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RESEARCH PAPER

Biogenesis of silver nanoparticles using selected plant leaf extract; characterization and comparative analysis of their antimicrobial activity

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ABSTRACT

Objective(s): To study the antimicrobial property of green synthesised silver nano metals with M.balbisiana, A.indica and O.tenuiflorm and their enhanced antibacterial activity, assessment of antimicrobial effect and to explore the possible mechanism of AgNPs synthesis in the active constitutions of selected temperate plant extracts.

Materials and Methods: Biosynthesis of AgNPs using plant extract was carried out and formation of AgNPs confirmed by perceptible observation, UV spectroscopy, Scanning electron microscope (SEM) and Dynamic light scattering (DLS) were used to characterize the AgNPs.

Results: Screening of the M.balbisiana, A.indica and O.tenuiflorm extracts was carried out using standard methods to find their constitutions. The antibacterial screening was carried out by agar well diffusion method against selected microorganisms. The absorption maxima of UV visible spectrum found in the range between 300 nm to 800 nm confirmed the formation of AgNPs. SEM images revealed relatively spherical shaped of AgNPs of biosynthesized AgNPs with mean diameter about 14.51±1.5nm in O.tenuiflourum, 09.10±1.50nm M.barbisiana and 11.00±1.50 in A indica. FTIR results expounded the functional groups of plant extract responsible for the bio-reduction of silver ions and their interaction between them.

Conclusion: These results showed with changes in plants constituents are may be responsible to form nanoparticles with different size and characteristics

Key words: *Antibacterial Activity, Azadirachta indica (Neem) leaf extract, Medicinal Plant Extract, Musa balbisiana (Banana), Ocimum tenuiflorum (Black Tulsi), Silver nanoparticles*

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INTRODUCTION

Green synthesis of nanoparticles is a current looming peak of the junction of biotechnology, microbiology and biotechnology has received acquired much focus due to flourishing need to develop eco-friendly technology in material synthesis [1]. A great deal of attempt has been made for the biosynthesis of inorganic material, particularly metal nanoparticles using microbes and plants [2]. Silver has many important applications. It is used as an antimicrobial agent, which is widely used in textile industry, domestic water purification systems, therapeutic devices, cosmetics, house hold electrical and electronic appliances [3] Besides their antimicrobial features, silver nanoparticles showed the preparation of sensors which is involved in the imaging purpose

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[4]. Because of their high conductivity, silver nanoparticles are used to make conductive inks, paste and gum in wide range of electronic devices [5]. Silver nanoparticles are also considered as a metal catalysts in several chemical and biochemical reactions like oxidation of styrene [6,7].Various strategies are employed for synthesis of silver nanoparticles [8]. Silver nanoparticles are synthesized by reduction in solutions by thermal decomposition, microwave assisted synthesis, and laser mediated synthesis and biological reduction method [9]. The novel is the most preferred way for the synthesis of nanoparticles as it offers one step, eco-friendly way of synthesis of nanoparticles. The *Musa barbisiana* (Banana) contains 18-33% of the fruits. They are good origin of polyphenolic compounds, carotenoids and other compounds which possess the favourable consequences on human health [10]. It also contains dietary fibre; proteins, essential amino acids, polyunsaturated fatty acids and potassium are present. Synthesis of AgNPs using *Musa barbisiana* (Banana) leaf extract is less cost effective process. *Osmium tenuiflourum* (Black Tulsi) is cultivated for religious and medicinal purpose and it's contain the essential oil and bioactive compounds. These bioactive compounds are for the synthesis AgNPs which has potential application in many fields [11]. In the present research green synthesis of silver nanoparticles were carried out using three different leaf methanoloic extracts of *Musa barbisiana* (Banana), *Osmium tenuiflourum* (Black Tulsi), *Azadirachta indica* (Neem). The *Azadirachta indica* (Neem) leaves are used a medicinal plant in auyrveda and siddha . It has rich sources of antimicrobial compounds. In recent years the novel metal nanoparticles have been synthesised using neem leaf extract. [12]. The traditional value of banana is used since ancient years. The whole part of plant is used for medicinal purpose. Hence, utilization of banana leaf for AgNPs synthesis is novel technology [13, 14]. The fixed of nanotechnology is most active researches in modern material science and technology. The major advantage of neem leaves based nanopaticle synthesis indicated that the reducing phytochemicals in leaf extracts of terpenoids. It was found as reducing component were served as capping and stabilizing agent during the nanoparticle developments, which can be analysed by FTIR studies [15]. The present investigation of our study is to synthesize silver nanoparticles using *Azadiacbhtai indica*, *Musa balbisiana* and *Ocimum tenuiflorum*. The synthesized AgNPs were characterized by FTIR spectrum analysis, XRD and TEM image analysis. To screened out antibacterial activity of silver nanoparticles by well diffusion method and also evaluated MIC analysis against selected microbes.

