Green Synthesis of Silver Nanoparticles from *Madhuca longifolia* and Its Antibiofilm Potential

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Abstract. The synthesis of silver nanoparticles using plant extract as a capping agent has been very easy, economical and environment friendly method. The *Madhuca longifolia* is one of the well-known trees for its various benefits from food industry to its medicinal applications. It is indigenous to India, Nepal, Sri Lanka and Myanmar. In the present aqueous extract was used from leaves of *M. lngifolia* has been used as a capping agent to form AgNPs by reducingsilver nitrate with the help of green synthesis route. UV-visible spectroscopicy gave maximaat 420 nmconfirmed the synthesis of *M. longifolia* AgNPs. Characterization was done by TEM, SEM, XRD and FTIR techniques. FTIR confirmed the presence of various phytochemicals and formation of nanoparticles. XRD confirmed the formation of crystalline structure of synthesized silver nanoparticles. The shape of silver nanoparticles was irregular and spherical. The reaction solution turned brown which is the primary indication of formation of AgNPs. Crystalline size was calculated and found to be 10–25 nm; and TEM showed the size of nanoparticles around 5–40 nm. The *M. longifolia* based Silver nanoparticles were evaluated for their antibacterial and antibiofilm activity on *Staphylococcus aureus* (Gram positive) and *Escherichia coli* (Gram negative) by disc diffusion and percentage inhibition methods was confirmed.

Keywords: *Madhuca longifolia* · Silver nanoparticles · TEM · SEM · XRD · FTIR

1 Introduction

Antibiotic resistance is frequently developed as a result of biofilm development. Different plants extracts have been explored to inhibit biofilm development to solve this problem. Because of the assumption that plant-based chemicals are harmless, have fewer side effects and are readily available. These plants-based compounds have a better chance

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of being developed into novel medicines and being utilized effectively to treat biofilm-associated illnesses. Biofilm development is one of the most common causes of bacteria gaining multidrug resistance. The biofilm life cycle is divided into four stages: bacterial attachments, microbial colony formation, bacterial growth and extracellular matrix production, and biofilm maturity, which is followed by bacterial dispersion to find new habitat. The proposed research focused on the production of silver nanoparticles utilizing *Madhuca longifolia* leaf extracts as green stabilizing and reducing agents [1]. Chemically synthesized nanoparticles may not be very useful in biomedical applications due to the toxic nature [2]. Silver nanoparticles synthesized by plant extract as reducing agents have been reported for many biomedical applications such as antioxidant [3, 4], antibacterial [2, 3, 5, 6] anticancer [2–4], gas sensing etc. The size of silver nanoparticles is important for antibacterial activity. It hsows that means smaller nanoparticles show active antibacterial activity due to their high surface area [7]. The sensitivity of Gram positive and Gram negative property of bacteria decide the antibacterial property of plant extract.

The Sapotaceae family includes *Madhuca longifolia.* This plant, usually known as Mahua, grows up to 20 m above from the ground. The tree is evergreen and has edible flowers. Wound healing, antitumor, antioxidant, anti-inflammatory and hepatoprotective are just a few of the ethnomedical applications of *M. longifolia. M. longifolia* flower extracts has been shown to have antimicrobial properties. Tannins, saponins, cardiac glycosides, anthraquinone glycosides, mucilage, proteins, terpenoids, and starch are the main phytochemicals found in *M. longifolia* [8].

The present paper provides the experimental results of the proposed work. These results show the analysis of silver nanoparticles (AgNPs) from *M. longifolia.* This analysis has been done at different magnification and sizes using various techniques. The produced nanoparticles are analyzed using distinct methods like Transmission Electron Microscope (TEM), Scanning Electron Microscopy (SEM), X-ray diffraction (XRD), and UV-Spectrophotometer. XRD verified the production of silver nanoparticles. UV-Viz Spectroscopy is used to monitor the optical characteristics. TEM and SEM are also utilized for validating surface morphology, particle size and distribution [9].

2 Materials and Methods

2.1 Materials

AR grade Silver nitrate ($AgNO_3$) was purchased from Merck India Limited (Mumbai, India). Leaves of *M. Longifolia* were obtained from tree present in the SHUATS campus. Mueller-Hinton (MH) Broth/Agar was used to ensure good culture growth to study the antimicrobial effects.

