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Synthesis, characterization and antimicrobial properties of green synthesised silver nanoparticles from stem bark extract of Pterolobium hexapetalum

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Abstract

Plant mediated synthesis of nanoparticles has wide application in biomedicine due to its novel properties and its eco-friendly nature. The present study deals with the biosynthesis of stable silver nanoparticles (SNPs) from the stem bark aqueous extract of *Pterolobium hexapetalum*. The synthesized nanoparticles are characterized by the colour change, observed from gray to dark brown indicates the formation of nanoparticles and UV–VIS surface plasmonresonance spectroscopy observed at 424 nm further confirmed the synthesized nanoparticles as SNPs. FTIR spectroscopic studies confirm that phenols, amines and halides of bark aqueous extract is mainly responsible for capping and stabilization of synthesized SNPs. The XRD data shows crystalline nature of nanoparticles and EDAX measurements reveals the presence of 67.32% Ag metal. Zeta potential at -21.0 mV the negative value indicates the high stability of nanoparticles. TEM microscopic analysis revealed that the size of synthesized SNPs ranging from 2 to 24 nm with spherical shape. Further, the antibacterial studies of synthesized SNPs show high activity towards *Staphylococcus aureus* with 17 mm diameter zone of inhibition followed by *Bacillus subtilis* with 7.25 mm

Keywords: *Bacillus subtilis, Staphylococcus aureus* Phenols, amines, zeta potential, diameter zone of inhibition

INTRODUCTION

Eco-friendly synthesis methods gain major research attention because it solves the problems associated with environmental pollution faced World-Wide. Utilisation of nontoxic solvents, closed reactors, 'green' techniques (biological methods, hydrothermal, ultrasound, magnetic, microwave, among others), and low temperatures are highly encouraged in order to attain a pollution free environment. Green-synthesized nanoparticles represent an innovative technique in the area of nanotechnology that is accomplished using plant extracts [1, 2], more suitable for a large-scale 'green synthesis' of the nanoparticles [3]. Nanoparticles are being considered as cluster of atoms between the ranges of 1to100 nm. Smaller the particle size has unique, chemical and physical properties and is very useful in biomedical science. Recent studies are focused towards synthesis of metals like, iron, copper, calcium, gold, palladium, zinc and silver nanoparticles using plant. Silver has been recognized its importance in chemistry, physics and biology due to its unique properties over the last few decades, synthesis and characterization of metal nanoparticles gained attention because of their peculiar properties compared to their bulk counterparts, having their high surface to volume ratio [4]. Sliver nanoparticles (SNPs) synthesized from medicinal plants has received, much attention in various biological activities like antibacterial [5] and antifungal [6].The reducing agents involved in the synthesis include various water soluble metabolites such as alkaloids, phenolic compounds, terpenoids, flavones, quinines, organic acids, polysaccharides, proteins and co-enzymes which are available in the plant extract [7].

Pterolobium hexapetalum (Caesalpiniaceae) is a medicinal herb (Fig.1) used by the Chenchu tribes of Nallamalai hills. Stem bark used for fever, cough, tooth ache, chest pain, dog bite (Rabies), vomits , heat boils; diahorrhoea, constipation and piles, bone fracture, jaundice, ulcer, skin infection, wound healing,; flowers against venereal diseases, skin infection [8]; fruit and seeds cure diarrhea , constipation , piles , cough and cold , treating ulcer [9]; leaves against delivery pains [10,11]. Stem bark decoction in case of whooping cough of infants and bark extraction dyspepsia in cattle [12]. *P.hexapetalum* is a characteristic dry deciduous straggling shrub on forest tree canopy with mass flowering known as "Bhoca" in the Nilgiris "Yerrachiki" in telugu, commonly known as Indian red wing. It is also a major source of nectar and pollen for honey bees which yield very sweet pleasant aroma honey [13]. *P.hexapetalum* leaf and stem bark alcohol , methanol, ethyl acetate , benzene and chloroform extracts reveals the presence of high quantities of alkaloids, flavonoids, phenols , glycosides, tannins, quinines and steroids. There is no reports of lignins, saponins and fixed oils. Effective antibacterial activity was observed on four selected bacterial strains with leaf and stem bark hot water and methanol extracts at 10mg/disc. MIC values on *Staphylococcus aureus* and *Bacillus subtilis* 0.312 mg. *Pseudomonas aeruginosa* 0.625 mg and Escherichia coli 1.25 mg [14].

Material and Methods

Medicinal plant material collection and Identification

Pterolobium hexapetalum was collected from Tirumala forest, during the months of July and December. The plant was authenticated by Prof. N. Yasodamma and voucher specimens BS 01, BS 02 were prepared as per the standard method [15] and deposited in the herbarium, Department of Botany.

