



Green Synthesis, Characterization, and Antimicrobial Activity of Silver Nanoparticles with Tuber Extract of *Cyanotis tuberosa* (Roxb) Schult. and Schult F.

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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 tuber aqueous extract of *Cyanotis tuberosa*. The synthesized nanoparticles are characterized by the colour change, observed from gray to brown indicates the formation of nanoparticles and UV–VIS surface plasmon resonance spectroscopy observed at 427 nm further confirmed the synthesized nanoparticles as SNPs. FTIR spectroscopic studies confirm that phenols, amines and halides of tuber 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 30.94% Ag metal. Zeta potential at -18.6mV the negative value indicates the high stability of nanoparticles. TEM microscopic analysis revealed that the size of synthesized SNPs ranging from 16 to 35 nm with spherical shape. Further, the antibacterial studies of synthesized SNPs show high activity towards *Klebsiella pneumonia* with 21mm diameter zone of inhibition followed by staphylococcus aureus, *Escherichia coli* and *Bacillus subtilis*.

Keywords

Zeta potential *Klebsiella pneumonia* crystalline, phenols, amines, characterization.

INTRODUCTION:

Nanoparticles are being considered as cluster of atoms between the ranges of 1 to 100 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 [1]. Among the biological routes, plant and plant materials mediated nanoparticle synthesis is more advantageous than microbes and animal products. This is due to the presence of broad variability of

biomolecules in plants that act as capping and reducing agents which in turn increase the rate of reduction and stabilization of silver nanoparticles [2]. Hence, among the metal nanoparticles, silver nanoparticles (SNPs) synthesized from medicinal plants has received, much attention in various biological activities like antibacterial [3] and antifungal [4]. 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 [5].

The present selected medicinal plant:

Species *Cyanotis tuberosa* belongs to the family Commelinaceae is very common in Grasslands. Erect

or diffuse herb, 15-30 cm tall, branch lets softly villous, roots tuberous; Leaves 20-30×1-2cm, linear to broadly ensiform, silky-villous beneath. Flowers minute, bluish-purple in axillary and terminal corymbose cymes. Capsules 0.1-0.3 cm long ellipsoid, hairy up word. (Fig.1 a,b) [6]. Root part is used in the treatment of continued fevers and worm infestation in cattle (Fig: I. plant photo). [7,8] Root paste of two spoons is administered twice a day for 15 days to treat liver problem and menstrual disorder by the Santal tribe. [9] Tubers are used by the gond tribe of Adilabad (dt) A.P to relieve cough. [10] Aerial part of this herb is used by the tribal people in North Eastern India as leafy vegetable. [11] *C. tuberosa* (Arugam pillai) mixed with curd as inchrulet at kovilhills, Iddapadi of Salem Dt, (TN).



Fig 1. (a) *Cyanotis tuberosa* whole plant



(b) Tubers

Earlier Phytochemical studies Like Alkaloids; anthraquinones, tannins, glycosides, etc. have been detected in both leaf and tuber. Histochemical localization revealed the presence of tannins, alkaloids, glycosides, lignin, etc. in different tissue zones of root. In aerial part, moisture content, total ash and acid insoluble ash are of 11.70±0.17%, 14.72±0.53% and 97±0.017%, respectively. In root part, moisture content, total ash and acid insoluble ash are 9.08±0.33%, 15.79±0.25% and 6.56±0.22%, respectively. Extractive values of root part in different solvents like water, methanol, ethanol, and benzene and petroleum ether are 17.44±2.62%, 7.61±0.95%, 5.33±0.44%, 1.88±0.21% and 1.33±0.33%, respectively. The aerial part, extractive values in the above solvents are 7.04±0.36%, 5.83±0.16%, 5.33±0.16%, 2.5±0.50%, 1.66±0.66%. [12].