2.2 Preparation of Plant Extracts from *Madhuca longifolia*

The dried leaves of *M. longifolia* were grounded on electric mixer and fine powder was prepared for extraction procedures. To get the extract, 10gm of dried Mahua powder was placed inside thick filter paper, made thimble and loaded into the main chamber in

Soxhlet extractor. Absolute alcohol (250 ml) was used as solvent for extraction at 80 °C for 3–4 h [10]. After the extraction 200 ml of leave extract was obtained and further concentrated with rotary evaporator and concentrated up to 100 ml final volume. Hence the final leave extract concentratedwas 0.25 g/ml. The extract was stored at 4 °C for future use.

2.3 Preparation of Silver Nanoparticles

The aqueous solution of leaf extract of *M. longifolia* and Silver Nitrate solutionsolution were mixed in the ratio of 1:4 and heated at 90 °C in water bath until color change observed [11]. A control of aqueous silver nitrate alone and diluted leaf extract with 5 ml type 1 water were also maintained under the same experimental conditions.

2.4 Characterization of Synthesized Nanoparticles from *M. longifolia*

Primary observation was done as the solution turned colloidal brown from yellowish color. After primary color change observation, UV-Vis spectrophotometer was used to study the information about silver nanoparticles synthesis in the range of 200–700 nm. Silver nanoparticles synthesized from the extract of *M. longifolia* were further characterized by using various characterization techniques. The morphology of silver nanoparticles from the *M. longifolia* leaf extract was monitored with TEM. Powdered form of silver nanoparticles from M.longifolia was observed under SEM. Similarly the powder form of AgNPs was used to determine the crystalline nature by using XRD using CuKα as X-ray source at 30 mA and 40 kV.

2.5 Antibacterial Activity of Synthesized Silver Nanoparticles

2.5.1 Agar Well Diffusion Method

The pathogenic bacterial culture was procured from Microbial Type Culture Collection (MTCC) and Gene bank, Gram –ve bacteria *Escherichia coli* MTCC687 and Gram +ve bacteria *Staphylococcus aureus* MTCC3160 were analyzed in this study. The antibacterial agar well diffusion method was performed by the method used by Patil *et al.,* [5]. Bacterial suspension with 100 μl of 0.5 McFarland-standard with optical density approx 0.08–0.1 at 625 nm was added on MHA agar plate and spread uniformly. Wells were prepared on agar plate with the help of 8 mm cork borer and 100 μl of AgNPs were added on each well with different concentrations ranging from 50–200 μg/ml. Plates were incubated for 24 h at 37 °C. After completion of incubation, clear zone was observed around each well which is called as zone of inhibition (ZOI). ZOI indicates bacterial growth inhibition and can be measured in mm. The experiment was performed in triplets' to confirm the antibacterial activity.

2.5.2 Antibiofilm Activity of Synthesized Silver Nanoparticles from *M. longifolia*

Assessment of antibiofilm activity of silver nanoparticles from the extract of *Madhuca longifolia* was done using microtiter plate (MTP) assay against two biofilm forming

microorganisms i.e. *Escherichia coli* and *Staphylococcus aureus*. 96-well flat bottom polystyrene micro titter plates method was used to estimate antibiofilm activity of AgNPs from *M. longifolia* [3]. Both microbial cultures were brought at OD550 = 0.02 (1.0×10^6 CFU/ml) and 100 μl from each broth were added to microtiter plate wells in triplicates. Then plates left for incubation at 37 °C for 4 h. Then 100 μl of nanoparticles were added to the respective wells after the incubation completed. Thus, the final concentration of tested nanoparticles was 1 mg/ml. After this the plate was incubated at 37 °C for 24 h without agitation. Negative control well was loaded with media but no test material (nanoparticles). Crystal violet staining method was used for quantification [12]. The assessment of anti-biofilm potential of silver nanoparticles from *M. longifolia* was done by adding 125 μl of ethanol to use for de-staining the wells. Then 100 μl aliquot of de-stained solution was transferred to 0.5 ml quartz cuvette and subjected to take the absorbance at 550 nm using UV-VIS-Spectrophotometer. The mean absorbance was determined and percentage inhibition of biofilm was noted using the following equation [13].

