano solution with Tamarind before and after addition of AgNO_3

Figure 5

UV-Visible absorption peak of 420 nm for AgNP's prepared with *Moringa oleifera* leaf extract

Figure 6

UV-Visible absorption peak of 430 nm for AgNP's prepared with Tamarindus indica fruit extract

Figure 7

Zeta potential distribution of AgNP's prepared with Moringa oleifera leaf extract

Results

	Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV): -15.5	Peak 1: -15.5	100.0	4.18
Zeta Deviation (mV): 4.18	Peak 2: 0.00	0.0	0.00
Conductivity (mS/cm): 1.45	Peak 3: 0.00	0.0	0.00
Result quality : See result quality report

Figure 8

Zeta potential distribution of AgNP's prepared with Tamarindus indica fruit extract

	Size (d.nm):	% Intensity:	St Dev (d.nm):
Z-Average (d.nm): 110.2	Peak 1: 134.2	100.0	66.73
PdI: 0.240	Peak 2: 0.000	0.0	0.000
Intercept: 0.964	Peak 3: 0.000	0.0	0.000
Result quality : Good

Figure 9

Size distribution by intensity of AgNP's prepared with Moringa oleifera leaf extract

	Size (d.nm):	% Intensity:	St Dev (d.n...
Z-Average (d.nm): 130.2	Peak 1: 149.1	100.0	56.88
Pdl: 0.193	Peak 2: 0.000	0.0	0.000
Intercept: 0.956	Peak 3: 0.000	0.0	0.000
Result quality : Good

Figure 10

Size distribution by intensity of AgNP's prepared with Tamarindus indica fruit extract

Figure 11

Plate showing the growth of *Staphylococcus aureus* colonies on MSA media

Figure 12

Gram staining for *Staphylococcus aureus*

Figure 13

Results of biochemical tests and catalase test for *Staphylococcus aureus*

Figure 14

Results of biochemical tests and catalase test for *Staphylococcus aureus*

Figure 15

ZOI of *Moringa oleifera* leaf extract, *Tamarindus indica* fruit extract, AgNO_3 solution, AgNP's for pure cultures of *Staphylococcus aureus*

Figure 16

ZOI of *Moringa oleifera* leaf extract, *Tamarindus indica* fruit extract, AgNO₃ solution, AgNP's for isolates of *Staphylococcus aureus* M. AgNP: *Moringa oleifera* silver nanoparticles T. AgNP: *Tamarindus indica* silver nanoparticles AgNO₃: Silver nitrate solution; DW: Distilled water T. extract: *Tamarindus indica* fruit extract M. extract: *Moringa oleifera* leaf extract

Figure 17

ZOI of antibiotics for pure cultures of *Staphylococcus aureus*

Figure 18

ZOI of antibiotics for isolates of *Staphylococcus aureus*