Synthesis of *P.hexapetalum* Stem bark SNPs

5 gms of stem bark dry powder was used for the extraction with 100 ml of milli *q* water on boiling water bath for 1 hour. Filter the content with whatman No. 1 filter paper and stored at room temperature for green synthesis of SNPs. 5 ml of plant extract was taken in 250 ml conical flask, titrated with 50 ml of 1mM Ag(NO3)2 at 60-80°C with the help of magnetic stirrer. The contents were centrifuged at 10000 rpm for 20 minutes to avoid the presence of any biological impurities. Further, it is used for characterization and antimicrobial studies.[16].

Characterizations of *P.hexapetalum* stem bark extract SNPs

UV–Vis absorption spectrum of SNPs was measured by using Nanodrop 800. Zeta potential analysed by HORIBA SZ-100, Fourier-Transform Infra Red (FT-IR) spectra of synthesized SNPs were analyzed in the range between 4,000 to 500 cm-1 with an IRAFFINITY-1,IR by ATR method. Crystalline nature of metallic silver nanoparticles were examined using an X-ray diffractometer (XRD) from Bruker, D8 advance, Germany. XRD-6000 equipped with Cu Ka radiation source using Ni as filter at a setting of 40 kV/30 mA. Transmission electron microscopy (TEM) technique was used to visualize the morphology of the AgNps. The 200 kV ultra-high-resolution transmission electron microscope (FEI-TECNAI G2 20 TWIN).TEM Grid were prepared by placing a 5 µL AgNp Solution on Carbon- Coated Copper grids and drying under lamp. [17-22].

Antimicrobial studies of *P.hexapetalum* stem bark extract SNPs

The antimicrobial activity of green synthesized silver nanoparticles from *P.hexapetalum* stem bark extract was analyzed against two Gram positive bacterial strains like *Bacillus subtilis* (MTCC-441), *Staphylococcus aureus* (MTCC-731) and Two Gram negative bacterial strains like *Escherichia coli* (MTCC-443), *Klebsiella pneumonia* (MTCC-741), Disc diffusion method [23] was followed for testing antimicrobial activity against green synthesized SNPs and comparative studies were made with plant stem bark extract as a positive control, 1mM $Ag(NO3)_2$ as negative control and Streptomycin as the standard. Sterile discs of 7mm size were prepared from whatman No.1 filter paper and 20 µl of each extract was loaded on separate discs with the help of micro pipette and allowed to air dry for one hour in aseptic conditions. Freshly prepared nutrient agar medium substrate for bacterial culture was poured into sterile Petri plates and allowed 30 minutes for solidification. The plates were swabbed with microbial cultures and placed the previously prepared discs; the

experiment was carried out in triplicates. The plates were incubated at 37 °C for 24 to 48 h then the zone of inhibition was measured

Results

Significant characters of selected medicinal plant of *Pterolobium hexapetalum*

Pterolobium hexapetalum was collected from Tirumala forest. *Pterolobium hexapetalum* is a straggling prickly shrubs, thorns recurved .Leave 2-pinnnate, leaflets oblong-oblanceoalte, entire obtuse, flowers white, in axillary or terminal racemes. Sepals and petals 5 each. Stamens 10ovary sessile, ovule1, style subulate, stigma dilated .pods samaroid, oblong winged reddish [24].

