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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 research, three distinct ratios of 1 mM silver nitrate to *Moringa oleifera* leaf extract 95:5, 90:10, and 85:15 were used to synthesize silver nanoparticles. The 90:10 ratio was chosen for further investigation based on having the largest peak, a decent size, and stability. To a silver nitrate solution, tamarind fruit extract was added until the solution's color changed from light brown to chocolate brown. The UV-Visible spectroscopy, Zeta potential, and size distribution by intensity were used to characterise the produced silver nanoparticles. The surface plasmon absorption band with maximal values at 420 nm and 430 nm respectively, indicated the existence of silver nanoparticles in the silver nano solution made with *Moringa oleifera* and *Tamarindus indica* fruit extract. Silver nanoparticles with sizes of 110.2 nm and 130.2 nm were produced from fruit extracts of *Moringa oleifera* and *Tamarindus indica*. Their zeta values were -12.5 mV and -15.5 Mv respectively. The agar well diffusion method was used to test the antibacterial effectiveness of nanosilver, and the silver nanoparticles demonstrated strong antibacterial activity against *Staphylococcus aureus*.

Keywords: Silver nanoparticles, *Moringa oleifera*, *Tamarindus indica*, characterization, antibacterial activity

1. Introduction
Nanotechnology is the ability to manipulate and reorganise matter at the atomic and molecular levels between about 1 and 100 nm, taking use of the unique characteristics and phenomena that exist there as opposed to those seen at smaller scales or in bulk behaviour [1]. The primary characteristic that sets nanoparticles apart is their higher surface area to volume ratio [2]. The noble metal nanoparticles display novel physicochemical characteristics that are not seen in bulk metals or individual molecules [3]. The properties that are greatly desired include control over size, composition, morphology, stability, and environmentally friendly synthesis. Applications in physics, chemistry, and biology involve noble metal nanoparticles like Ag, Au, Pt, and Pd [4].

A strong candidate for the nanostructured component of antibacterial and anticancer applications is silver nanoparticles. Because of its numerous uses in medicine, water treatment, and catalysis, AgNPs are in high demand. A promising type of active food packaging that extends the shelf life of foods and lowers the danger of infections is AgNP-based antimicrobial packaging. Physical methods, chemical approaches, and bio-assisted procedures can all be used to create nanoparticles [5]. The selection of synthesis process determines the size and form of AgNPs. Sodium borohydride, hydrazine, ascorbic acid, tri sodium citrate, and polyols were utilized in well-established procedures for the chemical reduction of silver ions. Despite their effectiveness, chemical procedures may be hazardous because of the chemicals utilized and the difficulty in getting rid of them. Chemical agents could pose a threat to the environment [6].

The use of certain philosophies to reduce or end the use or manufacturing of dangerous chemicals is known as "green synthesis." Metal nanoparticle biosynthesis has been suggested as a straightforward, affordable, high-yield, and environmentally benign method of producing

these materials [7]. Various green synthesis techniques involve the use of fungus, microbes, and plants. Plants can be thought of as nano-factories that synthesize metallic nanoparticles in a safe and advantageous manner, with the potential for large-scale production. In addition to being environmentally friendly, plants also produce at a higher rate than other biological models. The presence of different polyphenols and other heterocyclic chemicals influences a plant's ability to reduce [8]. AgNPs that use botanical extracts from plants such *Murraya koenigii* leaf [9], Mangosteen leaf [10], *Mangifera indica* leaf [11], *Jatropha curcas* [12], *Cinnamomum zeylanicum* leaf [13], *Camellia sinensis* [14] and *Aloe vera* [15] among others. Utilizing plant and fruit extracts has the benefit of forming stable nanoparticles without molecular aggregation even after longer periods of storage.

