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Studies on the Synthesis, Characterization and Antibacterial Properties of Green-Synthesized Silver Nanoparticles from Whole Plant Aqueous extracts of *Gynura lycopersicifolia* DC

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Abstract

Today green synthesis of silver nanoparticles (SNPs) from plants is an utmost emerging field in nanotechnology. In the present study, reported the synthesis of SNPs from aqueous whole plant extract of *Gynura lycopersicifolia*. These green-synthesized nanoparticles are characterized by colour change pattern, and the broad peak obtained at 445 nm, with UV–Vis surface Plasmon resonance studies confirm the synthesis of SNPs. FT-IR spectroscopic studies shows the presence of phenols and proteins mainly responsible for capping and stability of synthesized SNPs. Crystallographic studies from XRD indicates, the SNPs are crystalline in nature owing to 50 nm size. EDAX analysis shows 62.53% weight percentage of Ag metal in the sample indicates the purity of sample. And TEM microscopic study reveals the nanoparticles are spherical in shape with size ranging from 10 to 50 nm. Antibacterial studies of the synthesized SNPs on clinically isolated bacterial strains showed effective susceptibility towards *Klebsiella pneumonia* with 26.5 mm diameter zone of inhibition, at 40µl concentration. It indicates that whole plant extract of *Gynura lycopersicifolia* is suitable for synthesizing stable silver nanoparticles which act as excellent anthelmintic, antiseptic, against depressions and also as best antibacterial agent, may be due to the presence of phenolic compounds, flavonoids, glycosides and alkaloids.

Keywords

Gynura *lycopersicifolia*, Silver nanoparticles, Antibacterial activity, *Klebsiella pneumonia,* Plasmon resonance, crystatallographic.

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INTRODUCTION

Nano particles synthesis leads to the developing interest in biological approaches which are free from the use of toxic chemicals as by products. Thus, there is an increasing demand for "green nanotechnology. Biological approaches for both extracellular and intracellular nanoparticles synthesis have been reported till date using microorganisms including bacteria [1]. Many species of Asteraceae family reported synthesis of SNPs and their biological activities. Species *Eclipta alba* and *E.prostrata* shows the presence of rich flavonoid group as isoflavones nearly ten classes of secondary metabolites [2] reported biflavonyls isolation, identification and characterization [3-8] synthesis of metallic and oxide nanoparticles are carried out in *Eclipta* [9-10]. In

another Species *Tridax procumbens* phytochemicals like alkaloids, carotenoids, flavonoids (catechins and flavones) and tannins; also rich in sodium, potassium and calcium [11]. Plant extract used against bronchial catarrh, dysentery, diarrhoea, preventing hair loss, check haemorrhage for cuts [12-13].

Pharmacological studies against anti-inflammatory, hepatoprotective, wound healing, immunomodulatory, antimicrobial, antiseptic, hypotensive and cardiac effects [14-16]. *Vernonia cinerea* is recommended in the Thai traditional medicine and in other countries against smoking cessation and relief of asthma, cough, malaria, urinary calculi and against arthritis [17]. Active compounds of showed anti-inflammatory, analgesic, antipyretic, antioxidant and antibacterial [18-19]. *Xanthium strumarium* used against malarial fever, asthma, rheumatism, leprosy, migraine, smallpox and cancer with good number of phytoconstitutes [20].

The selected medicinal plant *Gynura lycopersicifolia* (Asteraceae) used for the synthesis of AgNps, is a slender, succulent, erect herb; leaves deeply, irregularly pinnatifid and glabrous, 6-8×10-13cm, with white hispid pubescent, auricled at base. Flowers on heads homogamous, 1.5×0. 5cm.light yellow disciform, receptacle flat; fruit Achenes, sparsely hispid between the ribs. (Fig-1. A & B). Grown near streams in moist forests on high hills, distributed in India and in Srilanka. Flowering and fruiting December-February [21] it is commonly called as Adavi Tametaaku Chettu. The leaves used for anthelmintic, antiseptic, and for fever. [22]. About 100 gm of leaf paste is made into pills of 5gm each is taken orally three times a day for patients suffering from post natal depression. [23].