Methods

Extraction of crude plant extract

The fresh leaves of *Musa barbisiana* (Banana*), Osmium tenuiflourum* (Black Tulsi), *Azadirachta indica* (Neem) were collected. The leaves were washed with the distilled water to remove the surface adherent materials. After the leaves were dried at room temperature for a week and kept in hot air oven at 60-65°C for 24 hr. The leaves were turned into fine powder and stored in desiccators for the preparation of crude plant extraction [16].

Solvent extraction of active compounds from Musa barbisiana (Banana), Osmium tenuiflourum (Black Tulsi), Azadirachta indica (Neem)

100g of oven dried powder of selected plant leaves were taken with double distilled water in soxhlet apparatus for extraction. The extraction process was carried out until the liquid was apparent for 6-7 hr. Then the plant extract was filtered through vacuum filtration for mixing with 1mM concentration of $AgNO_3$ for biosynthesis of nanoparticles [17].

Synthesis of silvernanoparticles (AgNps)

The fresh leaf of *Musa barbisiana* (Banana), *Osmium tenuiflourum* (Black Tulsi), *Azadirachta indica* (Neem). 10 g of oven dried leaf was mixed with 100mL of sterilized double distilled water. This mixture is boiled for 5 min in water bath for 70-80 ° C for 6 hr. The extract was filtered through what man filter paper no1 and stored at -15 °C. Then 20ml of each plant extract was added separately to 80mL of silver nitrate solution keeping its concentration at 1mM. As a result, a brown-yellow solution was formed, indicating the formation of silver nanoparticles after the incubation of 12-15 hr at room temperature. It showed that aqueous form of silver ions might be reduced by aqueous extract of plant active compounds to generate stable silver nanoparticles in water. The synthesized AgNps were lyophilized for further evaluations.

Characterization of synthesised silver nanoparticles

The formation of silver nanoparticles was confirmed by UV Vis spectral analysis with Shimadzu UV visible spectrophotometer (UV-1800, Japan). FTIR spectra of were recorded on Perkin Elmer 1750 FTIR Spectrophotometer. The particle size and surface morphology was analysed using Transmission electron microscope (TEM), operated at an accelerated voltage of 120 kV. Photoluminescence studies were evaluated by using eclipse Fluorescence spectrophotometer (Agilent technologies).

Phytochemical Screening of plant extracts

Phytochemical tests for selected plant extract were Alkaloids, saponins, flavonoids, tannins, protein, carbohydrate, glycosides phenols, steroids, terpenoids carried out on the aqueous and methanol extracts reconstituted in respective solvents using standard protocols [16, 17].

Antimicrobial Screening of the synthesized silvernanoparticle

The bacterial strains used for present investigation are *Escherichia coli* NCIM 293*, Bacillus subtilis* NCIM 2718*, Staphylococcus aureus* NCIM 5021*,* and *Klebsiella pneumoniae* NCIM 5546 were obtained from MTCC.

A loop full of culture was inoculated into nutrient broth and incubated at 37°C for 24 hr to obtain a bacterial culture. This procedure was carried for the selected bacterial cultures to obtain inoculums of particular broth cultures. The well diffusion method was used to assay the plant materials for antimicrobial activity. The bacterial lawns of selected microbes were plated with Mueller Hinton Agar media. Cork borer was used to bore wells in the agar plate. The synthesized silver nanoparticles extract of different concentrations (25µl, 50µl, 75µl, and 100µl) were separately dispensed into the wells of different plates using a micropipette. Water is used as a negative control and suitable antibiotic suspension with 1000µL were used as positive control against specific test organisms. The plant extract and synthesized AgNPs were loaded in the well at different concentration and allowed to diffuse for 30 mins at room temperature (for well diffusion). The plates were incubated at 37°C for 24 hours and the zones of inhibition were measured.

Determination of minimum inhibitory concentration

The MIC of the extract was determined according to the macro broth dilution technique (NCCLS 2000) from the prepared plant extracts 25µl, 50µl, 75µl, and 100µl was taken. It is added to the series of test tubes containing 10ml of nutrient broth. About 0.1ml of microbial culture is also added to each and every tube. The tubes were incubated at 37ºC. After overnight or 48 hours the turbidity is measured using spectrophotometer in terms of optical density at 540nm.

FTIR analysis

FTIR (Fourier Transform Infrared Spectroscopy) analysis was done by using Shimadzu 8400s spectrophotometer in the mid-infrared of 4000-400 cm^{-1}. The FTIR spectrum acts as a valuable analytical tool that enables the perseverance of both the type and power of interactions that occur within potential biomolecules containing various functional groups after synthesis of AgNPs.

