

Green Synthesis of nano silver particles and elucidation of their antibacterial activities.

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ABSTRACT:

Silver nanoparticles have a great significance globally due to their antimicrobial properties. The present study deals with the conversion of particulate silver into its nano form in presence of reductase enzyme produced by the extracts of various natural products, collected from wastes like used green tea leaves, shoe flower and marigold petals, banana and onion peels. Freely available natural resources like leaves of tulsi, neem, grass, banana, blackberry and thankuni were also used. The study also deals with the elucidation of its antibacterial effects against different Gram positive and Gram negative bacterial strains like *Bacillus subtilis*, *Klebsiella variicola*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Key words: Green synthesis, nanosilver, antibacterial, nano particles.

Introduction

Nanotechnology is an important field of modern research dealing with synthesis, strategy and manipulation of particle's structure ranging from approximately 1 to 100 nm in size, wherein all the chemical, physical and biological properties are found to change.

Nano-silver is referred to as silver (Ag) suspension in pure de-ionized water [Reddy, 2015] where approximately 80% of the silver remain in the form of metallic silver nanoparticles with the remaining silver in ionic form and silver nanoparticles (AgNPs) are of particular interest due to their peculiar properties and wide applications (Padalia et al, 2015). Due to its ultra-fine size, it shows remarkable transparency and antibacterial performance, broad-spectrum and long lasting bactericidal effect (Zhang et al, 2013). In spite of their antibacterial and antibiotic characteristics, physical and chemical methods of nanoparticle production include the use of harmful toxic chemicals and involve reaction steps that increase high cost depending on high energy requirements (Ciceket et al, 2015).

In order to make the process cost effective and eco-friendly as well, green synthesis of nano silver particles was found to be the only option left for the scientists (Njagiet al, 2011). Green synthesis methods include biological conversion of particulate silver into silver nanoparticles by the bio-organics of plants extracts, microbial enzymes or polysaccharides. Among the various biological methods of silver nanoparticle synthesis, the use of plant extracts is found to be potentially advantageous over microorganisms due to the convenience in preparation, ease of improvement, the less biohazard for the former and elaborate process of maintaining expensive cell cultures at highly aseptic conditions for the latter (Valli & Vaseeharan, 2012 and Kumar & Yadav, 2009).

The present study deals with the green synthesis of silver nanoparticles using various natural resources and detection of its toxicity on different gram positive and gram negative bacterial colonies.

Materials and methods

Collection of natural sources for enzyme production: Dry petals of shoe flowers (*Hibiscus rosa-sinensis*) and marigold (*Calendula officinalis*), peels of banana (*Musa acuminata*), and dried peels of onion (*Allium cepa*) were collected from market dumps. Widely available natural resources like leaves of tulsi (*Ocimum tenuiflorum*), neem (*Azadirachta indica*), grass (*Cynodon dactylon*), banana (*Musa acuminata*), blackberry (*Syzygium cumini*), thankuni (*Centella asiatica*) were collected.

Preparation of reductase enzyme from plant extract: The different natural products collected were thoroughly washed, dried and were chopped into fine pieces. 10 grams of each of the chopped material was then boiled in separate beakers filled with 100 ml of distilled water for 15 minutes with occasional stirring (Nestor, et al, 2008). After cooling, they were filtered thoroughly through Whatmann's No. 1 filter paper and the filtrate was used as the extract.

Preparation of nano silver particles: 10 mM aqueous AgNO₃ solution was added to the plant extract in a ratio of 1:10 and was incubated at room temperature for nearly one hour for conversion of particulate silver into its nano form (Ramteket et al, 2013).

Analysis of nano particles: The synthesized silver nanoparticles indicated by colour changes were monitored through scanning in a UV-visible spectrophotometer (Shimadzu, Japan) within 300-500 nm wavelength range. AgNO₃ solution (10mM) and plant extracts without AgNO₃ were also scanned separately at the aforementioned range. (Logeswari *et al*, 2013).