Gynura lycopersicifolia

Fig1. A: Whole plant

Fig 1.B. Infloroscence

MATERIAL AND METHOD

Collection of Plant material and Synthesis of SNPs;

Gynura lycopersicifolia whole plant collected from Jaapali area of Tirumala, Chittoor District, Andhra Pradesh, India; Herbarium specimen was identified and deposited (Voucher No.KP:05) in the Department of Botany, Sri Venkateswara University, Tirupati.(24) Whole plant dry Powder of 5gm was extracted with 100 ml of milli *q* water kept on boiling water bath at 37^0C 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 contents 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. [25]

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 IR-AFFINITY-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, 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 was prepared by placing 5 µL, AgNp Solution on Carbon- Coated Copper grids and drying under lamp. [26-27]

Antibacterial activity of SNPs

The antibacterial activity of green synthesized silver nanoparticles from whole plant extract was analyzed against two Gram positive bacterial strains *Bacillus subtilis* (MTCC-441) may causes conjunctivitis and irridocheroiditis [28] *Staphylococcus aureus* (MTCC-731) Causes wound, infections, dermatitis, osteomycitis pneumonia and skin syndrome (SSSS) [29] and two Gram negative bacterial strains *Escherichia* coli (MTCC-443) Causes diarrhoea. Haemorrahage colitis, urinary tract infections, gasteroentitis, pyogenic infection [30] *Klebsiella pneumonia* (MTCC-741) causes Pneumoniae, thrombophlebitis, urinary tract infections, cholecystis, diarrhoea, skin infection meningitis, pyogenic [31] Pneumonia, thrombophlebites, Urinary tract infection, cholecystitis, diarrohea meningitis Pyogenic skin infections.

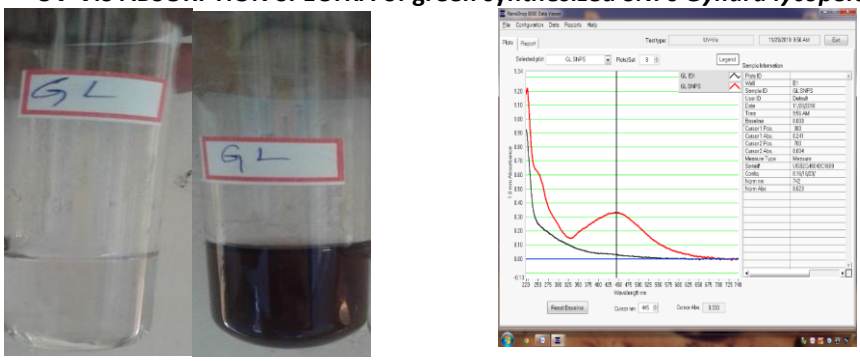
Disc diffusion method [32] was followed for testing antimicrobial activity against green synthesized SNPs and comparative studies were made with whole plant aqueous extract as a positive control, 1mM $AgNO_3$ as negative control and *Ciprofloxacin* as the standard. Sterile discs of 7 mm size were prepared from whatman No.1 filter paper and 10 µl of each extract (plant and Ag NO_3) different concentration of SNPs (10µl,20µl,40µl) 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 media for bacterial culture substrate was poured into sterile Petriplates 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 hr then the zone of inhibition was measured with scale in mm.

RESULTS AND DISCUSSION

Synthesis and characterization of Silver Nano particles:

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 445 nm, it is due to surface Plasmon resonance of silver nanoparticles in the reaction Mixture (Fig.2). The SNPs obtained posses a negative Zeta potential value (Fig.3). Zeta potential is an essential parameter for the characterization of stability in aqueous nanosuspensions. A minimum of ± 30 mV Zeta potential value is required for the indication of stable nanosuspension [33]. The SNPs *G.lycopersicifolia* Zeta potential at-22.4mV,negitive value indicates the high stability of nanoparticles, due to the electrostatic repulsion. Nanoparticles. FTIR spectrum was analysed between 4000 to 500 cm–1; the broad peaks obtained at 3280 cm–1 assigned for O-H (Stretch) bond of phenols; 1585 cm–1 assigned for C-C (Stretch) bond of aromatic; 1371C-H (Bend) bond of alkanes; 1093C-N (Stretch) bond of aliphatic, amines; 823 N-H (wag) and 517 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 [34].These FTIR studies suggested that the hydroxyl groups of phenols and amide 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 leaf extract was analyzed by X-ray diffraction analysis. An intensive peak at 38.22; 44.74; 65.58 and 77.38 of 2θ 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 *Gynura lycopersicifolia*.



(a) **(b)**

Fig.2 a Synthesized SNPs of *Gynura lycopersicifolia* mixture Colour change grey to dark brown (b) UV- VIS analysis of synthesized SNPs shows peak at 445 nm.

ZETA POTENTIAL ANALYSIS

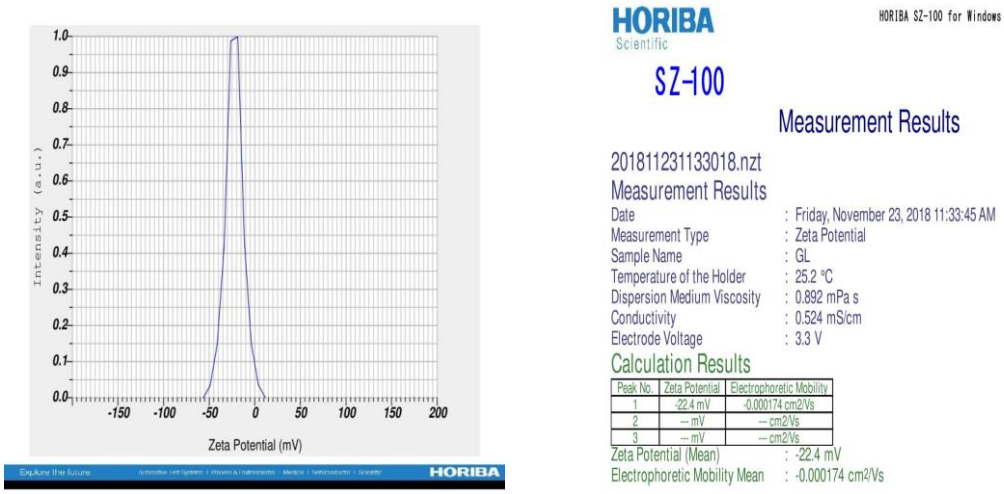
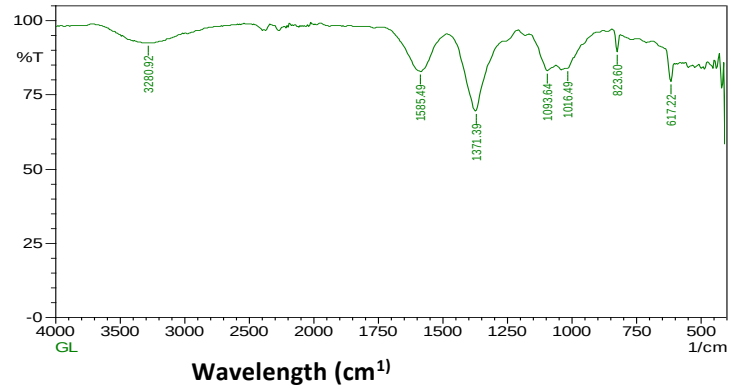


Fig.3 a Zeta potential of green synthesized SNPs extract of *Gynura lycopersicifolia.*

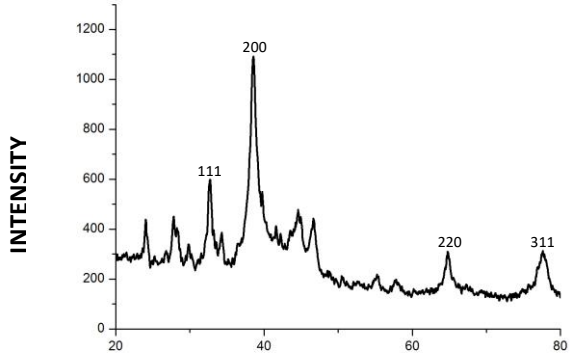
FTIR SPECTRAUM



Wavelength (cm$^{1)}$

Fig.4 FTIR spectra of green synthesized SNPs from extract of *Gynura lycopersicifolia.* (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.