X-Ray Diffraction analysis

X-Ray Diffraction pattern were documented in powdered thin film of AgNPs using X-ray diffractometer (Bruker Biosciences Corporation, Billerica, MA, USA) at 1 step / second . The powdered thin film of AgNPs was irradiated with Cu-Kα radiation and the analysis was carried out at 20°–0° (2θ) with a step size of 0.001°.

Transmission electron microscope (TEM) and Dynamic Light scattering (DLS)

In Transmission electron microscope analysis the bioreductive formation and existence of AgNPs was analyzed by locating a drop of green synthesized AgNPs solution on carbon-coated TEM grids and dryed in room temperature. The sizes of synthesized nanoparticles were measured by Dynamic light scattering.

Statistical analysis

The statistical significance of the antimicrobial activity of different plant extract and synthesized AgNPs at various concentration results was calculated using one-way analysis of variance through student's t-tests. Statistically significant variation were calculated using the Duncan's new multiple range test at P>0.05. The graphs were examined using Origin (v6.0; Origin Lab, Corporation, Northampton, MA, USA).

RESULTS

Synthesis of nanoparticles

The silver nanoparticles were synthesized the aqueous extract of *Musa balbisiana* (banana leaf), *Ocimum tenuiflorum* (tulasi leaf), and *Azadiachta indica* (neem leaf) Fig. 1 (a)-(c). The development of pale yellow colour in the sample is due to the reduction of silver ion nanoparticles. During exposure to the plant extract with silver nitrate, the active compounds in the plant extract reduced with Silver nitrate solution, which is visually notable by colour change. Different volumes of aqueous solution of three different plants extract were added to different concentration of silver nitrate solution to obtain the maximum amount of silver nanoparticles synthesis.

Phytochemical analysis

The *Azadiachta indica*, *Musa balbisiana* and *Ocimum tenuiflorum* leaf extract was used for qualitative photochemical analysis. Table 1 shows as Alkaloides, Saponins, Flavonoids, Tannins, Steroides, Glycosides, Phenol compounds and Terpenoides.

Fig 1. Synthesized AgNPs of (a) Musa balbisiana , (b) Ocimum tenuiflorum and (c) Azadiachta indica

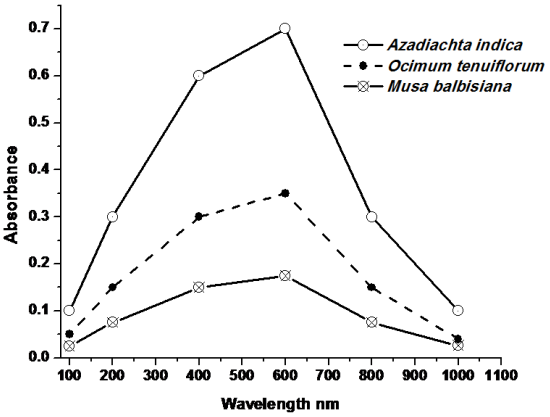


Fig 2. Absorption spectra of AgNPs observed in selected plants with 1 mM aqueous solution of silver nitrate. UV Vis spectral analys

FTIR analysis

The role of *Azadiachta indica*, *Musa balbisiana* and *Ocimum tenuiflorum* leaf extract as a reducing and capping agent which were presence of some functional groups was confirmed by FTIR analysis of AgNPs. A broad band between 3454cm^{-1} is due to the N-H stretching vibration of group NH_2 and OH the overlapping of the stretching vibration of attributed for water and of *Azadiachta indica*, *Musa balbisiana* and *Ocimum tenuiflorum* leaf extract molecules. The band at 1636cm^{-1} corresponds to amide C=O stretching and a peak at 2083cm^{-1} can be assigned to alkyne group present in phytoconstituents of extract Fig. 2 and Table 2.The observed peaks at 1113cm^{-1} denote –C-O-C- linkages, or –C-O- bonds. The observed peaks are mainly attributed to flavanoids and terpenoids excessively present in plants extract.

X-Ray Diffraction analysis

The X-Ray diffraction pattern for the green synthesized AgNPs using *Azadiachta indica*, *Musa balbisiana* and *Ocimum tenuiflorum* extract was compared and analysed with standard data. The main peaks were found at 38°, 46°, 65°, and 78° (2θ values) corresponds to the reflection of (111), (200), (220), and (311) planes respectively. It confirms the crystalline phase index of the AgNPs. The polycrystalline nature of biosynthesized AgNPs inferred from XRD data suggest smaller crystallite sizes than the particle sizes observed in TEM images.