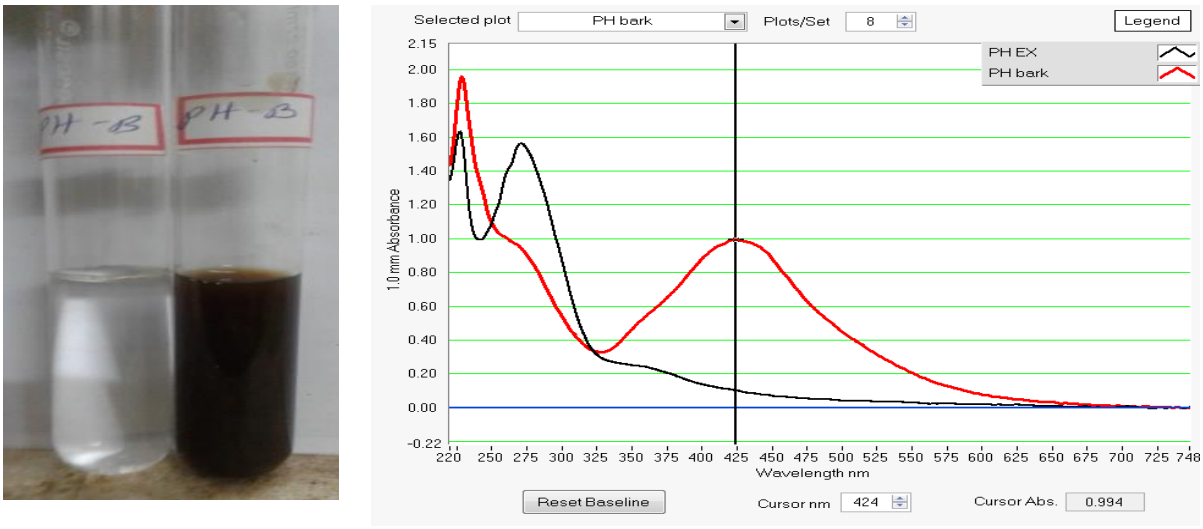
Habit Stem bark Bark inner side

Fig.1 ***Pterolobium hexapetalum***

Synthesis and Characterization of stem bark AgNPs of *P. hexapetalum* :

UV–visible spectral analysis:

The formation of *P.hexapetalum* stem bark extract silver nanoparticles was monitored by UV-VIS absorption spectra. The colour change from grey to Dark Brown is observed and a typical absorption peak obtained at 424 nm, it is due to surface Plasmon resonance of silver nanoparticles in the reaction Mixture (fig.2 a,b).

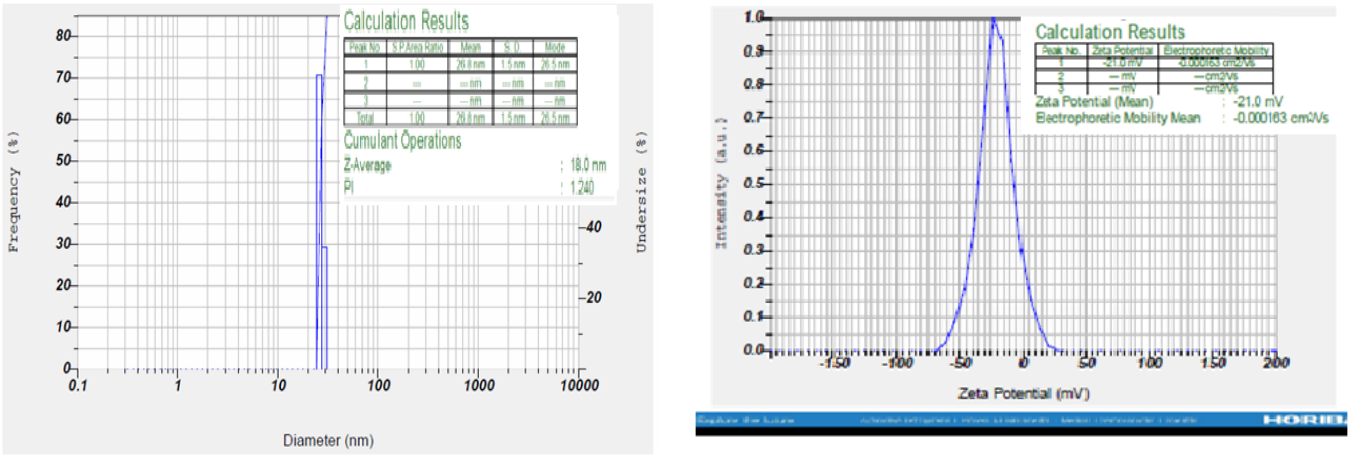


(a) (b)

Fig:2 (a) UV-VIS analysis of *P.hexapetalum* synthesized SNPs shows peak at 424 nm. (b) Colour change grey to dark brown.

Particle size and Zeta potential analysis of *P.hexapetalum* stem bark AgNPs:

The particle size of the biosynthesized *P.hexapetalum* stem bark AgNPs was detected by the intensity and laser diffraction method using the biosynthesized colloidal solution in which the AgNPs are polydispersed in mixture solution. The distribution of AgNPs are in the range of 10 nm to 100 nm in size with and the average size of synthesized AgNPs was found to be 26.6 nm (Fig.3: a&b) with and PI value of 1.240 (polydisperse index). Further the zeta potential analysis of AgNPs was detected to be -21.0 mV, due to its high negative zeta potential it prevent the AgNPs from agglomeration in the medium, leading to long term stability, because of the electrostatic repulsive between the AgNPs. Zeta potential is an essential parameter for the characterization of stability in aqueous nanosuspensions minimum of $\pm$ 30 mV Zeta potential values is required for indication of stable nanosuspension [25]. Zeta potential at-21.0mV, negitive value indicates the high stability of Nanoparticles. So, these results clearly indicated that the particles are fairly stable due to the electrostatic repulsion.



(a) (b)

Fig.3: (a) Particle size (b) Zeta potential of SNPs from Bark aqueous extract of *Pterolobium hexapetalum.*

Fourier Transform infra-Red (FTIR) analysis:

FTIR spectrum was analysed between the scan ranges from 4000 to 500.