Almost everywhere in the nation, *Moringa oleifera* (drumstick) is grown for its leaves and fruits, which are utilised as vegetables. Almost all of the plant's components have been used in traditional medicine. Additionally, the plant's leaves have been found to have anticancer, hypotensive, cardioprotective, wound-healing, and therapeutic for eye ailments properties [16]. By reducing the silver ions in the solution of silver nitrate with the aqueous extract of *M. oleifera* leaf, the first-time synthesis of Ag nanoparticles was created by [17]. *Tamarindus indica*, also referred to as "Imli," is a plant in the Fabaceae family. This is a fairly widespread fruit plant found in many parts of India. The fruit *Tamarindus indica* has tartaric acid, sugar, and vitamins and has a sweet-and-sour flavour. It is typically utilised as the primary ingredient in traditional medicine and cuisine. *Tamarindus indica* fruit extract was used to create AgNPs without the use of an external surfactant, capping agent, or template, according to a report [18]. At low concentrations, the biologically created nanoparticles were extremely hazardous to a variety of pathogenic microbes. Strong bactericidal and inhibitory actions as well as a wide range of other antimicrobial properties are all present in silver ions [19]. Recent microbiological and chemical studies suggested that the interaction of Ag+ with thiol groups was crucial for the inactivation of bacteria [20]. It is discovered that bulk silver catalyses the total destructive oxidation of microorganisms in an oxygen-charged aqueous medium [21].

The preferred metal is silver nanoparticles because they have the potential to effectively kill micro-organisms [22]. The broad spectrum of target areas that the silver nanoparticles affect include both extracellular and intracellular locations. Antibiotic resistance is typically easier to develop in microbes than silver resistance [23]. The objective of this effort was to characterise, evaluate and green synthesise the antibacterial properties of silver nanoparticles.

2. Materials and Methods

2.1 Materials

The *Moringa oleifera* leaves and *Tamarindus indica* fruits were procured from the grounds of the college of Veterinary Science, Rajendranagar, Hyderabad, India, and a solution of 1 mm silver nitrate was made.

2.2 Preparation of plant extract

As shown in (Fig.1), freshly collected *Moringa oleifera* leaves were rinsed in tap water to get rid of stuck-on dust particles. The remaining moisture is then removed by drying it at room temperature. The dried *Moringa oleifera* leaves were ground into powder after the moisture was removed. Adding 20 g of *Moringa oleifera* leaves that have been finely pulverised to 100 ml of double-distilled water and boiling the mixture for 10 minutes. For later usage, the extract was chilled and filtered. Similar to this, 100 ml of double-distilled water was added to 10 g of tamarind fruit (Fig. 2), which was then mixed thoroughly and heated for 20 minutes. Whatman No. 1 filter paper was used to filter the resulting extract, and the filtrate was collected and kept for later use.

Fig 1: *Moringa oleifera* fresh leaves

Fig 2: *Tamarindus indica* fruit

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

For the synthesis of silver nanoparticles, several ratios of *M. oleifera* leaf extract to 1 mm silver nitrate 95:5, 90:10, and 85:15 were added to a conical flask and maintained at 25°C on a magnetic stirrer for one hour. The characteristic colour change from pale yellow to brown (Fig. 3) associated with the bioreduction of Ag+ to Ag0 with the action of moringa leaf extract may be attributed to the surface Plasmon resonance (SPR) of silver nanoparticles. Similar to this, 100 ml of a 1 mm silver nitrate solution was put in a conical flask. *Tamarindus indica* fruit extract was then gradually added until the solution's colour changed from light brown to a chocolate brown hue (Fig. 4).

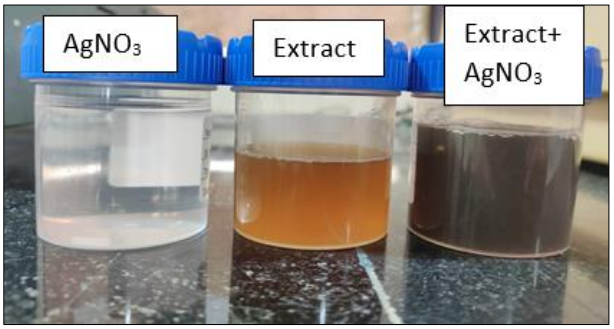


Fig 3: Silver nano solution with Moringa before and after addition of $AgNO_3$.