X-RAY DIFFRACTION ANALYSIS



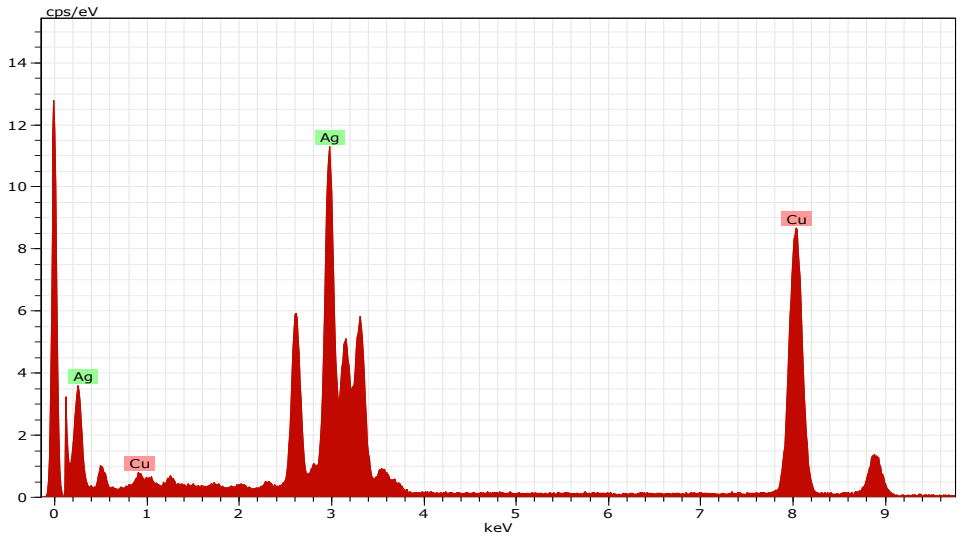
2 θ (Theta)

Fig.5 XRD pattern of green synthesized SNPs from extract of *Gynura lycopersicifolia.* (Intensive peak at 38.22; 44.74; 65.58 and 77.38 of 2θ degrees of X-axis corresponds to 111, 200, 220 and 311 Bragg Reflections of Y-axis.)

TEM with EDAX analysis provides further insight into the morphology and size of the nanoparticles along with the presence of different metal concentrations in the sample. 50 nm resolution studies of green synthesized SNPs show spherical shape with 11-20 nm size of nanoparticles. EDAX analysis was performed to know the percentage of Ag present in the sample. Spectra shows the presence of silver 62.53%;absorption peak along with different elements Copper 37.47 % (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 [35]. Higher resolution studies with TEM analysis, to know the size, morphology and agglomeration pattern at 20 nm resolution studies reveals the nanoparticles are 7-10 nm in size owing spherical shape without any agglomeration observed between the particles (Fig 7 a, b, c, d).

Antimicrobial activity:

These green synthesized silver nanoparticles of *Gynura lycopersicifolia* shows antibacterial activity with 26 mm diameter zone of inhibition on *Klebsiella pneumonia*. (Fig 8, 9 and Table: 1) followed by *B.subtilis* 19 mm; and on S.aureus and *E.coli* with 18 mm respectively at 40 µl concentration. It is observed very poor activity with plant extract and with Ag $(NO3)^2$. The control *Ciprofloxacin* shows 40 mm diameter zone of inhibition an average against all selected microbes. The SNPs shows less significant effect on Gram positive, may be due to the presence of thick layers of peptidoglycon (polypeptide proteins) when compared to the Gram-negative bacteria and the penetration of SNPs through cell membrane is easy which are more susceptible.