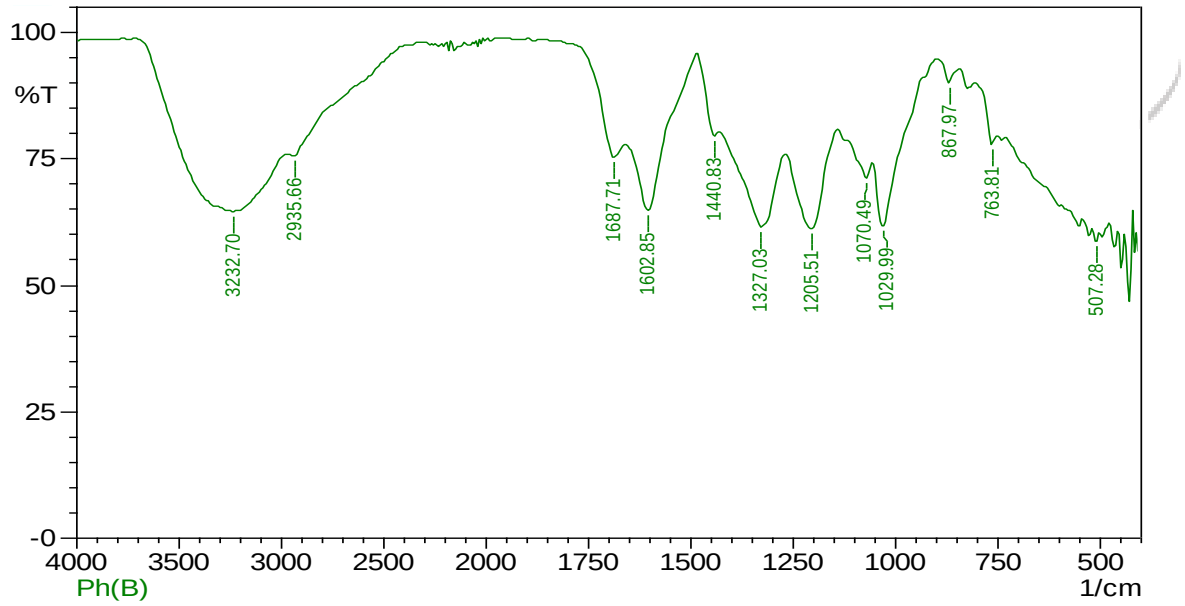


Fig.4 FTIR spectra of SNPs from stem bark extract of *Pterolobium hexapetalum.*

FTIR Spectrum results at 3232.63 cm^{-1} assigned for O-H (Stretch) bond of Alcohol/phenols; 2935.66 cm^{-1} for C-H (Stretch) bond of primary amines; 1687.71 cm^{-1} for -C=C- (Stretch) bond of alkenes ; 1602.85 cm^{-1} for N-H (Bend) bond of primary Amines; 1440.83 cm^{-1} for C-H (Bend) bond of alkanes; 1327.03 cm^{-1} for C-N (Stretch) bond of aromatic; 1205.51 and 1070.49 cm^{-1} for C-N (Stretch) bond of a aliphatic amines; 867.97 cm^{-1} for C-H (Oop) bond of aromatic; 763.81 cm^{-1} assigned for C-cl (Stretch) bond of alkyl halides; 507.28 cm^{-1} for C-H (Bend) bond of alkynes (fig.4). These FTIR studies suggested that the hydroxyl groups of

phenols and amide groups of proteins forming a layer to the nanoparticles and acting as capping agents to prevent agglomeration and providing stability to the medium.

XRD Analysis:

The nature of the nanoparticles synthesized from bark extract was analyzed by X-ray diffraction analysis. The XRD for plant derived SNPs of *P.hexapetalum*, an intensive peaks at 13.53; 23.93; 27.97; 29.57;32.40; 38.32; 44.24; 46.39; 64.68 and 77.46 of 2θ degrees of X-axis corresponds to 202, 021,110, 120, 012, 022, 032, 132, 221 and 143 Bragg Reflections of Y-axis (JCPDDS No: 84-1261) (fig 5). These Bragg reflections confirm that the nanoparticles are crystalline in nature.