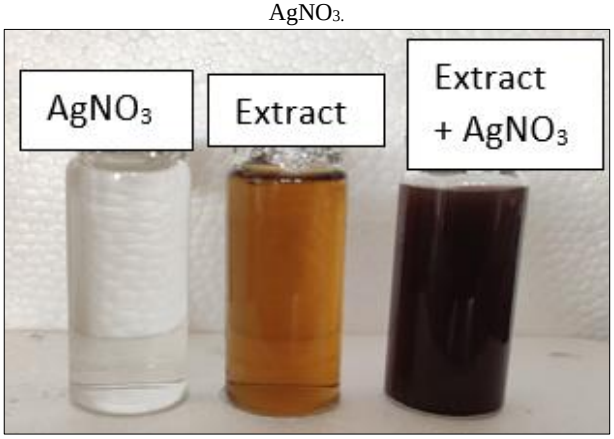


Fig 4: Silver nano solution with Tamarind before and after addition of $AgNO_3$.

2.4 Characterization of Silver nanoparticles
For verification of nanoparticle production, size, and shape, silver nanoparticles will be characterized.

2.4.1 UV-Vis Spectral Analysis
Thermo Fisher spectrophotometer measurements of reaction mixture wave lengths in the UV-vis spectrum at a resolution of 1 nm (from 300 to 600 nm) in a 2 ml quartz cuvette with a 1 cm path length were used to confirm the presence of silver nanoparticles.

2.4.2 Zeta Potential Analysis
After the produced sample was dissolved in deionized water, it was ultrasonically processed. Following filtration and centrifugation for 15 min, at 25 ºC and 5000 rpm, the supernatant was recovered from the solution. A computer-controlled particle size analyzer was used to study the particle distribution in the liquid after the supernatant was diluted 4–5 times (ZETA sizer Nano series, Malvern instrument Nano Zs).

2.4.3 Bacterial culture and antibacterial activity
One hundred mastitis milk samples from various dairy farms in and around Hyderabad were isolated for the *Staphylococcus aureus* organism, which was then verified by a number of biochemical tests (Table 1), the catalase test, and Gram staining. The MTCC Chandigarh provided the pure culture. The effectiveness of green produced AgNPs was evaluated using the agar well diffusion method by conducting an antibacterial sensitivity test on pure cultures and isolates of *Staphylococcus aureus*. The disc diffusion assay with Muller-Hinton (MH) agar and in accordance with CLSI recommendations was used to determine the isolate's antimicrobial susceptibility. Five colonies of the isolate were injected into MH broth, and tubes were then incubated at 37^0C for 2–8 h to reach a turbidity comparable to 0.5 on the Mac Farland scale. In order to seed *S. aureus* on the surface of a petri dish containing MH agar, a sterile swab was first introduced, pressed against the tube well to remove any surplus liquid, and then rotated at least twice. Using sterile forceps 4 discs (Table 2) impregnated with antimicrobials and 50 µl of AgNP's of *M. oleifera*, *Tamarindus indica*, $AgNO_3$, extracts and distilled water were placed at equal distances from each other on the surface of inoculated agar plate. The plate was then inverted and incubated for 24 hours at 37^0C. After incubation, disc readings were taken, and a ruler was used to measure the diameter of the inhibition halos. The interpretation was produced in accordance with the zone size interpretation table given by the disc maker. *Staphylococcus aureus* pure culture and isolates from milk with mastitis were examined for their antibacterial activity.

Table 1: Biochemical tests

S. No	Tests	Typical reactions of *S.aureus*
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	Concentration (µg/unit)
1.	Tetracycline	30
2.	Ampicillin	10
3.	Ciprofloxacin	5
4.	Ceftriaxone	30

3. Results
3.1 UV-Visible Spectroscopy
The presence of silver nanoparticles was confirmed by UV-Visible spectroscopy, which was used to characterise the silver nanoparticles. The absorption spectrum of the brown silver nano solution made with *Moringa oleifera* and *Tamarindus indica* revealed a surface plasmon absorption band with maximum values of 420 nm (Fig. 5) and 430 nm (Fig. 6), respectively.