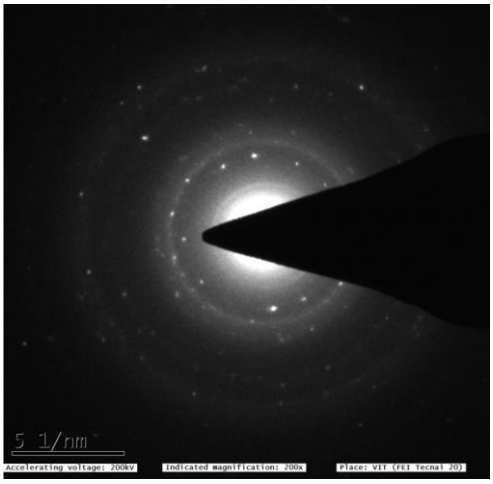


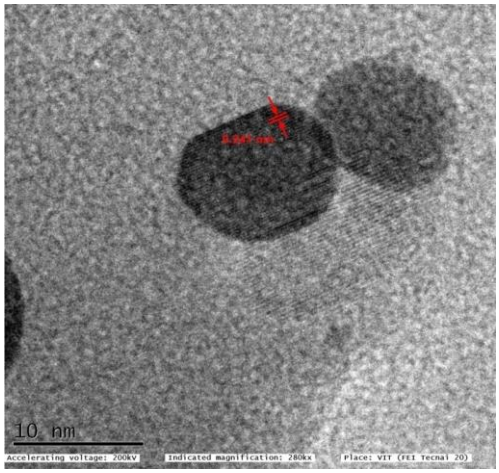
Energy (keV)

Spectrum: Spectrum 443- GL

Element Series	Net unn. C	norm. C [wt.%]	Atom. C [wt.%]	[at. %]	Error (3 Sigma) [wt.%]
Copper K-series	54662	37.47	37.47	50.43	3.49
Silver L-series	76867	62.53	62.53	49.57	18.85
Total:		100.00	100.00	100.00	

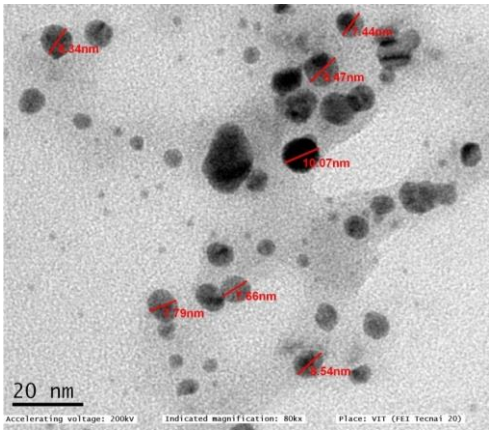
Fig 6 EDAX analyses of green Synthesized SNPs shows 62.53 weight percentages.

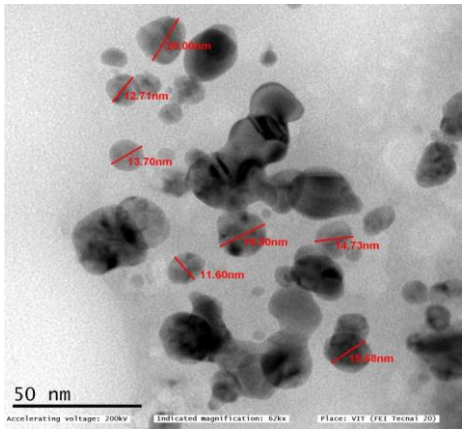




(a) **(b)**

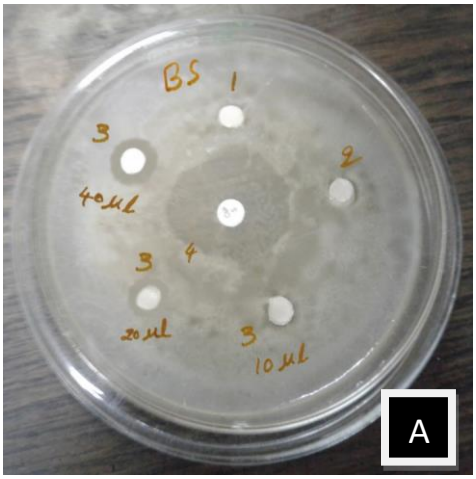
Fig 7 (a) Selected area electron diffraction (SAED) of green synthesized SNPs of *Gynura lycopersicifolia* (b) 10 nm resolution studies of green synthesized SNPs.