Percentage Inhibition (%) = [O.D (Negative Control) – O.D. (Sample)/O.D. (Negative Control)] × 100

3 Results and Discussions

3.1 Green Synthesis and Characterization of Silver Nanoparticles from *M. longifolia*

Silver nanoparticles from *M. longifolia* were prepared by green synthesis method and characterized with the help of UV-VIS spectroscopy, Transmission electron microscopy (TEM), Fourier transform infrared spectroscopy (FTIR), X-Ray Diffraction (XRD), Scanning Electron Microscope (SEM).

*M. longifolia*aquous leave extract functioned as reducing agent which reduced metallic silver to nanosilver and therefore the modification in color was observed. As a primary indication AgNPs exhibited brown color in aqueous solution. Krithinga et al. 2015 [14] also reported the striking colors from colorless to yellowish brown as a primary indication of formation of AgNPs.

3.2 UV-Vis Spectroscopy Analysis

The ultraviolet-visible spectroscopy of the reaction media is used to track the decrement of pure Ag^+ ions. In a UV-Vis spectrophotometer, the UV-Vis absorption range of the sample is considered in the wavelength from 200 to 700 nm. The perceived color of the chemical involved is immediately reflected by its absorption in the visual spectrum. Figure 1 indicates the UV-vis absorption analysis between absorption unit and wavelength of silver nanoparticles. The absorbance maxima of silver nanoparticle was measured at 420 nm (Fig. 1).

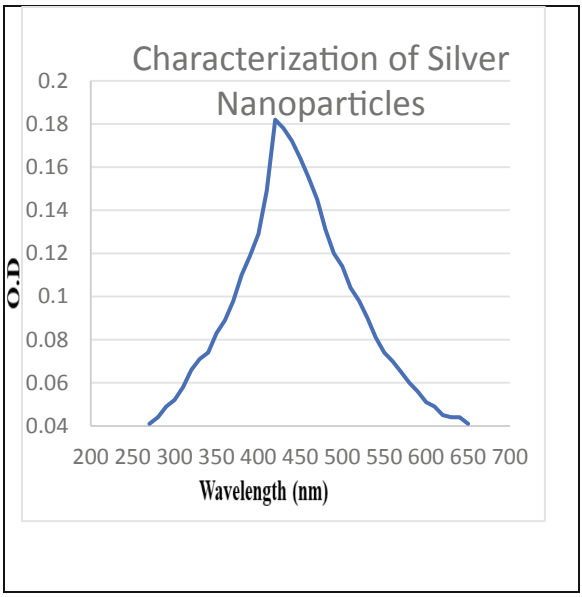

Fig. 1. UV-Vis spectrophotometer analysis of *M. Longifolia* AgNPs

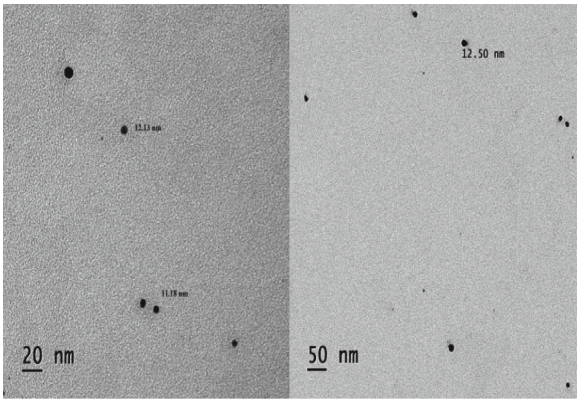

Fig. 2. TEM images at different magnification of *M. longifolia* AgNPs at resolution (a) 20 nm, (b) 50 nm and (c) 100 nm

3.3 TEM Analysis of *M. longifolia* AgNPs

TEM (Transmission electron microscopy) was applied to evaluate the morphology of *Madhucalongifolia* mediated AgNPs. Figure 2 shows a TEM micrograph taken from aqueous AgNPs placed on carbon covered copper grids. The clusters of sphericaly shaped nanoparticles with size distribution ranges from ± 5–40 nm at resolution of 20 nm, 50 nm and 100 nm.