Antibacterial activity

The antibacterial activity assays of the synthesised AgNPs were done on, *E. coli, B. subtilis, S. aureus* and *K. pneumoniae*, by standard well diffusion methods. To find out Minimum inhibitory concentration (MIC) of (range between 25 to 100µg/mL) synthesized AgNPs and

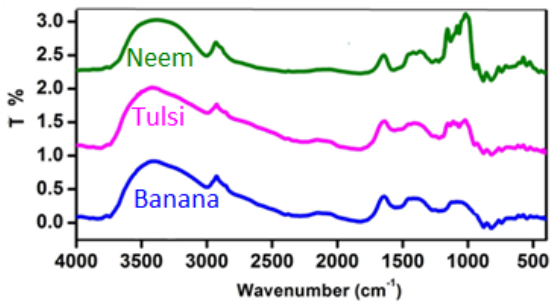


Fig 3. FTIR spectra of Musa balbisiana, Ocimum tenuiflorum and Azadiachta indica leaf AgNPs

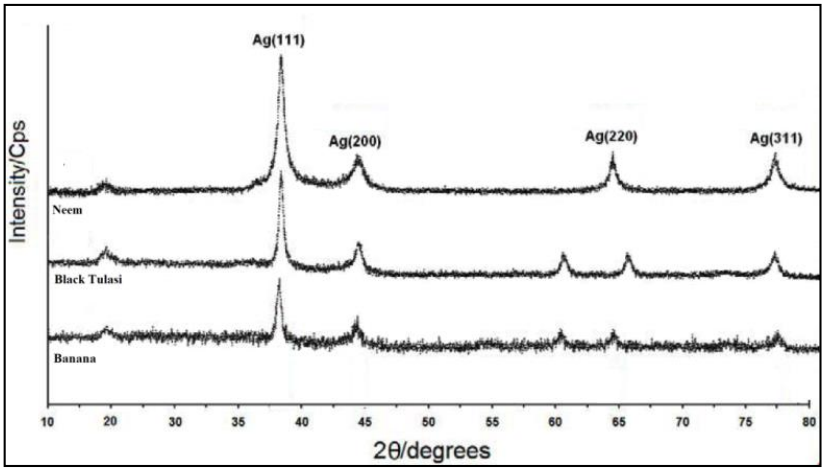


Fig 4. XRD pattern of Musa balbisiana , Ocimum tenuiflorum and Azadiachta indica leaf AgNPs

crude leaf extract. LB broths were prepared and 10µL overnight grown bacterial cultures were inoculated in the tubes under aseptic condition. The tubes were incubated at 37°C for 24 h and the lowest concentration of crude leaf extract and green synthesized AgNPs that inhibited the growth of bacteria was considered as MIC for the selected microbes [18-20]. The green synthesised silvernanoparticle has tremendous clinical advantages such as, microbicidal, wound healing, other medical and electronic applications, and makes this method potentially for the large scale synthesis. In vitro toxicity studies of silver nanoparticles on wide range of pathogen opens a door for novel antibacterial agents. The green synthesized nanoparticles were characterized by FTIR spectra analysis. The FTIR spectrum confirms the presence silver nanoparticles along with vibration of functional group changes in the band range between 4000 to 500cm^{-1} (Table 1). Further the antibacterial activity of crude leaf extract and synthesised silver nanoparticles was

Table 1. Photochemical Analysis of Musa balbisiana (banana leaf), Ocimum tenuiflorum (tulasi leaf) and Azadiachta indica leaf extract

Plant Leaf	Flavonodies	Terpenoides	Glycosides	Steroids	Phenols	Alkaloids	Saponins	Tannins	Catachine	Anthroquinone
Musa balbisiana	+	+	+	+	-	-	+	-	-	-
Ocimum tenuiflorum	-	-	-	-	+	+	-	+	-	-
Azadiachta indica	-	-	-	-	+	+	+	+	-	-