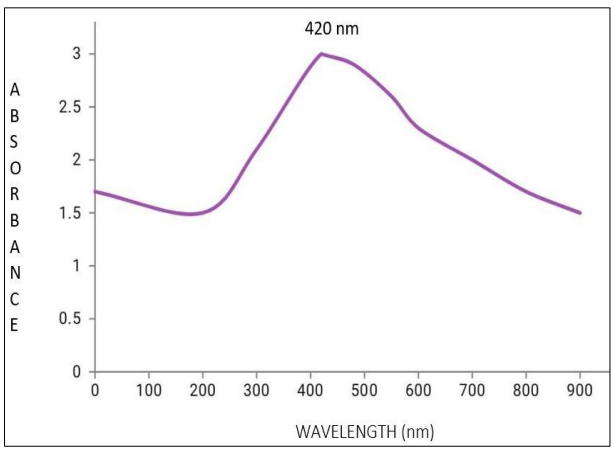


Fig 5: UV-Visible absorption peak of 420 nm for AgNP's prepared with *Moringa oleifera* leaf extract

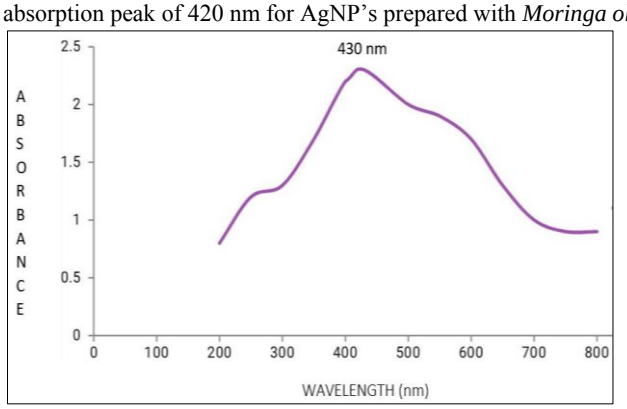


Fig 6: UV-Visible absorption peak of 430 nm for AgNP's prepared with *Tamarindus indica* fruit extract.

3.2 Zeta Potential and Size distribution by intensity

The colloidal suspension's nanoparticle's potential stability is gauged by their zeta potential. Silver nanoparticles made from *Moringa oleifera* and *Tamarindus indica* have zeta potential values of -12.3 mV and -15.5 mV. (Fig. 7, 8). These zeta potential values show that the produced silver nanoparticles have good stability. AgNPs with *Moringa oleifera* and *Tamarindus indica* have silver nanoparticle sizes of 110.2 nm and 130.2 nm, respectively (Fig. 9, 10).

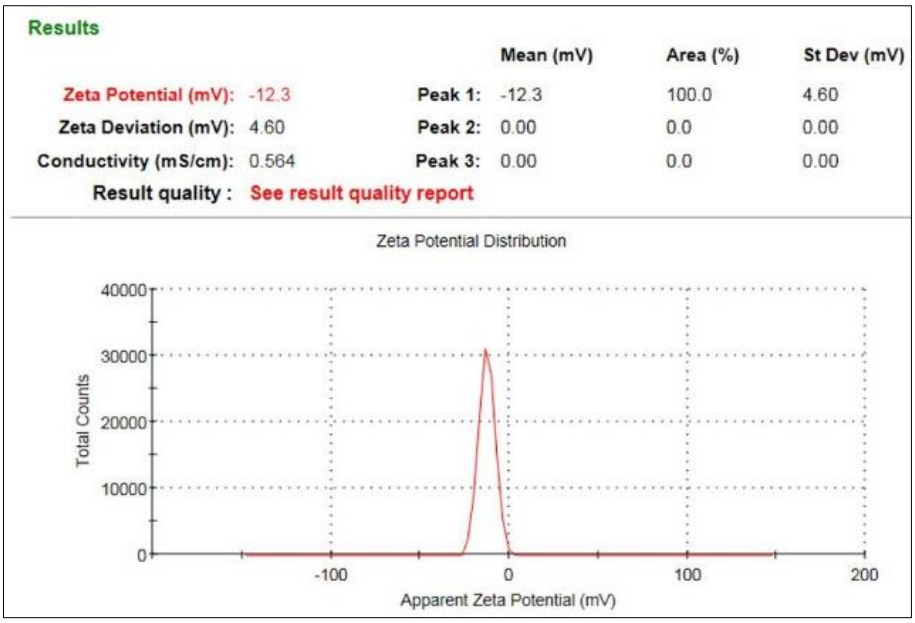


Fig 7: Zeta potential distribution of AgNPs prepared with *Moringa oleifera* leaf extract