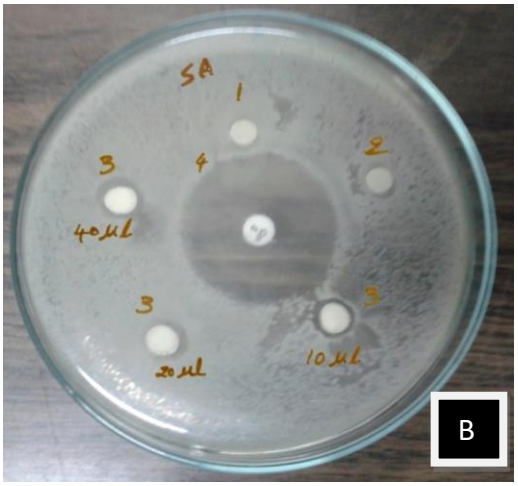




(c) **(d)**

(c) 20 nm resolution studies of green synthesized SNPs of *Gynura lycopersicifolia* shows spherical shaped nanoparticles with 7-10 nm (d) 50 nm resolution SNPs with 11-20 nm size.





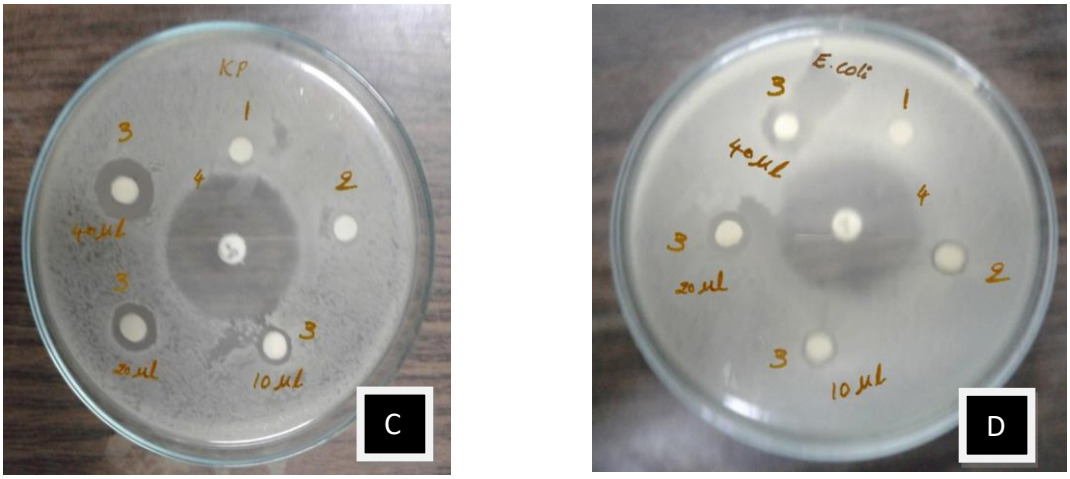


Fig.8 Antibacterial activity of Synthesized SNPs from whole plant extract of *Gynura lycopersicifolia.* (A) *Bacillus subtilis,* (B) *Staphylococcus aureus,* (C) *Klebsiella pneumoniae* (D) *Escherichia coli* (1) Plant extract (2) Ag $(NO_3)_2$ (3) SNPs (4) Ciprofloxacin.