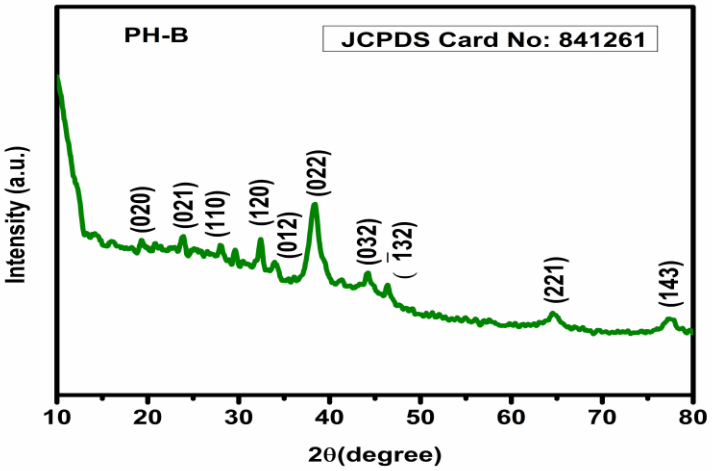
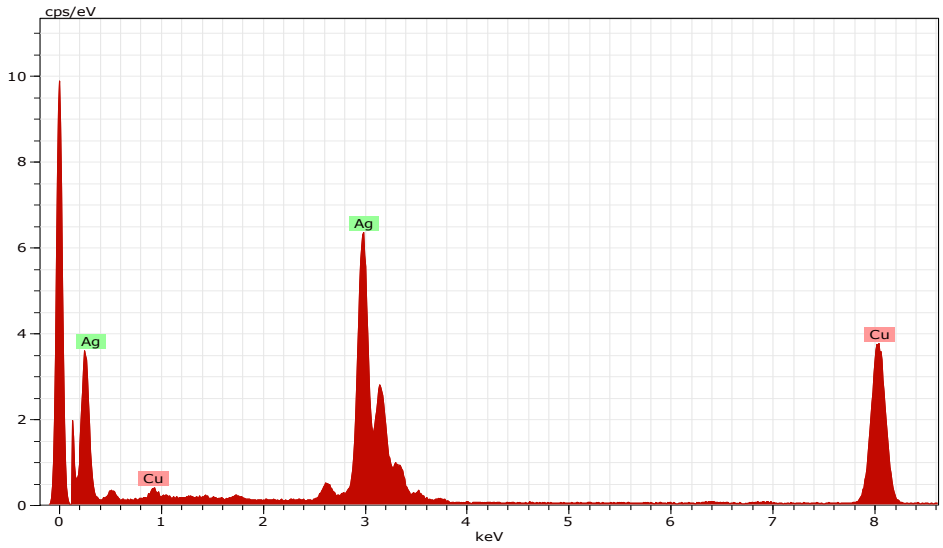


Fig 5: XRD pattern of SNPs from stem bark extract of *Pterolobium hexapetalum*

TEM with EDAX analysis: *P.hexapetalum* bark extract SNPs

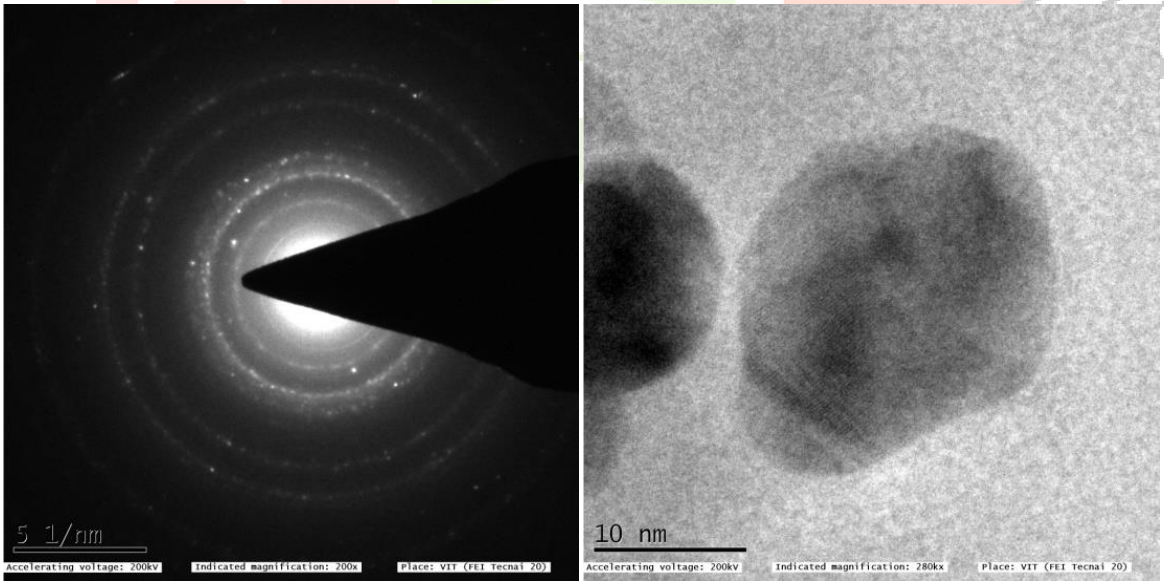
TEM with EDAX analysis provides further insight into the morphology and size of the nanoparticles along with presence of different metal concentrations in the sample. The EDAX spectra shows strong silver (67.32 %) absorption peak along with different elements with their weight percentage Copper (32.68 %) (fig.6) and the results indicated that the reaction product has high purity of SNPs .Presence of C, N and O in the sample analyzed by EDAX indicates proteins as a capping material towards these silver nanoparticles [26]. Higher resolution studies with TEM analysis, to know the size, morphology and agglomeration pattern of nanoparticles at 50 nm resolution studies of nanoparticles on TEM analysis reveals the nanoparticles are 2-24 nm in size owing spherical shape without any agglomeration observed between the particles (fig. 7).