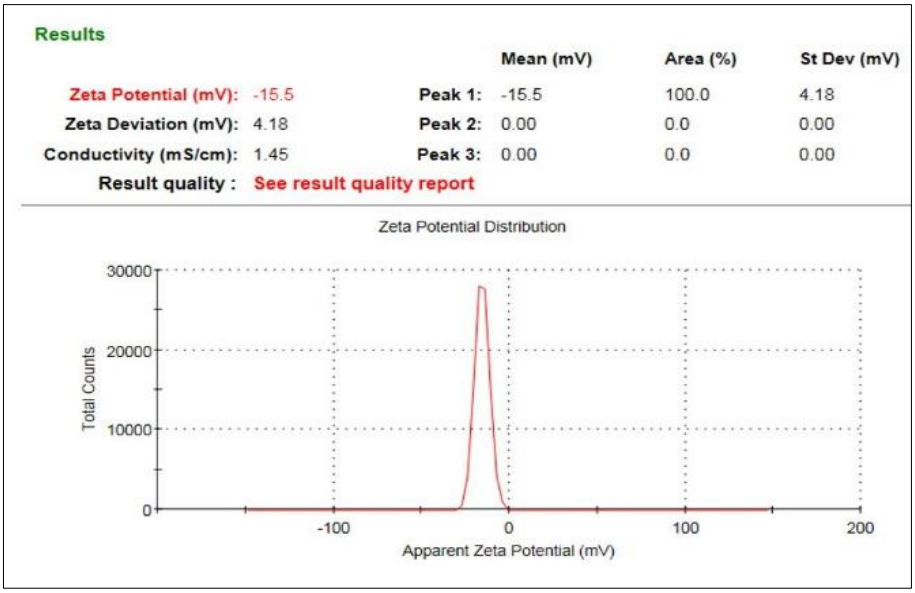


Fig 8: Zeta potential distribution of AgNPs prepared with *Tamarindus indica* fruit extract

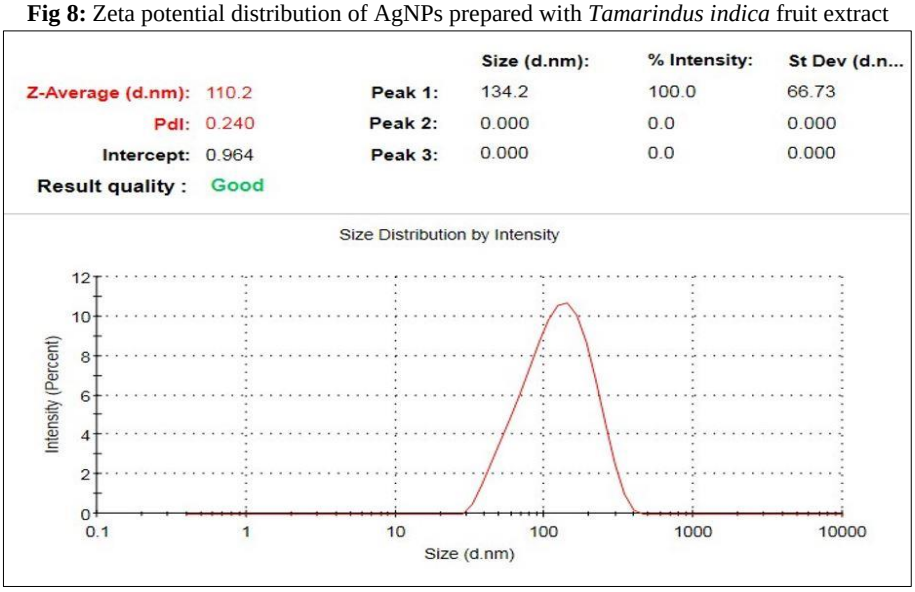


Fig 9: Size distribution by intensity of AgNP's prepared with *Moringa oleifera* leaf extract