MATERIAL AND METHODS

Collection of Plant material and Synthesis of SNPs;

Cyanotis tuberosa was collected from Jaapali area of Tirumala, Chittoor District of Andhra Pradesh, India; Herbarium specimen was identified and deposited (Voucher No. RK:01) in the Department of Botany, Sri Venkateswara University, Tirupati as per standard method. [13] Tuber Powder of 5gm was extracted with 100 ml of milli q water kept on boiling water bath at 37°C 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 tuber extract was taken in to 250 ml conical flask, titrated with 50 ml of 1mM Ag (NO₃)₂ at 60-80°C stirred well 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 antibacterial studies. [14]

Characterization of silver nanoparticles

UV-Vis absorption spectrum of SNPs was measured by using Nanodropp 800. Zeta potential analysed by HORIBA SZ-100, Fourier-Transform Infra Red (FT-IR) spectra of synthesized SNPs were analyzed in the

range of 4,000 to 500 cm^{-1} with an IRAFFINITY-1, IR by ATR method. Crystalline nature of metallic silver nanoparticles was examined using an X-ray diffractometer (XRD) from Bruker, D8 advance, Germany. XRD-6000 equipped with Cu, K α 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 was prepared by placing 5 μL Ag Np solutions on Carbon- Coated Copper grids and drying under lamp. [15-16]

Antibacterial activity of SNPs

The antibacterial activity of green synthesized silver nanoparticles from tuber extract was analyzed against two Gram positive bacterial strains *Bacillus subtilis*(MTCC-441), *Staphylococcus aureus*(MTCC-731)and two Gram negative bacterial strains *Escherichia coli*(MTCC-443), *Klebsiella pneumonia*(MTCC-741), Disc diffusion method [17] was followed for testing antibacterial activity against green synthesized SNPs and comparative studies were made tuber aqueous extract as a positive control, 1mM AgNO $_3$ as negative control and *Streptomycin* as the standard.

Sterile discs of 7 mm size of whatman No.1 filter paper was leaded with 10 μl of each extract (plant and Ag (NO $_3$) $_2$) and SNPs with the help of micro pipette and allowed to air dry for one hour in aseptic conditions. Freshly prepared nutrient agar media 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 hr then the zone of inhibition was measured with scale in mm.

RESULTS AND DISCUSSION

Synthesis and characterization of Silver Nanoparticles:

The formation of 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 427nm, it is due to surface Plasmon resonance of silver nanoparticles in the reaction mixture (fig.2). The SNPs obtained posses a negative. Zeta potential is an essential parameter for the characterization of stability in aqueous nanosuspensions minimum of ± 30 mV values is required for indication of stable nanosuspension [18]. Zeta potential at-18.6mV (fig.3), indicates the high stability of Nanoparticles. So, these results clearly indicated that the particles are fairly stable due to the electrostatic repulsion. The FTIR spectrum was analysed between 4000 to 500 cm^{-1} .Here the broad peaks obtained at 3311 cm^{-1} assigned for O—H (Stretch) bond of phenols; 1635 cm^{-1} assigned for N—H (Bend) bond of primary amines and 553 cm^{-1} assigned for C-Br (Stretch) of alkyl halides (fig.4). Results also supported as found in *Sophora Interrupta* leaf aqueous extract mediated synthesis of silver nanoparticles [19].These FTIR studies shows at the hydroxyl groups of phenols, amides and alkyl halides groups of proteins forming a layer around the nanoparticles and acting as capping agents to prevent agglomeration and providing stability to the medium. The nature of the nanoparticles synthesized from tuber extract was analyzed by X-ray diffraction analysis. Observed an intensive peak at 38.64; 44.32; 65.22 and 77.24 of 2 θ degrees of X-axis corresponds to 111, 200, 220 and 311 Bragg Reflections of Y-axis (Fig 5). These Bragg reflections confirm that the nanoparticles are crystalline in nature.