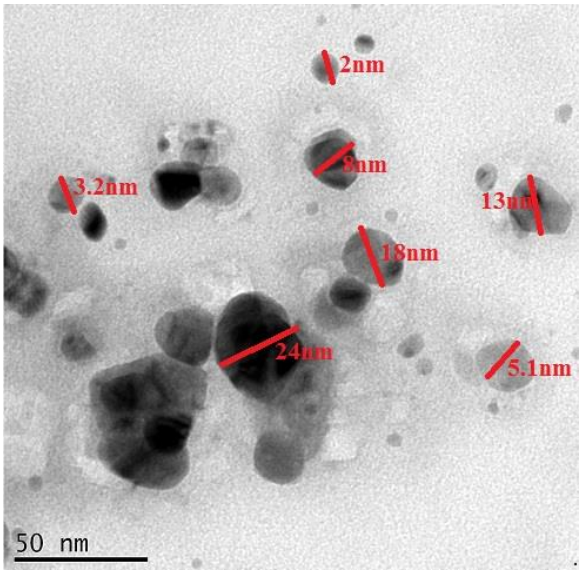
Spectrum: Spectrum 445-PhB

Element	Series	Net unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error (3 Sigma) [wt.%]
Copper	K-series	54316	32.68	45.18	3.05
Silver	L-series	94282	67.32	54.82	20.29
Total:			100.00	100.00	

Fig.6 EDAX pattern of SNPs from stem bark extract of *Pterolobium hexapetalum*



(a) (b)



(C)

Fig.7 (a) Selected area electron diffraction (SAED) of *P.hexapetalum* stem bark extract SNPs, (b) 10 nm of SNPs. (C) 50 nm resolution studies of SNPs shows mostly spherical shaped with 2-24 nm size.

From these microscopic studies with TEM analyses reveals these green synthesized silver nanoparticles from *Pterolobium hexapetalum* stem bark extract shows the size range from 2 to 24 nm. having spherical shape without any agglomeration between the particles

Antimicrobial activity of *P.hexapetalum* bark extract SNPs

These green synthesized silver nanoparticles were assessed for antimicrobial activities against two gram positive and Two gram negative bacterial strains. Among the bacteria the highest inhibition zones were observed on *Staphylococcus aureus* (17.00mm) followed by *Bacillus subtilis* (7.25mm) (Fig 8, 9 and Table 1).