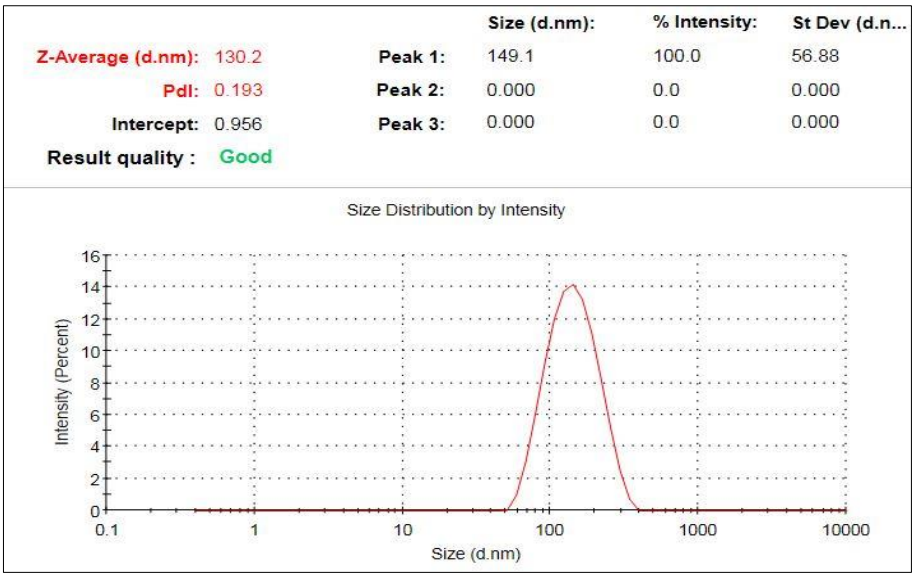


Fig 10: Size distribution by intensity of AgNP's prepared with *Tamarindus indica* fruit extract

3.3 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.