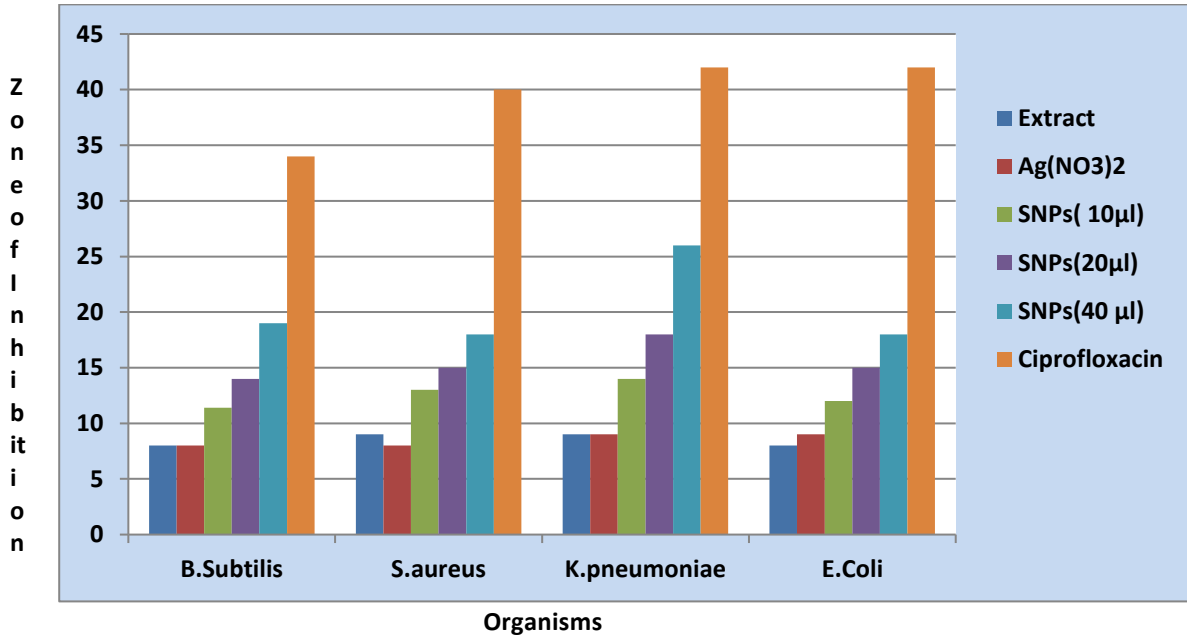


Fig 9 Zone of inhibition of different extracts of *Gynura lycopersicifolia* on clinically isolated bacteria

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

Extracts 10 µl /Disc	Diameter Zone of Inhibition in mm			
	B. subtilis	*S. aureus*	*K. pneumoniae*	*E. coli*
Plant Extract	8.0±0.32**	9.0±0.32**	9.0±0.32**	8.0±0.32**
Ag $(NO3)^2$ SNPs	8.0±0.32**	8.0±0.32**	9.0±0.32**	9.0±0.32**
10 µl	11.4±0.32**	13±0.57*	14±0.57*	12±0.32**
20 µl	14±0.32**	15±0.57*	18±0.57*	15±0.32**
40 µl	19±0.32**	18±0.57*	26±0.57*	18±0.32**
Control Ciprofloxacin	34.0±0.32	40.0±0.32	42.0±0.57	42.2±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)

All the results were compared with other Asteraceae members SNPs synthesis and their biological activities. *E. alba* silver surface plasma resonance was observed at 419 nm and the reaction Process takes below 6 hours; Then Nanocrystalline nature of nanoparticles almost spherical in shape with diameters range of 2-6 nm [36]. SNPs of *T. procumbens* shows peak between 410-430 nm, with a mean size ranges between 13.51-17.24 nm. FTIR results shows the presence of primary and secondary amines, alcohols, ethers, phenols, alkenes, aldehyde carboxylic acid and lactones; and the antifungal activity with leaf and stem extracts of TNPs (*T. procumbens)* at 50 ppm concentration antifungal inhibition on *Aspergillus niger* and on *A.flavus* Shows more effective with 85% and 60% respectively. Whereas antibacterial activity with leaf and stem TNP's inhibits at 30 ppm shows against *E. coli,* and at 40 ppm against *Vibrio cholera* by both food poisoning and agar disc diffusion method comparing with aqueous, methanol and ethanolic extracts of the plant leaf and stem and also with the standard drugs like *Tetracycline*, *Penicillin* and *Streptomycin* observed effective inhibition equally to that of *Tetracycline* with leaf and stem SNPs, showing the highest inhibition 30.08 mm diameter zone against *V. cholerae* and 17.5mm on *E.coli* [37].