UV-VIS ABSORPTION SPECTRA of green synthesized SNPs *Cyanotis tuberosa*

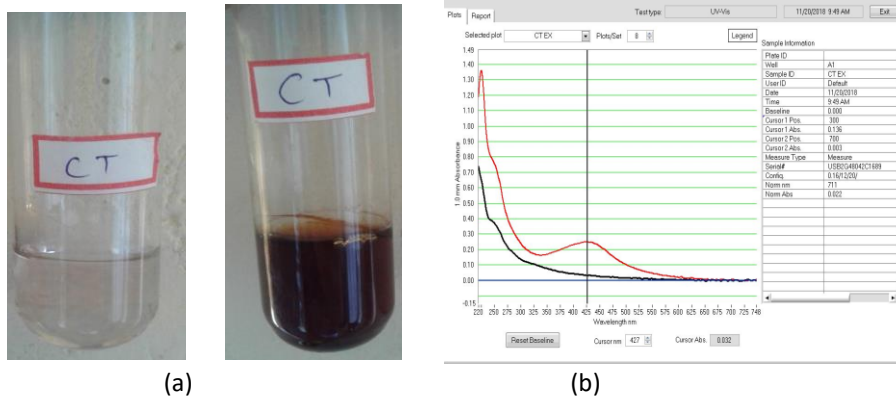


Fig.2 (a) Synthesized SNPs *Cyanotis tuberosa* mixture Colour change grey to dark brown (b) UV-VIS analysis of synthesized SNPs shows peak at 427 nm.

TEM with EDAX analysis at 50 nm resolution studies of green synthesized SNPs shows spherical shape, between 16-35 nm size and also reveals the presence of silver 30.94% with absorption peak along with Copper 28.18 % and Carbon 40.88 (fig.6) and the results indicated that the reaction product has high

purity of SNPs. TEM analysis, at 20 nm resolution reveals the nanoparticles are spherical shape without agglomeration observed between the particles (fig.7 a, b, c, d). Analysis reveals that the size range between 16-35 nm at 50 nm resolution.

Zeta Potential

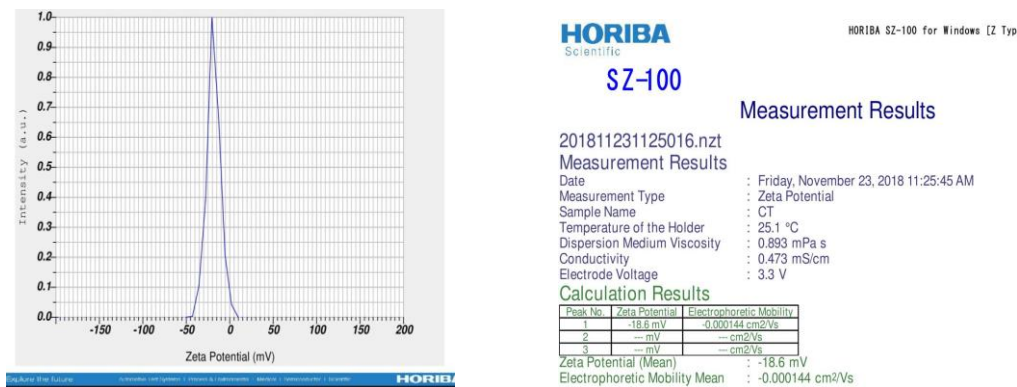


Fig.3 Zeta potential of green synthesized SNPs extract of *Cyanotis tuberosa*.