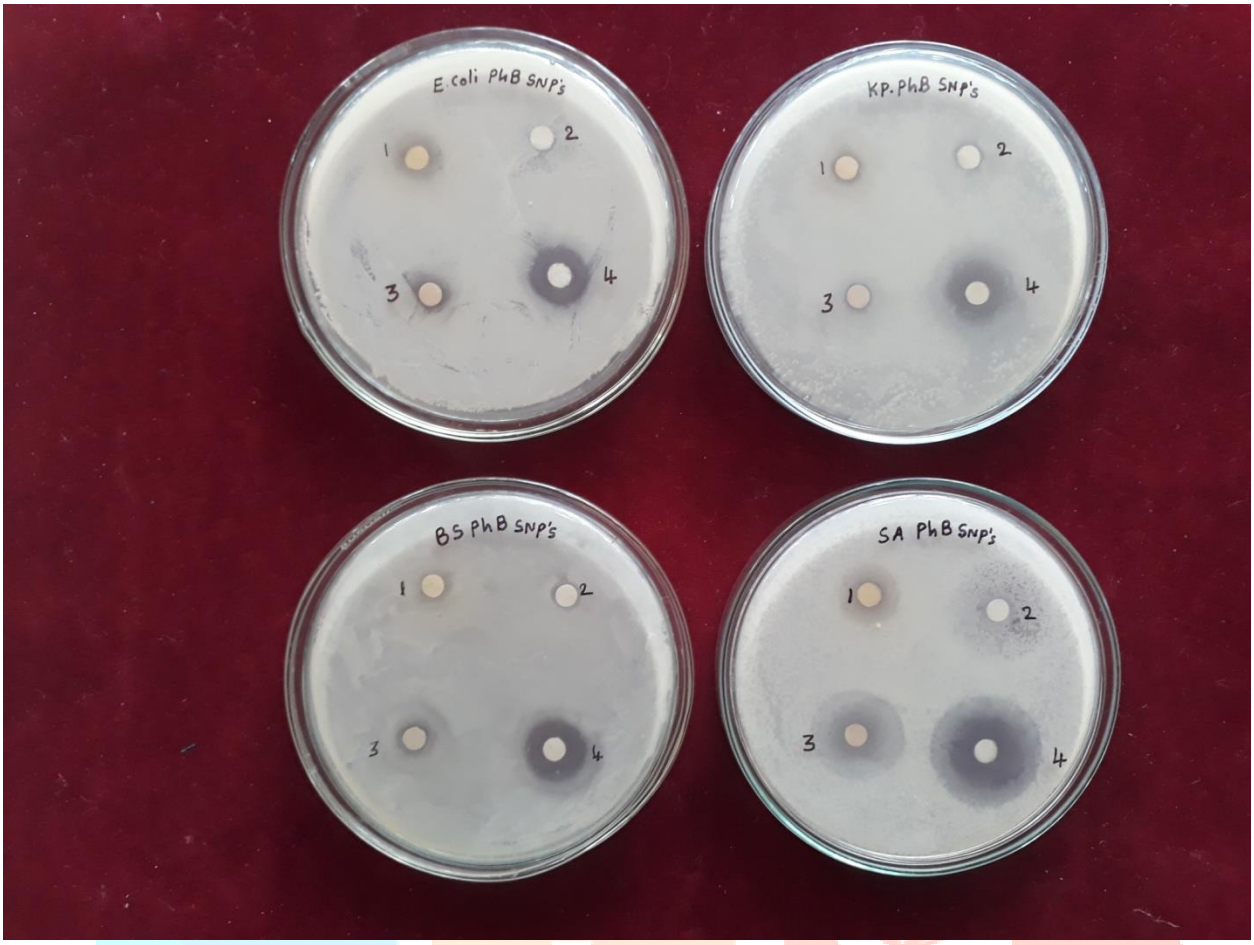


Fig: 8 Antimicrobial activity SNPs of *Pterolobium hexapetalum* bark extract

E.coli K.pneumonia, B.subtilis. S.aureus (1) Ag (NO_3) (2) Plant extract (3) SNPs (4) Streptomycin.

Table 1 Antibacterial Effect of different extracts and silver nanoparticles of *P.hexapetalum* bark extract

	Zone of inhibition(mm)			
Organism	**Plant Extracts**	**$Ag(NO_3)_2$**	**SNPs**	**Streptomycin**
E.Coli	9.75 ± 0.25	6.5 ± 0.29	7.25 ± 0.25	8.5 ± 0.29
KP	7 ± 0.41	6.5 ± 0.29	6.5 ± 0.29	18.25 ± 0.48
BS	6.5 ± 0.29	7 ± 0.41	7.25 ± 0.48	12 ± 0
SA	6.5 ± 0.29	6.75 ± 0.25	17 ± 0	24 ± 0

All the data are expressed as mean ±S EM: **p<0.01,* p<0.05 as compared to Control group, n=3: (One –way ANOVA followed by Dunnett's test)