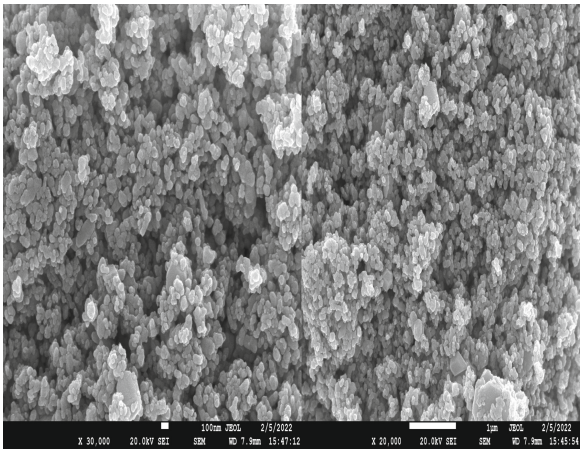

Fig. 3. SEM image of *M. longifolia* Silver Nanoparticle

3.4 SEM Analysis of Silver Nanoparticles from *M. longifolia*

Irregular shaped clusters were visible in the Fig. 3, scanning electron microscopy image (SEM) of silver nanoparticles from *M. longifolia.* The size distribution of AgNPs was approximately 0.1 μm length and width approximately 10–25 nm at 50000× magnification.

3.5 XRD Analysis of Silver Nanoparticles from *M. longifolia*

The crystal shape of silver nanoparticles from *M. Longifolia* was confirmedwith the help of XRD analysis. Figure 4 represents the XRD diffracted intensity from 25° to 75° at 2 theta angles. The nanoparticles exhibit a spherical shape, according to the XRD pattern. Figure 4 shows the XRD analysis of AgNPs from *M. longifolia.* The XRD peaks) was compared with pure crystalline silver structure published by JCPDS (Joint Committee on Powder Diffraction Strandards file no. 04-0783. The pattern of diffraction showed cubic phase Ag with intensive peaks at 2θ value of 36.24°, 44.78°, 62.55° and 67.22° which can be corresponded to the (111), (200), (220) and (311) planes of the face centered cubic structure (FCC), respectively. ML-AgNPs crystalline nature was confirmed by XR Diffractrometer.

3.6 FTIR Analysis of Synthesized Silver Nanoparticle from the Extract of *M. longifolia*

The participation of the plant extract as a capping agent was confirmed by FTIR analysis of the silver nanoparticles from *M. longifolia* (Fig. 5). The FTIR spectra of the *M. longifolia* extract shows several peaks at 515.30 cm^{-1}, 1057.63 cm^{-1}, 1202.46 cm^{-1}, 1620.10 cm^{-1}, 3206.76 cm^{-1}. Other peaks at 428.03 cm^{-1}, 436.98 cm^{-1}, 469.94 cm^{-1} represents the formation of nanoparticles. The peak at 3206.76 cm^{-1} represents the O-H stretch and H-bonded alcohols and phenols. The absorptions peak 1620.10 cm^{-1}