Table 2. FTIR Functional group vibrations in selected plants

	Functional group vibration in selected plants leaf extract				
Band cm^{-1}	*Azadiachta indica*	Band cm^{-1}	*Musa balbisiana*	Band cm^{-1}	*Ocimum tenuiflorum*
526	C-Br stetch, Alkyl halides	420	C-Cl stretch, Alkyl halides	439	C-Br stretch, Alkyl halides
574	C-Br stetch, Alkyl halides	441	C-Cl stretch, Alkyl halides	526	C-Br stretch, Alkyl halides
611	- C(triple bond) C-H: C-H bond, Alkanes	476	C-Cl stretch, Alkyl halides	576	C-Cl stretch, Alkyl halides
711	C-H rock, Alkanes	522	C-Br stretch, Alky halides	709	-C(triple bond)C:H C-H bend, Alkynes
763	N-H wag, Primary, secondary amines	574	C-Cl stretch, Alkyl halides	763	C-H rock, Alkynes
856	C-Cl stretch, Alkly halides	617	-C(triple bond) C:H C-H bend, Alkynes	880	C-Cl stretch, Alkyl halides
929	O-H bend, Carboxylic acids	763	O-H rock, Alkanes	929	O-H bend, Carboxylic acid
1020	C-N stretch, Aliphatic amines	856	C-Cl stretch, Alkyl halides	1018	C-N stretch, Aliphatic amines
1084	C-N stretch, Aliphatic amines	929	O-H bend, Carboxylic acid	1082	C-N stretch, Aliphatic amines, Alkyl halides
1111	C-N stretch, Aliphatic amines	1020	C-N stretch, Aliphatic amines	1157	C-H wag(-CH_2x), Aliphatic amine
1159	C-H wag (-CH_2X)	1084	C-N stretch, Aliphatic amines	1242	C-N stretch, Alkanes
1413	C-H bend, Alkanes	1112	C-H wag(CH_2x), Alkyl halides	1367	C-H rock, Alkanes
1637	N-H bend, Primary amines	1153	C-H wag(CH_2x), Alkyl halides	1419	C-H bend, Primary amines
2150	-C(triple bond) C-S Stretch, Alkynes	1452	C-H bend, Alkanes	1647	N-H bend, Alkanes
3419	-C (triple bond) C-H: C-H stretch, Alkynes (terminal)	2152	-C(triple bond) c- stretch, Alkanes	2929	C-H stretch Primary, secondary amines, amines
		2924	C-H stretch, Alkanes	3392	N-H stretch, Primary, secondary amines, amines
		3423	N-H stretch, Alkanes	439	C-Br stretch, , Alkanes
		420	C-Cl stretch, Primery, secondary amines,	526	C-Br stretch, , Alkanes

compared against *B. subtilis, E. coli, S. aureus* and *K. pneumoniae.* The study revealed the green synthesized AgNPs possess more zone of inhibition which is comparable with organism specific standard antibiotics. Thus the present study emphasizes the synthesis of silver nanoparticles with crude extract of showed potent antibacterial effect on selected microbes (Fig. 3).

DISCUSSION

The present research has proved potential clinical applications of nano biotechnology in medicinal sciences. Generally nanoparticles have wide applications in an early diagnosis and curing of diseases caused by multidrug-resistant pathogens. AgNPs have been identified broad range of usage in various industries for manufacturing devices. In the field of pharmaceuticals AgNPs are used in the preparation of ointment and creams for burn and wound infection-prevention/healing. Global attempts have been made to exploit the synthesis of NPs with less cost effective, biocompatible and free from toxic byproducts. Currently, many reports are available on the use of different plants for AgNPs synthesis.

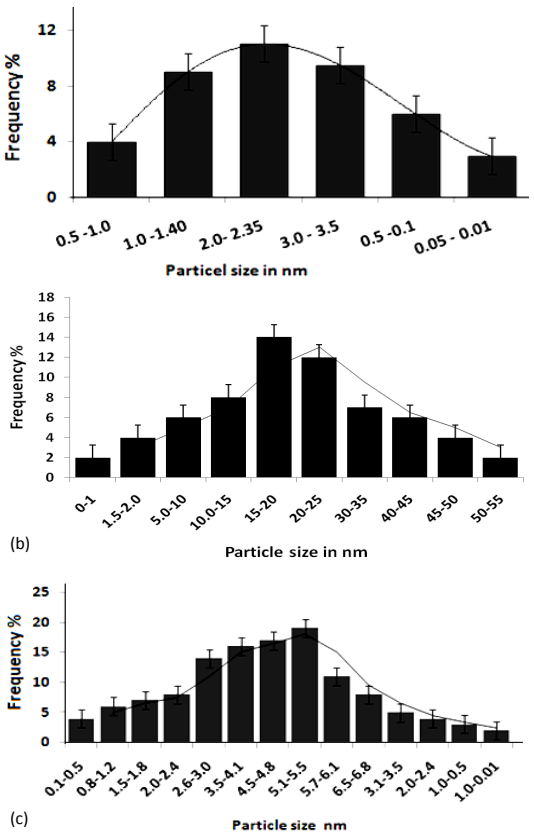


Fig 5. Particle size distribution of green synthesized AgNPs in (a) of Musa balbisiana extract, (b) Ocimum tenuiflorum extract, and (c) Azadiachta indica extract

Green synthesis of AgNPs using plant extract is due to the bio reduction of metal ion in to zerovalance metal nanoparticles. The bio reduction also mediated by different plant bio chemicals such as reducing and non-reducing sugars, proteins, terpenoids, lipids and other poly phenolic compounds of the plant extracts. *Osmium tenuiflourum* (Black Tulsi) contains different types of active compounds among euginol is the major compounds which is act as a reducing agent in the formation of AgNPs by bio reduction with silver nitrate [21].The development of AgNPs after bio reduction of Ag^{+} which was confirmed by the change of color with addition of leaf extracts of *Osmium tenuiflourum* to 1mM $AgNO_3$ solution.