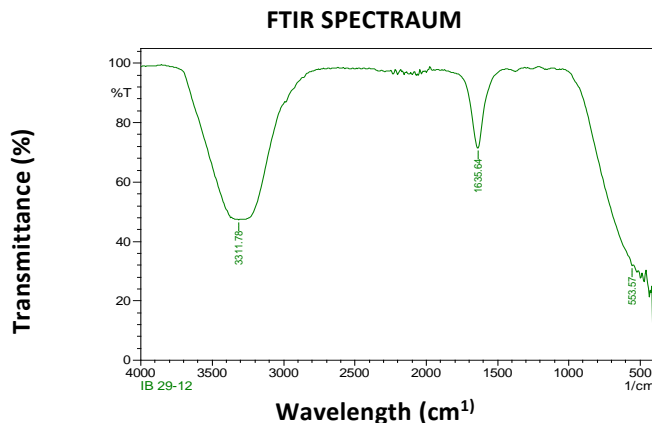


Fig.4 FTIR spectra of green synthesized SNPs from extract of *Cyanotis tuberosa*. (3311 cm⁻¹ assigned for O—H (Stretch) bond of phenols, 1635 cm⁻¹ assigned for N—H (Bend) bond of primary amines and 553 cm⁻¹ assigned for C-Br (Stretch) of alkyl halides.

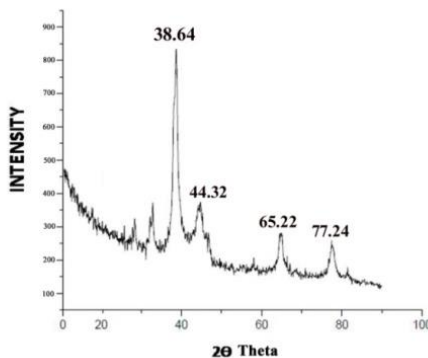
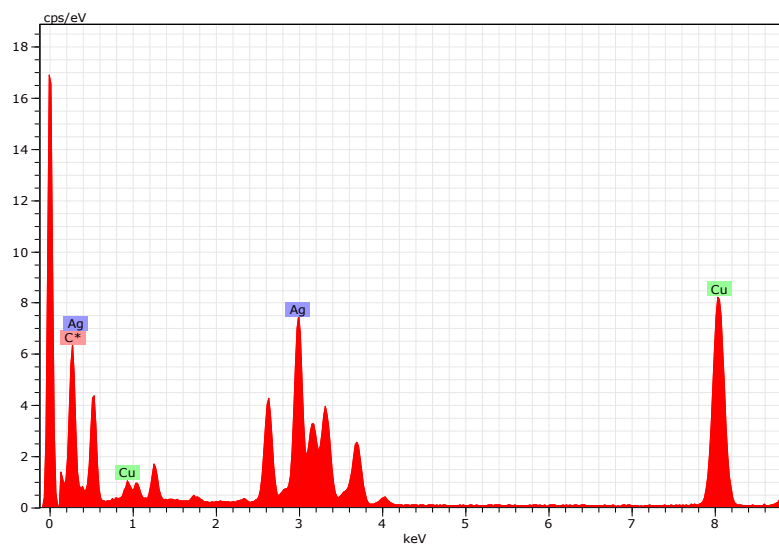


Fig.5 XRD pattern of green synthesized SNPs from extract of *Cyanotis tuberosa* (Intensive peak at 38.64; 44.32; 65.22 and 77.24 of 2θ degrees of X-axis corresponds to 111, 200, 220 and 311 Bragg Reflections of Y-axis.)



Spectrum: ct

Element Series	Net un.	C norm.	C Atom.	C Error (3 Sigma)	
	[wt.%]	[wt.%]	[at.%]	[wt.%]	
Carbon K-series	9597	40.88	40.88	82.33	3.96
Copper K-series	51924	28.18	28.18	10.73	2.64
Silver K-series	4846	30.94	30.94	6.94	3.22

Total: 100.00 100.00 100.00					

Fig 6 EDAX analyses of green Synthesized SNPs of *Cyanotis tuberosa* shows 30.94 weight percentage