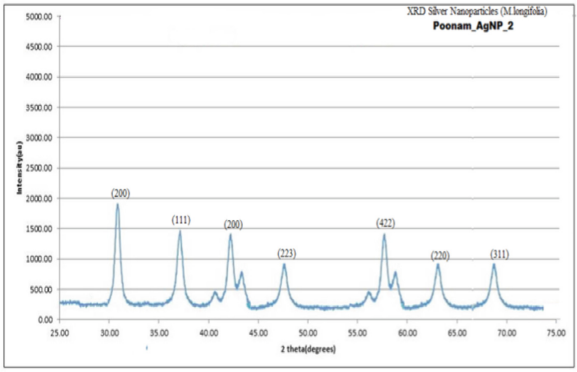

Fig. 4. XRD analysis of silver nanoparticles from *M. longifolia*

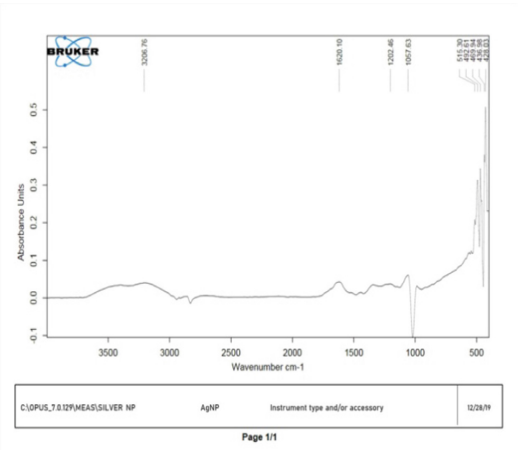

Fig. 5. FTIR analysis of silver nanoparticles from *M. longifolia*

represents N-H bonds corresponds to the bonding ofI° amines. The absorption peaks 1202.46 cm^{-1} and 1057.63 cm^{-1} represents the C-N stretch which corresponds to the aliphatic amines. The absorption peak situated at 515.30 cm^{-1} represents the presence of C-Br stretch corresponded to alkyl halides.

3.7 Antibacterial Effect of Synthesized Silver Nanoparticles from *M. longifolia*

Antibacterial activity of AgNPs from *M. longifolia* was tested against two pathogenic strains of bacteria (*E. coli* and *S. aureus*). Synthesized AgNPs were showed potential antimicrobial acivity against test bacteria. The parameters and readings are collected in the Table 1. Well number 1 and 6 are positive and negative control respectively. The well contains methanol as negative control showed no zone of inhibition (Fig. 6); and well of positive control contains 5 μg/ml ciprofloxacin antibiotic. Hence zone of inhibitions

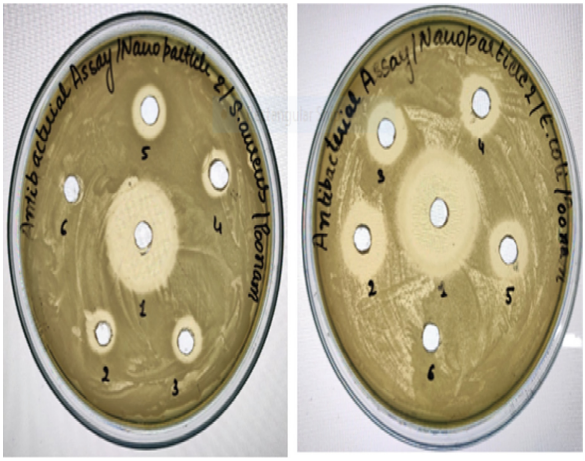

Fig. 6. Antimicrobial activity test of AgNPs from M. longifolia against (a) *S. aureus* (b) *E. coli*

Table 1. Antibacterial study of AgNPs from *M. longifolia*

S. No.	Sample	Zone of Inhibition (mm)	
		E. coli	*S. aureus*
1	Positive Control	16	15
2	50 µg/ml NP	10	8
3	100 µg/ml NP	11	9
4	150 µg/ml NP	13	12
5	200 µg/ml NP	14	13
6	Negative Control	0	0

measuring 16 mm and 15 mm for *E. coli* and *S. aureus* respectively. As the increase in concentration of silver nanoparticles the ZOI isalso increasing. Hence the prepared silver nanoparticles from *M. longifolia* showed good susceptibility against the bacteria.

In the Fig. 6, wells are labeled as well 1 - positive control (antibiotic); well 2 - 50 µg/ml NP, well 3 - 100 µg/ml NP, well 4 - 150 µg/ml NP; well 5 - 200 µg/ml and well 6 - negative control (methanol).

3.8 Antibiofilm Potential of Silver Nanoparticles Form *M. longifolia*

The test organisms used in the study were *E. coli* and *S. aureus*. The formation of biofilm of the test organisms were confirmed and detected by using crystal violet method.

Table 2 shows the moderate production of biofilm of both the bacterial strains.

In Table 3 the test sample is AgNP-ML (Silver nanoparticles *M. longifolia*), Negative control (media with test organism-*E. coli* but no test material). The percentage of

Table 2. Confirmation of formation of biofilm

S. No.	Test Organism	O.D. at 550 nm			Mean ± S.D.	Category
		R1	R2	R3		
1	Control	0.88	0.86	0.85	0.863 ± 0.0152	–
2	*Staphylococcus aureus*	1.77	1.81	1.74	1.733 ± 0.0351	Moderate Producing
3	*Escherichia coli*	1.82	1.89	1.83	1.846 ± 0.037	Moderate Producing

Table 3. Antibiofilm effect of silver nanoparticles from *M. longifolia* against *E. coli*