The pale brownish yellow color maximum wavelength (λ_{max}) range of *Osmium tenuiflourum* extract based synthesis AgNPs is in the range between 400 – 800 nm Fig. 4. It may be transfer of charges between medium and the developed nanoparticles. The developed aggregates of *Osmium tenuiflourum* silver nanoparticles were confirmed with the fall of peak in range between 111 to 311nm in XRD analysis [22]. The particle size of AgNPs synthesized with *Osmium tenuiflourum* average diameter is 14.51 ± 1.5nm (Fig 5(a)). The synthesized AgNPs with *Osmium tenuiflourum* is highly stable due the presence of protein which can be act as a capping agent and bioreduction process. Yet further analysis is required to find the respective bio molecule involved in bioreduction process. AgNPs synthesis with *Musa barbisiana* (Banana) leaf extract is not well studied. The *Musa barbisiana* leafs has rich amount of pentosans and soluble starch along with proteins and chlorophyll [23]. Development of AgNPs from 1mM with *Musa barbisiana* leaf extract shows a yellowish brown color with maximum wavelength (λ max) was observed in the range between 400 - 800nm Fig 2. Appearance of this color is due to the vibration in the silver nanoparticle followed by excitation of surface Plasmon vibration [24].These vibration are due to the collective oscillation of free electrons available in the reduced AgNPs, such observable changes on biomass color due to the formation of AgNPs have been reported in earlier [25].Mechanism of silver nanoparticles formation with carbohydrate (Starch) under this experimental condition is amylase in the aqueous

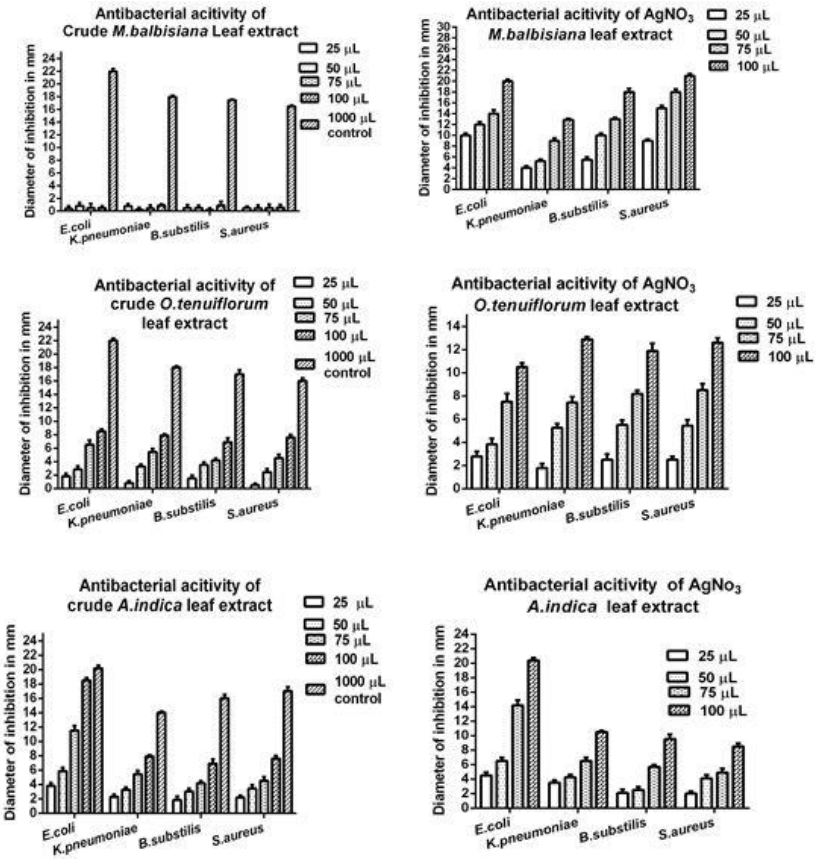
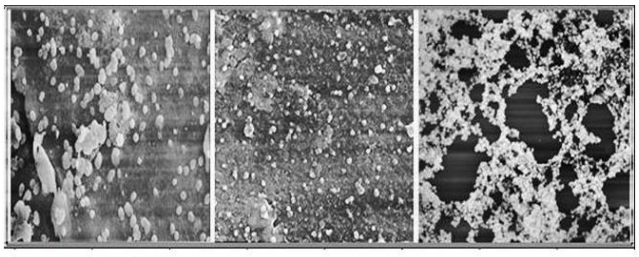