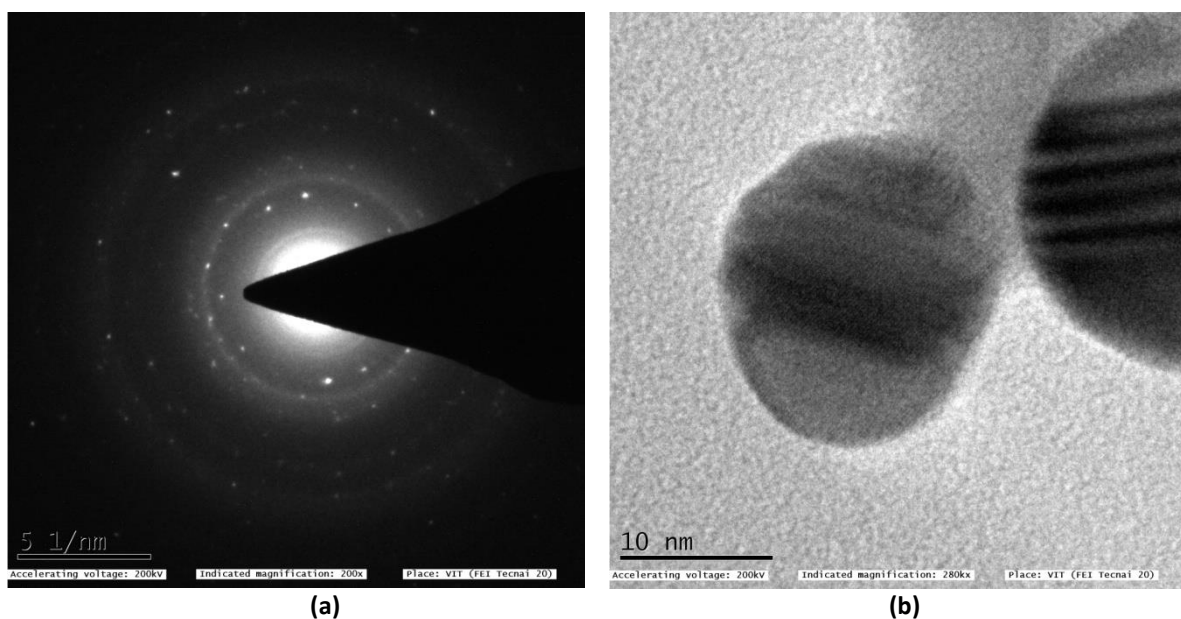
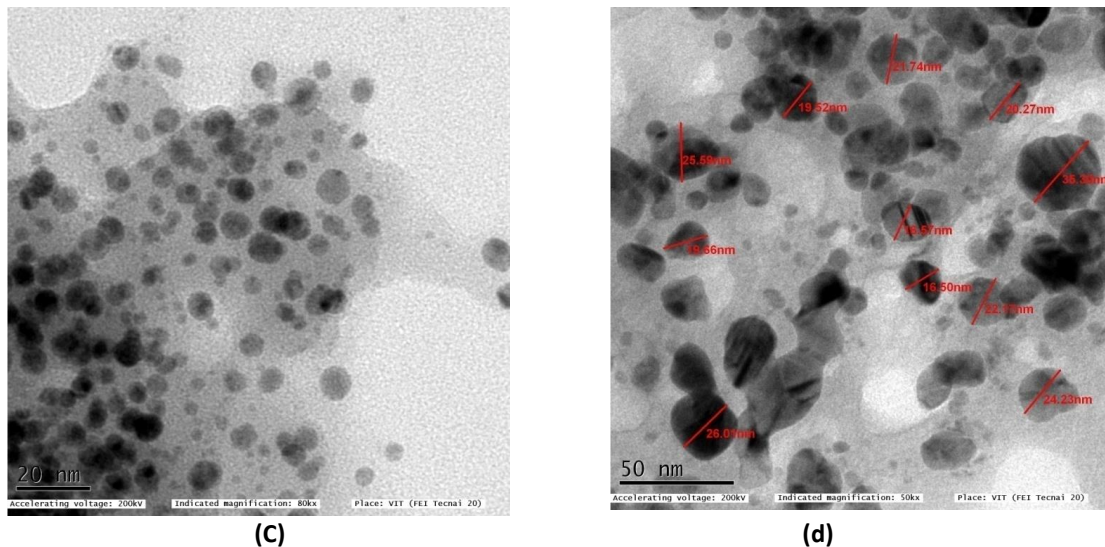