Preparation of bacterial cultures: The bacterial strains, namely *Bacillus subtilis*, *Klebsiella variicola*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were grown separately in 100 ml Erlenmeyer flask, each containing basal medium (BM) composed of (g/l): peptone 0.9; (NH₄)₂HPO₄ 0.1; KCl 0.1; MgSO₄ · 7H₂O 0.1 and glucose 0.5 (pH 7.0), incubated at 30°C for 42 hours.

Determination of antibacterial activities: Each of the indicator bacterial strain was grown separately on spread plate. Each petri-plate was divided into two halves and at each half, one well was prepared through scooping out of the agar from the plate. To observe the effects of the synthesized nanoparticles on the indicator bacteria, nano silver particle suspension synthesized from a natural source (50 µl) was poured into the “experimental” well, and 50 µl of distilled autoclaved water was pipetted into the control wells. The same method was applied and repeated for all the four bacterial agar plates. The petri-plates were incubated for 24 hours (Ciceket *et al*, 2015). The presence of any transparent colony or the zone of inhibition or ‘halo’ around the experimental well would denote bacterial lysis. The halo diameter and well diameter was measured and the ratio between the two indicates the level of antibacterial activity.

All the experiments were done thrice.

Results and discussions:

Nanosilver production in presence of various plant extracts: A noticeable change in the colour of the plant extracts namely, petals of shoe flower, marigold, leaf and peel of banana, onion peel, tulsi leaf, neem leaf, grass, thankuni, blackberry leaves were found for all the sources tested after incubation with 10 mM AgNO₃ solution. In each case, a brownish tinge was noticed that indicated the conversion of particulate silver into its nanoform (Fig 1). The colour of the extracts changed to greenish brown and reddish brown due to excitation and surface plasmon vibration of the silver nanoparticles being produced from particulate silver in the reducing environment provided by the extract.

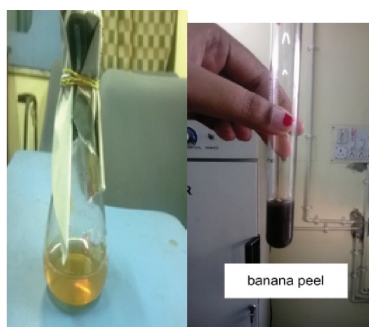
Analysis of nano silver production: As indicated by the sharpest peak of the silver nanoparticles at a range of 350-450 nm in UV-visible spectrophotometer dried leaf of tulsi was proved to be the best source for production of nanosilver than the others (Fig 2). It was followed by banana leaves, banana peels, and shoe flower petals; while grass, onion peel, neem, blackberry, thankuni and marigold petals gave relatively lower peaks. However, neither the scan for particulate silver solution nor the prepared natural extracts without AgNO₃ solution showed desirable peak within the 300 - 500 nm wavelength range. The relatively more potent nano-silver synthesizing plant sources were tulsi leaves, banana leaves, banana peels, and petals of shoe flower and were used for subsequent studies.

Analysis of antibacterial activity of nanosilver: To study the antimicrobial ability of nanosilver on two Gram positive strains - *Bacillus subtilis* and *Staphylococcus aureus* and two Gram negative strains - *Klebsiella variicola* and *Pseudomonas aeruginosa* were used as indicator strains, of which maximum susceptibility was shown by *Staphylococcus aureus*. Among the bacteria tested, Gram positive strains gave larger halos than the gram negative strains possibly due to the presence of tightly packed lipopolysaccharide (LPS) at the outer membrane of Gram-negative bacteria conferring effective resistive barrier against nanoparticles. It was mention worthy that no such bactericidal halo could be seen around the well containing plant extracts without nano silver particle (data not shown).