Fig 6. Antibacterial activity of (a) selected organism against Musa balbisiana crude leaf extracts and synthesized AgNps in agar well diffusion assay, (b) selected organism against Ocimum tenuiflorum extract crude leaf extracts and synthesized AgNps in agar well diffusion assay, and (c) selected organism against Azadiachta indica crude leaf extracts and synthesized AgNps in agar well diffusion assay



(a).*M.balbisiana* leaf AgNps (b).*O.tenuiflorum* leaf AgNps (c).*A. indica* leaf AgNps

Fig 7. TEM image of synthesized AgNPs using (a) Musa balbisiana leaf (b) Ocimum tenuiflorum leaf, and (c) Azadiachta indica leaf

solution of starch converted in to small molecules. The crude *Musa barbisiana* leaf extract with 1mM $AgNO_3$ solution under hot condition turns amylase to β-D-glucose. Where β-D-glucose act as a reducing agent during the formation of AgNPs. The aldehyde terminal presents in soluble starch take part in the bio reduction of silver nitrate. Also this soluble starch may act as a stabilizer for the formation of stable AgNPs in *Musa barbisiana* leaf extract. The formed aggregates of *Musa barbisiana* leaf silver nanoparticles were confirmed with the development of peak in range between 111 to 311nm in XRD analysis with average particle size of 09.10 ± 1.50 Fig. 5(c). For the synthesis of AgNPs in aqueous solution of *Azadirachta indica* (Neem) extract, 1mM silver nitrate solution mixed with *Azadirachta indica* extract. The change of color from green to brownish yellow is due to the excitation of surface plasmon vibrations in the developed silver nanoparticles [26]. The maximum

wavelength (λ_{max}) range of *Azadirachta indica* extract based synthesis of AgNPs is found in the range between 400-800 nm Fig. 2. The reported principle active compounds in the *Azadirachta indica* extract were quercitin, neemazal and nimbecedine etc., [27]. UV Visible spectrum of the *Azadirachta indica* based AgNPs synthesis study also exhibit the XRD analysis with average particle size of 11.00 ± 1.50 Fig. 5(b). In order to compare the antimicrobial activity of synthesized AgNPs, agar well diffusion assay was performed with selected bacterial cultures. The different concentration of crude leaf extract and synthesized AgNps were used to compare the anti-bacterial activity. The zone of inhibition was higher in synthesized AgNps than crude extract in all different concentration Fig 6-6b**.** FTIR spectroscopy was applied to compare the molecular conformation changes in the crude extract and synthesized AgNps of *Azadirachta indica*, *Musa barbisiana* and *Osmium tenuiflourum* (Fig. 3). Corresponding structural changes assignments are listed in Table 1. The major peaks were observed at 1012, 1080, 1614, 1654cm^{-1} in all the three selected plant leaf extract were assigned to the stretching vibration of the O–H and aliphatic C–H stretching bonds of the alkyl group, respectively. The bands between 3421cm^{-1} and 2900cm^{-1} were attributed to stretch of –OH groups and C–H stretching, which existed in pentosan in *Musa barbisiana*, euginol in *Osmium*