Fig 7 (a) Selected area electron diffraction (SAED) of green synthesized SNPs, (b) 10 nm resolution studies of green synthesized SNPs



(c) 20 nm resolution studies of green synthesized SNPs. (d) 50 nm resolution studies of green synthesized SNPs show mostly spherical shaped nanoparticles with 16-35 nm size

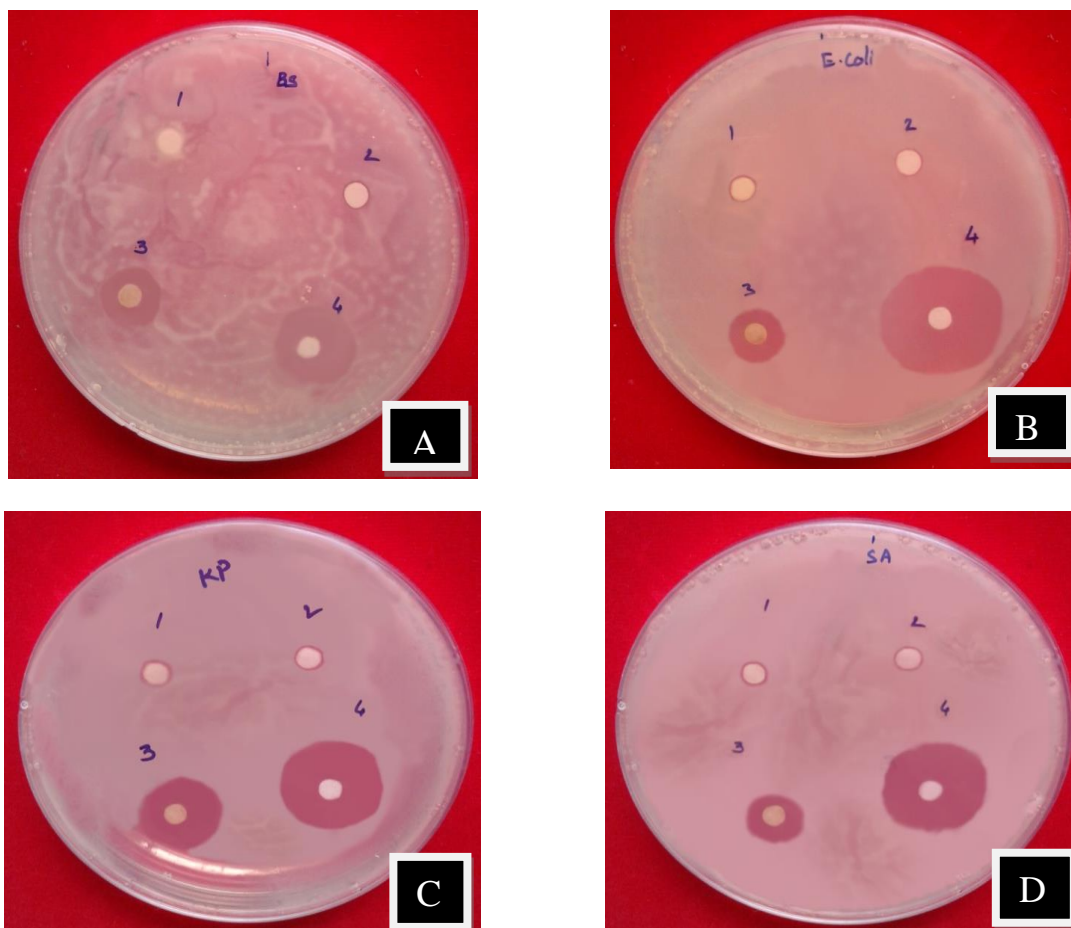


Fig.8 Antibacterial activity of Synthesized SNPs from tuber extract of *Cyanotis tuberosa* (A) *Bacillus subtilis*, (B) *Escherichia coli* (C) *Klebsiella pneumoniae* (D) *Staphylococcus aureus* (1) Plant extract (2) Ag (NO₃)₂ (3) SNPs (4) Streptomycin

Table 1 Effect of different extracts and green synthesized silver nanoparticles on clinically isolated bacterial Strains.