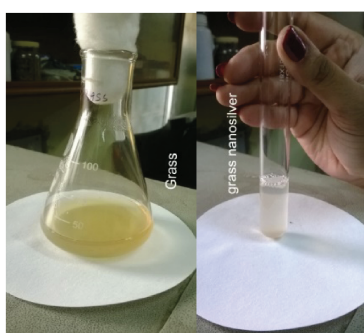
Conclusion :

Banana peels and tulsi leaves were found to be effective for nanosilver production by Ibrahim, 2015 and Ramteke *et al*, 2013 respectively, whereas shoe flower leaves were tested for the production of nano silver by Reveendran *et al*, 2016. But the present paper deals with all these plant sources taken from market dumps (banana leaf and banana peel) and temple wastes (tulsi leaf and petals of dried shoe flower and marigold) that would add economy in the nano silver production. Moreover, synthesized silver nanoparticles revealed good antimicrobial activity against the selected Gram positive and Gram negative bacterial strains.

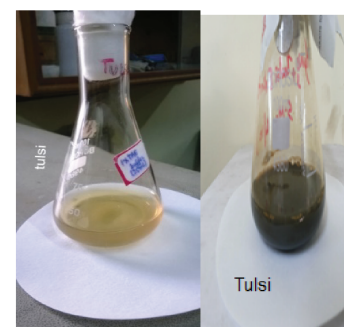
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Banana peel