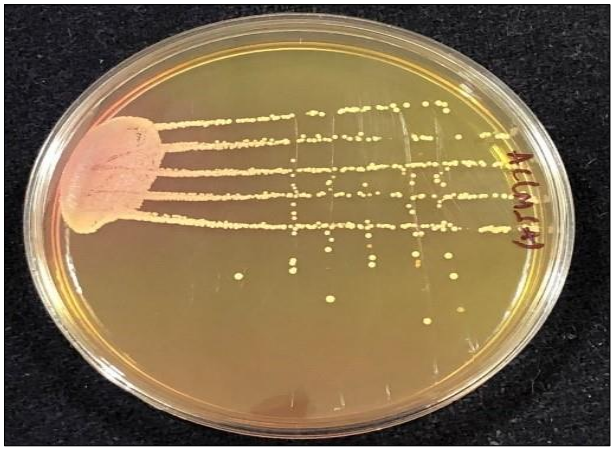


Fig 11: Plate showing the colonies of *S.aureus* on MSA

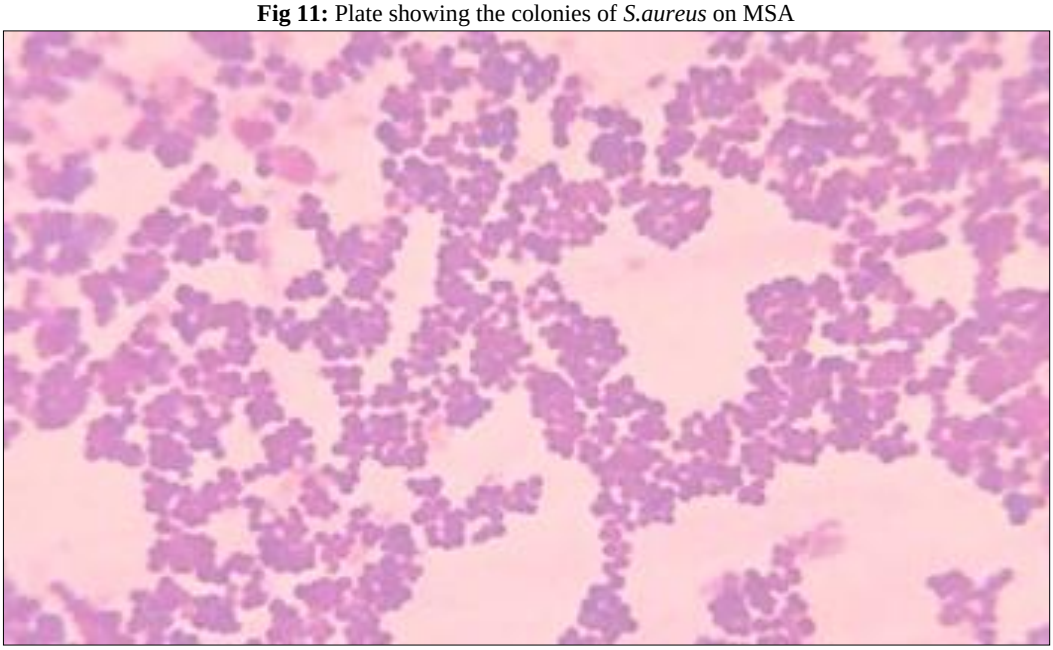


Fig 12: Gram staining for *S.aureus*

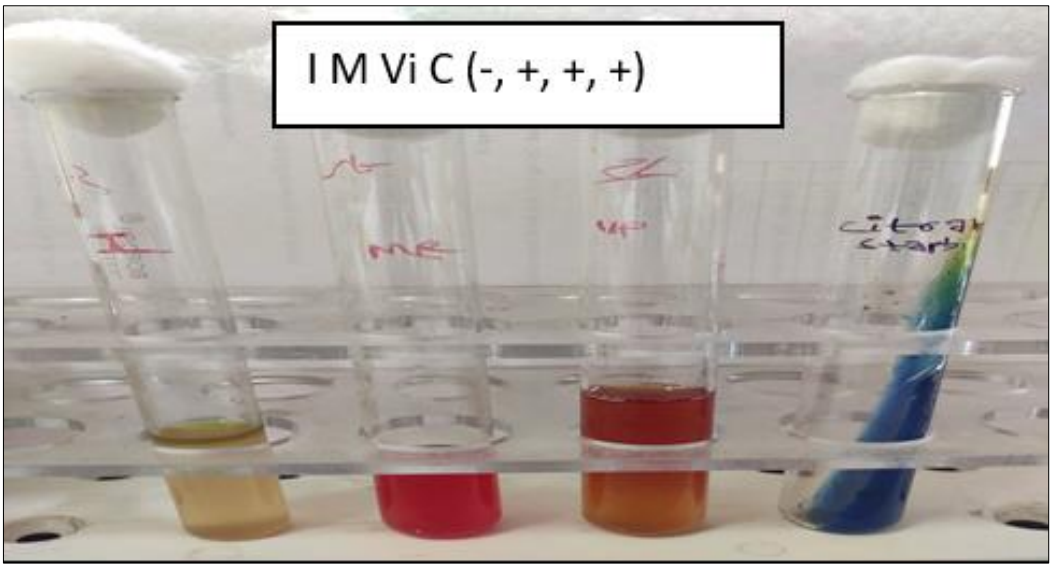


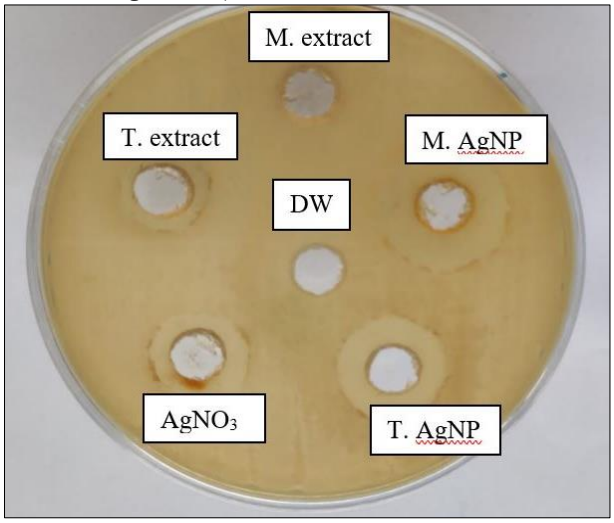
Fig 13: Results of biochemical tests (I M Vi C) for *Staphylococcus aureus*

Fig 14: Results of Catalase test for *Staphylococcus aureus*

3.4 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.).