Extracts 10mg/Disc	Diameter Zone of Inhibition in mm			
	<i>B. subtilis</i>	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>S. aureus</i>
Plant Extract	7.3±0.32**	7.6±0.32**	7.2±0.32**	7.1±0.32**
Ag (NO ₃) ₂	8.2±0.32**	7.8±0.32**	8.2±0.32**	7.9±0.32**
SNPs	16.2±0.32**	15.8±0.57*	21±0.57*	17.2±0.32**
Control- Streptomycin	26.3±0.32	28.2±0.32	26.8±0.57	25.3±0.32

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)

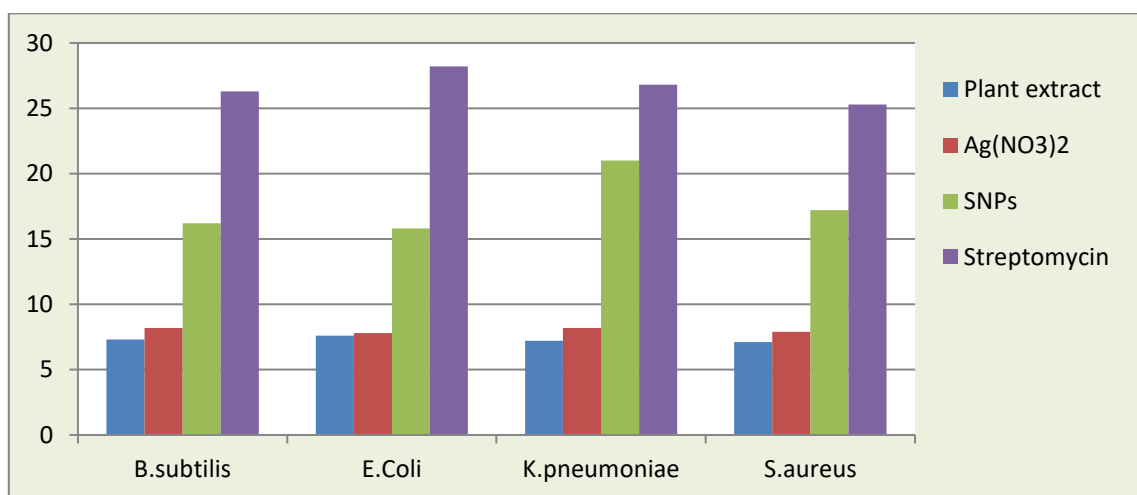


Fig 9 Zone of inhibition of different extracts of *Cyanotis tuberosa* on clinically isolated bacteria

Antimicrobial activity:

Silver nanoparticles of *Cyanotis tuberosa* were assessed for antibacterial activity against two gram positive and two-gram negative bacterial Strains shows the highest inhibition on *Klebsiella pneumoniae*, with 21mm diameter zone of inhibition (Fig. 8, 9 and Table 1). The SNPs shows less effect on gram positive. The gram-positive bacteria having thick layers of peptidoglycon (polypeptide proteins) when compared to the gram-negative and the penetration of SNPs through cell membrane is easy in case of gram-negative bacteria which are more susceptible. Least inhibition observed against *E. coli* with 15.8mm.

CONCLUSIONS

The present study showed simple rapid and economical synthesis of silver nanoparticles. Silver nanoparticles of *C. tuberosa* might have been used in various medicinal applications. Further the above silver nanoparticles revealed to possess an effective antibacterial property against *Klebsiella pneumoniae*. Earlier studies of *C. tuberosa* also support the presence of alkaloids, anthraquinones, tanins, glycosides, with high percentage of aqueous

extractive value in tuber support the effective activity of SNPs against *Klebsiella pneumonia* which may shows better therapeutic activity towards fevers and on worm infestations in support of herbal treatments.

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