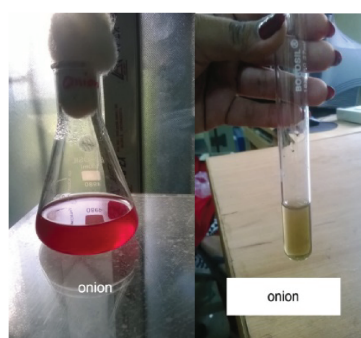


Grass



Tulsi

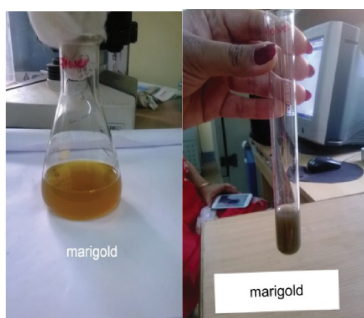
Fig



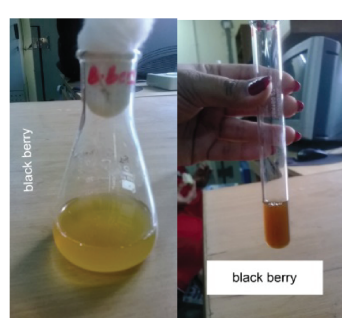
Onion



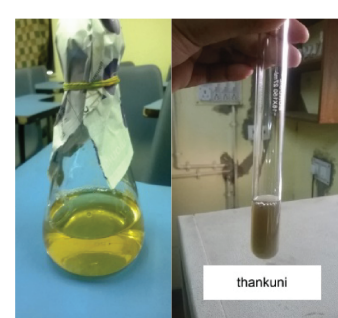
Shoe flower



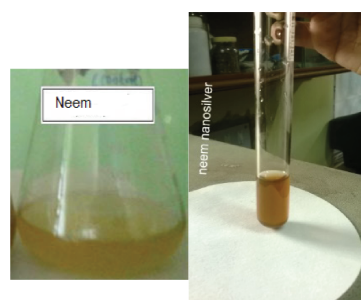
Marigold



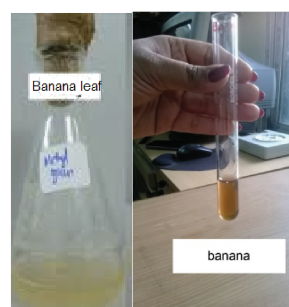
Black berry



Thankuni



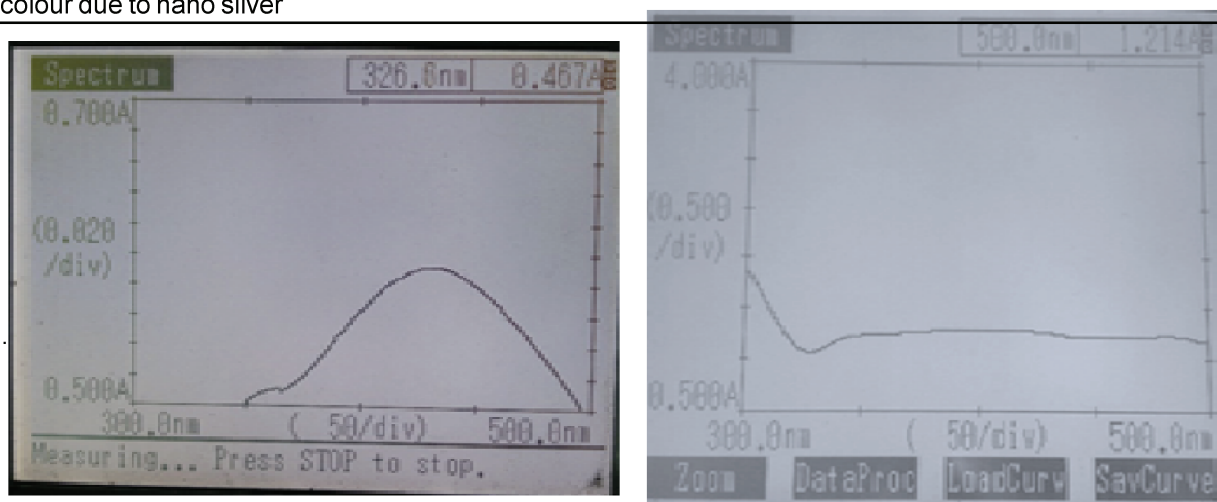
Neem



Banana Leaf

1. Biosynthesis of silver nanoparticles using different plant sources. Left Column: Control extract, Right Column: changed colour due to nano silver

formation.



A

B

Fig 2. Absorbance peaks for (A) nanosilver particles (B) particulate silver in spectrophotometer.