S. No.	Sample	Concentration of sample	Observed O.D at 550 nm			Mean ± S.D.	Percentage Inhibition (%)
			R1	R2	R3		
1	AgNPsML	1 mg/ml	0.530	0.549	0.553	0.544 ± 0.012	63.39
2	AgNPsML	500 mg/ml	0.982	0.986	0.987	0.985 ± 0.002	33.71
3	AgNPsML	250 mg/ml	1.28	1.26	1.119	1.219 ± 0.087	17.96
4	Negative Control	–	1.49	1.52	1.45	1.486 ± 0.035	–

Table 4. Antibiofilm effect of silver nanoparticles form M. longifolia on *S. aureus*

S. No.	Sample	Concentration of sample	Observed O.D at 550 nm			Mean ± S.D.	Percentage Inhibition (%)
			R1	R2	R3		
1	AgNPsML	1 mg/ml	0.593	0.589	0.568	0.583 ± 0.013	54.80
2	AgNPsML	250 mg/ml	0.839	0.828	0.835	0.834 ± 0.005	35.34
3	AgNPsML	500 mg/ml	0.891	0.895	0.921	0.902 ± 0.016	30.07
4	Negative Control	–	1.263	1.212	1.235	1.237 ± 0.026	–

inhibition shows different percentage at different concentration of AgNPS-ML samples. The highest percentage inhibition is 63.39% against E. coli biofilm.

In Table 4 the test sample is AgNPs-ML (Silver Nanoparticle from *Madhuca longifolia*), Negative control (media with test organism-S. aureus but no test material). The percentage inhibition showed different percentage at different concentration of the sample. The highest percentage of inhibition is 54.80% of AgNPsML against S. aureus biofilm. At 250 mg/ml and 500 mg/ml the percentage inhibition is 35.34% and 30.07%.

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percentage inhibition showed different percentage at different concentration of the sample. The highest percentage of inhibition is 54.80% of AgNPsML against *S. aureus* biofilm. At 250 mg/ml and 500 mg/ml the percentage inhibition is 35.34% and 30.07%.

4 Discussion

Silver nanopartciles were prepared with green route using *Madhuca longifolia* as the capping agent. The prepared silver nanoparticles from *Madhuca longifolia* were then characterized with the help of various techniques such as UV-vis spectroscopy, TEM, SEM, FTIR, XRD. UV-viz spectroscopy helped to confirm the plasma resonance. The maxima peaks of M. longifolia mediated silver nanoparticles were recorded at 420 nm, confirms the formation of AgNPs. SEM and TEM confirm the size and morphology of the particles. SEM shows the crystalline and spherical particles approximately ± 10–25 nm. According to results from TEM the size of NPs was found around ± 5–40 nm. Crystal shape of silver nanoparticles from *M. longifolia* was confirmed by using XRD analysis. FTIR analysis of silver nanoparticles from *M. longifolia* confirmed the presence of different functional groups which showed the capping property of the extract for silver nanoparticles. Other peaks were confirmed in the FTIR confirming the formation of silver nanoparticles. After the characterization techniques the synthesized silver nanoparticles were checked for their antimicrobial and antibiofilm effects on bacterial culture. For antimicrobial assay very popular agar well diffusion method was implemented. Antibiofilm assay was done after confirming the formation of biofilm of bacterial culture. The biofilm formation assay confirmed moderate formation of biofilm. *E. coli* (Gram –ve) and *S. aureus* (Gram +ve) bacterial culture was studied to check the antibiofilm and antimicrobial activity.

5 Conclusion

By using plant extract in green synthesis of nanoparticle, not only simple and cost effective, but it is an ecofriendly approach. In the present research work the efforts were made to synthesize antibacterial silver nanoparticles from the plant extract of *Madhuca longifolia.* The dried and powdered leaves of *Madhuca longifolia* were used for reduction of silver nitrate into silver nanoparticles (AgNPs). Silver nanoparticles from *M. longifolia* were characterized by SEM, TEM, XRD, FTIR and UV-visible spectroscopy. UV-viz spectroscopy confirmed the surface plasma resonance of green synthesized AgNPs. The biosynthesized MLNPs were found to have significant antimicrobial and antibiofilm effects against the pathogenic bacteria *Escherichia coli* and *Staphylocossus aureus.*

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