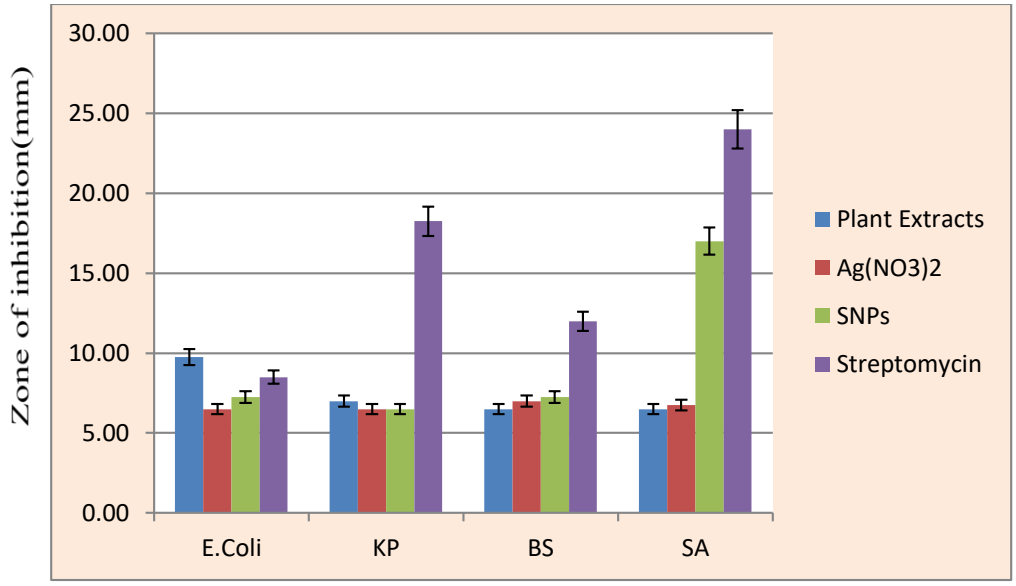


Fig 9 Antibacterial activity: Zone of inhibition of *P.hexapetalum* SNPs and different extracts of bark

Discussion

Antibacterial activity of leaf *P.hexapetalum* on *B.subtilis*, *S.aureus*, *P.aeruginosa* and *E.coli* at 10 mg /ml with hot water and methanol extracts proved more effective twice to that of the control drug Gentamycin at 10mg /ml followed by stem bark extracts equal to that of the control drug, as the diameter zone of inhibition 21 to 22mm against all selected bacterial strains with leaf extracts;13 to 15mm with stem bark extracts; 13-20 mm with drug control Gentamycin. MIC concentration ranges from 0.312 to 1.25 mg compared to Gentamycin 10mg against all bacterial strains [14]. Antifungal activity of *P.hexapetalum* leaf, fruit, flower, and stem bark aqueous, methanol and benzene extracts at 10mg/ml proved their effective inhibition on *A.niger* and *C.albicans* ranging with 30-35 mm zone of inhibition MIC ranging from 0.156 to 0.625mg compared to that of control drug Nystatin nearly double the activity. Antibacterial and anti fungal activities of *P.hexapetalum* were compared with earlier reports of the Caesalpiniaceae members like *Bauhinia*, *Cassia*, *Caesalpinia*, *Hardwickia*, *Peltophorum* and *Tamarindus*, *P.hexapetalum* antifungal activity at 10mg/ml was supported to that of the *Bauhinia purpurea* and *B.rufescens* bark methanol extracts at 100-150 mg/ml on *C.albicans* nearly with equal zone of inhibition and also 12-20 mg/ml of MIC values [27 &28]. *B. tomentosa* flower ethanol extracts at 5mg/ml on A.niger and C.albicans showed equally effective activity to that of *P.hexapetalum* flower extracts , MIC values ranges from 0.312 to 1.25 [29]. *Cassia alata* leaf methanol extracts and *C.fistiula* leaf ethanol and hydro alcoholic extracts on *A.clavatus* and on *C. albicans* showed remarkable inhibition at 20mg/ml and also on the other fungi [30, 31, 32]. *C.nigricans* leaf petroleum ether +ethyl acetate extracts in combination on *C.albicans* at 2-1MI^{-1} showed effective inhibition [33]. Similarly *C.bonducella* isolated compound on *C. albicans* at 200-600 mg/ml [34].

The silver nano particles derived from *Cassia auriculata* extract exhibited maximum inhibitory effects against the pathogen *S. boydii* (22 mm) and *S. dysentriae* (21mm). It also controlled the pathogens *K. vulgaris*,

S. aureus and *S. typhi* effectively (18 mm) [35]. *Tamarindus indica* Leaf AgNPs with 18 mm and 14 mm wider ZOIs on *E. coli* and *Staphylococcus aureus* respectively. Results proved the significant antibacterial effect of synthesized AgNPs on bacterial strains of both gram positive and negative group [36]. The synthesized AgNPs of *Cassia tora* showed against highest activity was observed on *Escherichia coli*.[37]. *Caesalpinia bonduc* AgNPs showed maximum antibacterial activity against *Pseudomonas aeruginosa* (120mm). Moderate activity was shown against *Salmonella typhi* (60mm), *Neisseria gonorrhea* (60mm), *Shigella dysenteriae* (50mm).The least activity was shown against *Vibrio cholerae* (35mm) and *Escherichia Coli* (40mm)[38].

Conclusions

The biosynthesized silver nanoparticles using ***Pterolobium hexapetalum*** stem bark aqueous extract proved excellent antimicrobial activity against *Staphylococcus aureus* with 17.00 mm diameter zone of inhibition which also proved with the presence of significant phytoconstituents in support of ethno medicinal uses and other biological activities proved as antibacterial, antipyretic and antifungal. Hence the biological approach appear to be cost efficient alternate to conventional physical and chemical method of silver nanoparticles synthesis and would be suitable for developing a biological process for large scale production .Theses silver nanoparticles may be used in effluent treatment process also for reducing the microbial load

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