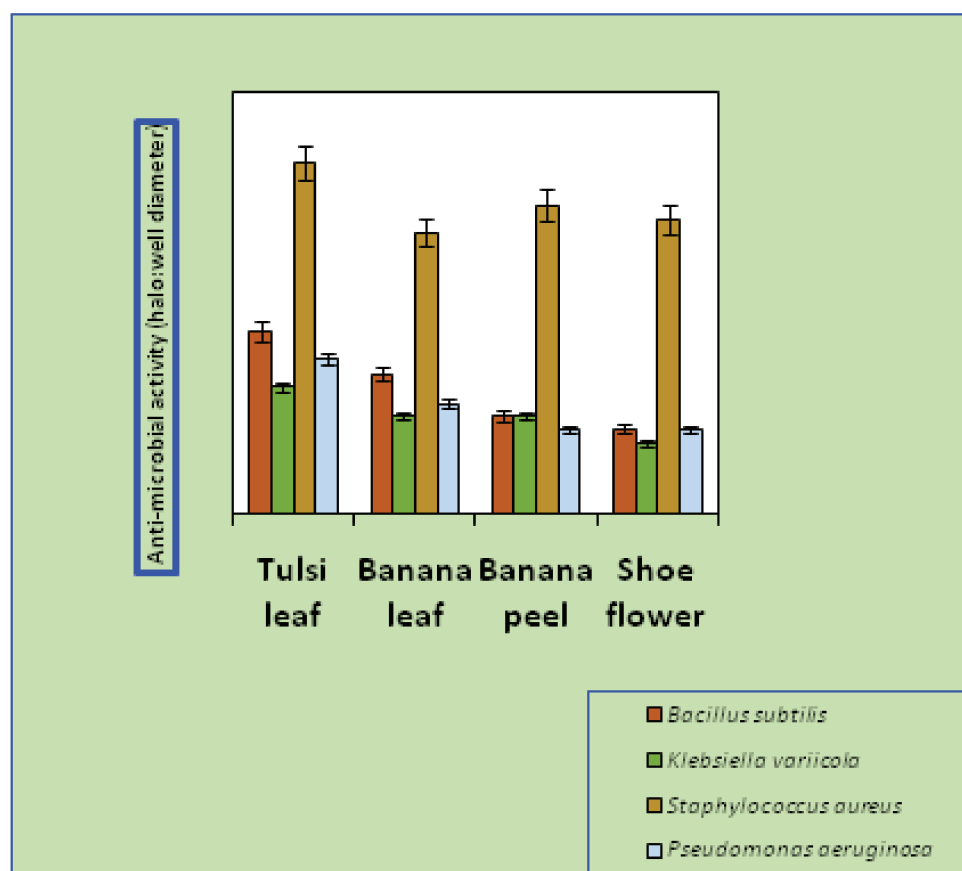


Fig 3. Antibacterial profile of nanosilver from various plant sources.

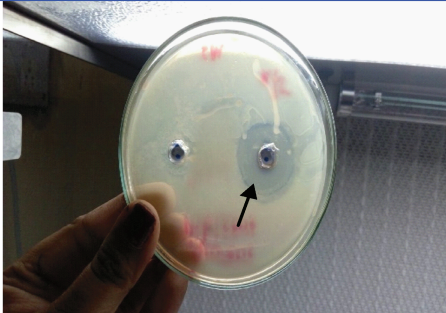
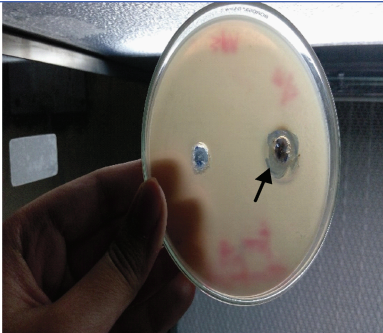
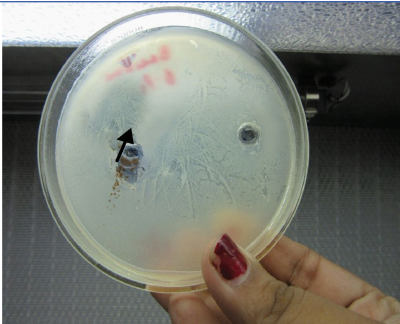
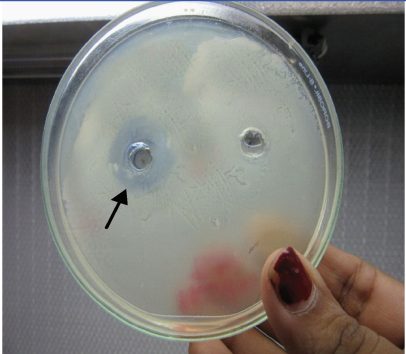
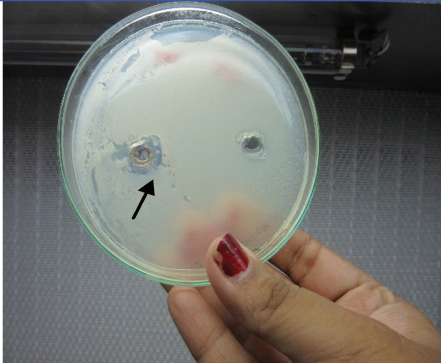
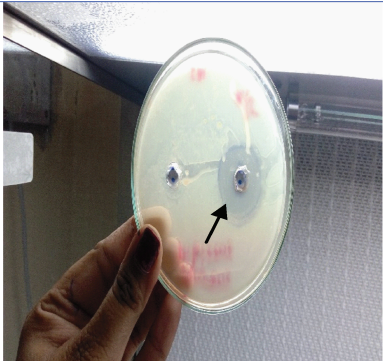
Sources	Gram positive bacteria	Gram negative bacteria
Tulsi Leaves		
Banana Leaves		
Banana Peels		
Shoe flower Petals		

Fig. 4. Appearance of halo surrounding the experimental well containing nano silver particles. The arrow indicates the appeared halo.

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