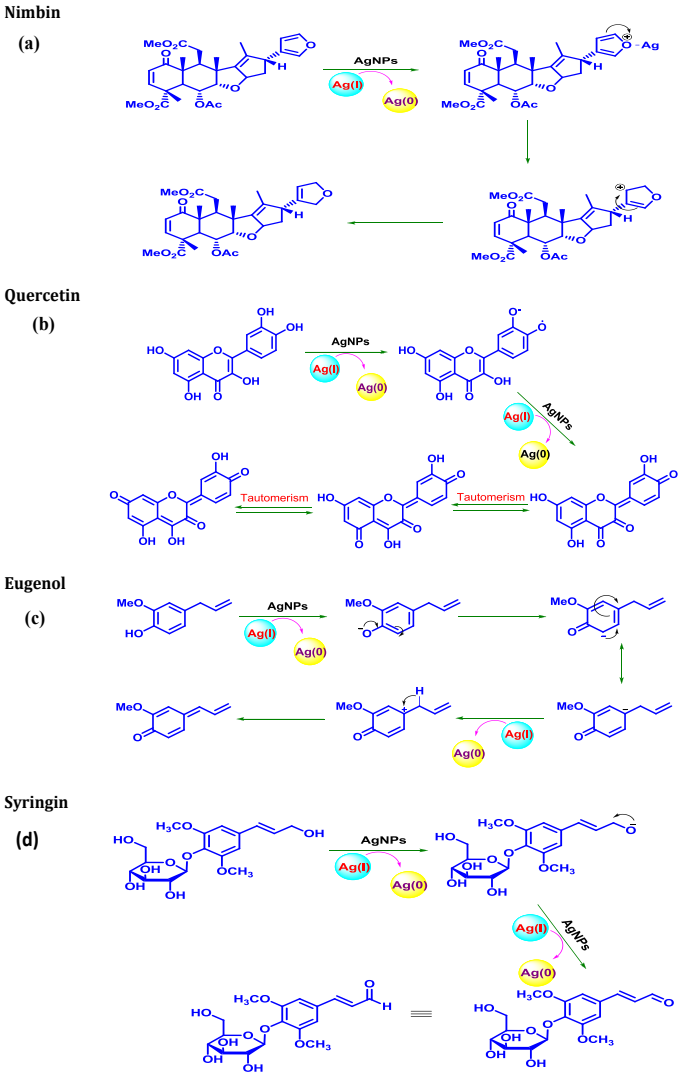


Fig 8. (a) Possible mechanism of reaction for the formation of silver nanoparticles in active compounds of selected leaf extract

tenuiflourum and Nimbidin in *Azadirachta indica* leaf [29]. The TEM image of biosynthesized AgNPs were showed in *Azadirachta indica*, *Osmium tenuiflourum* and *Musa barbisiana* were showed in Fig. 7. The formation of various size of AgNPs is depends on the active constituents present in the plant extract. Were those active compounds act as reducing agent and are oxidized by $AgNO_3$, resulting in the formation of AgNPs. The possible reaction could be summarised as:

$AgNO_3$ $\quad Ag^+ + NO^+_3$

$e^- + Ag^+$ $\quad Ag$ + by products

Azadirachta indica has wide range of active compounds, but in leaves of *Azadirachta indica* mainly contains cyclic trisulphide and cyclic tetra sulphide (nimbin and quercetin). In *Osmium tenuiflourum* majorly hold eugenol. Syringin and quercetin are foremost active constitutions of *Musa barbisiana* leaf extract. The exact molecular mechanism of biosynthesis of AgNps with nimbin, quercetin, eugenol and Syringin are not studied well. Here we try to figure out the promising reaction mechanism of AgNps synthesis in the selected plant extracts Fig.8.

The methanolic leaf extraction of Azadirachta indica contains active constituent called Nimbin which have a furan unit in the molecule. By the subsequent addition of silver nanoparticles reduce the furan moiety (i.e. conversion of dihydrofuran), moreover the Ag(I) to Ag(0) role has been shown in the aforementioned Scheme 8a.

The active compound of Quercetin in methanolic Azadirachta indica leaf extract underwent reduction by adding silver nanoparticles. The hydroxyl group present in the Quercetin which generate oxygen radical with silver nanoparticles (i.e) Ag(I) to Ag (0). Further the oxygen radical migrate to the ring system. Therefore this protocol generates keto-enol tautomerism. Ultimately, the keto- formation as illustrated in Scheme 8b.

The methanolic Osmium tenuiflourum leaf extract have Eugenol (active phenolic component) was reduced by silver nanoparticle. This hydroxyl group present in Eugenol has been converted in to corresponding benzoquinone surrogate, The transformation of Ag(I) to Ag (0) leads to the keto-enol tautomerism in the Eugenol and its mechanistic pathway shown in Scheme 8c.

The active compound of syringin has been extracted from the Musa barbisiana leaf which was reduced by silver nanoparticles [the primary alcohol present in syringin has been converted in to corresponding aldehyde by reduction of Ag(I) to Ag (0)]. The mechanistic pathway has depicted in Scheme 8d.

CONCLUSION

In Hindu mythology *Musa barbisiana, Osmium tenuiflourum* and *Azadirachta indica* plant leafs are sacred and traditionally used in siddha and ayuerdic sciences. This is the first report of comparative synthesis of AgNPs in above three temperate region plants. The highly stable monodisperse AgNPs silver nanoparticles are synthesized using selected leaf extract, among well-known medicinal plants are *Osmium tenuiflourum* and *Azadirachta indica*. The synthesis AgNPs are found to be efficient in terms of microbial growth inhibition even in low concentration for both gram positive and negative organisms. It can be concluded that the synthesized AgNPs of size various in each plant extract.ie. (14.51 ± 1.5nm in *Osmium tenuiflourum*, 09.10 ± 1.50nm *Musa barbisiana* and 11.00 ± 1.50 in *Azadirachta indica*). Investigation on the antibacterial activity of AgNPs against *E.coli*, *K. pneumoniae*, *B. subtilis* and *S. aureus* reveals high potential of *Osmium tenuiflourum* extract stabilized AgNPs to be used as antimicrobial agent in the field of medical, food and cosmetic purpose. The biosynthesis of AgNps with different plant will produce various sizes of nanoparticles. However, formation of molecular mechanism of biogenesis of AgNps with traditional plants is yet to study well.

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CONFLICT OF INTERESTS

The authors confirm that this article content has no conflict of interest.

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