M.AgNP: *Moringa oleifera* silver nanoparticles
T.AgNP: *Tamarindus indica* silver nanoparticles
$AgNO_3$: Silver nitrate solution
DW: Distilled water
T. extract: *Tamarindus indica* fruit extract
M. extract: *Moringa oleifera* leaf extract

Fig 15: ZOI of *Moringa oleifera* leaf extract, *Tamarindus indica* fruit extract, $AgNO_3$ solution, AgNP's for pure cultures of *Staphylococcus aureus*

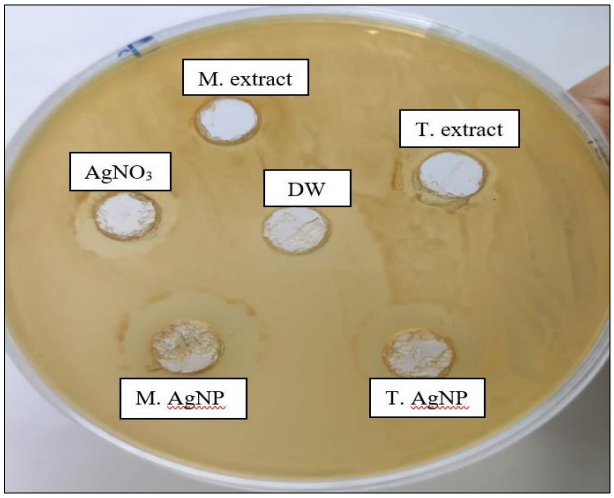


Fig 16: ZOI of *Moringa oleifera* leaf extract, *Tamarindus indica* fruit extract, $AgNO_3$ solution, AgNP's for isolates of *Staphylococcus aureus*

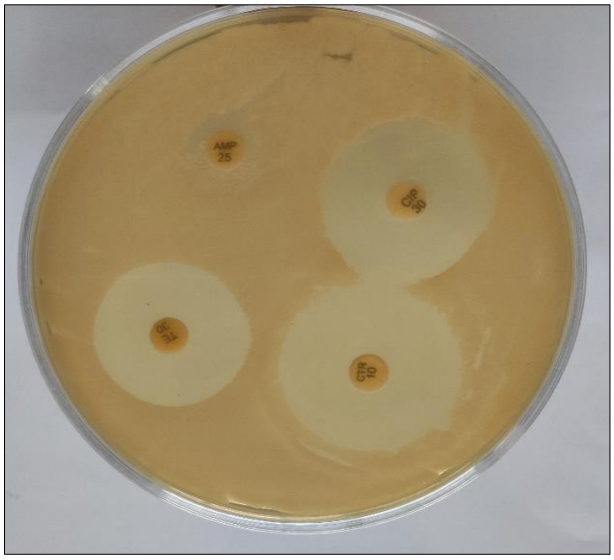


Fig 17: ZOI of antibiotics for pure cultures of *Staphylococcus aureus*

Fig 18: ZOI of antibiotics for isolates of *Staphylococcus aureus*

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 Inhinition (mm)	
		On Pure cultures	On isolates (Mean ± SE)
1	$AgNO_3$ Solution	14	13.00±0.25
2	*Moringa oliefera* leaf extract	2	1.02±0.11
3	*Tamarindus indica* fruit extract	3	2.00±0.14
4	*Moringa oliefera* AgNPs	26	24.00±0.29
5	*Tamarindus indica* AgNPs	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

4. Discussion

4.1 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].

4.2 Characterization of silver nanoparticles

4.2.1 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].

4.2.2 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].

4.2.3 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 $AgNO_3$ 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 $AgNO_3$ solution against *Staphylococcus aureus* with zone of inhibition of 2 mm [27]. Slightly higher ZOI (15 mm) than the present study was reported for $AgNO_3$ 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 $AgNO_3$ 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 synthesized 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.

5. 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.

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