Ag NPs of *V. cinerea* were synthesized between 30 to 60 min; exhibited absorption peak at 430nm. SNPs are stable for more than 6 months at room temperatures (29-30^{o}c); SEM analysis showed the particle size ranging 5 to 50 nm; *V.cinerea AgNps are associated* with more characteristic tannins. Antibacterial activity of *V.cinerea* AgNPs at 160 mg/ml concentration against isolated *xanthomonas comprestris pv .malvacearum* from cotton plant shows 13.00 mm diameter zone of inhibition at 80mg/ml of MIC values: compared to the control drug *Chloramphenical* exhibited 22.34 mm zone of inhibition. Whereas crude extracts of *V.cinerea* has not shown any action.[38].

AgNps synthesis of *X. strumarium* leaf extract at 450 nm of UV -VIS spectrum shows the peak for reduction. FTIR spectrum shows the presence of characteristic group of alkyl haloids, amines as bioactive compounds confirmed the presence of alkaloids, flavonoids and terpenoids as capping and stabilizing agents. SEM micrographs revealed the spherical shape of Ag NPs, EDAX analysis showed the NPs are more stable for a long duration. TEM images confirm the size between 20-50 nm XRD characterized the crystalline nature. Antibacterial activity by both disc-diffusion and Agar-well methods observed maximum inhibition at 60 ppm on *E. coli* between 14-17mm zone of inhibition, lowest on *Pseudomonas aeruginosa* between 9-10 mm zone of inhibition. [39].

The phytochemical studies of selected medicinal plant reported in the Percentage extraction of *G.lycopersicifolia* leaf showed highest in ethanol 9.6 % followed by Ethyl acetate 7.2% and in aqueous 6.4%; Qualitative phytochemical analysis of leaf resulted in the presence of Alkaloids, Proteins and Amino acids ,Phenolic compounds, Flavonoids and Saponins in Ethanolic extract ; Whereas Phenolic compounds are absent in Ethyl acetate extracts in addition to the above Glycosides are Present; in aqueous extract only Phenolic compound and Saponins are Present ;in common Phenolic compounds were observed.

Anthelmintic activity of leaf *G.lycopersicifolia* ethyl acetate and ethanolic extracts are equally effective in the time taken for paralysis of worms at 30mg/ml as 36.2 min and time taken for the death of worms 43.3 min when compare to Albendazole the standard as 9.0 min and 35.6min in the time for paralysis and death of the worms respectively: ethanol extracts also shows most equally as 35.6min for Paralysis and 45.0min for the death of worms. [40].

DISCUSSION

Phenolic compounds play on important role as chemo-preventive agents; inhibition of colon cancer, antiallergenic, anti-inflammatory, and antimicrobial and antioxidant Glycosides increase blood flow to the effused area, reduced thyroid function [41].Alkaloid; act as Anticancerous, reducing fever and Pain [42].most of the Alkaloids works on central nervous system [43].Glycosides; used in the treatment of congestive heart failure and cardiac arrthonia[44].Saponins; act as Spermicidal Cardiovascular Spasmolytic ,Expectorant, Antihistaminic, antitussive activity and fungicidal properties [45] Flavonoids; effective as Antibacterial, Anti-inflammatory, antiviral, antithrombic and vasodilators . [46].

CONCLUSIONS

The biosynthesized silver nanoparticles using G.*lycopersicifolia* whole plant extract proved excellent antimicrobial activity against *Klebsiella pneumonia* with 40 µl of concentration with 26 mm diameter zone of inhibition. Hence the biological approach appear to be cost efficient alternate to conventional physical and chemical method of silver nanoparticle synthesis and would be suitable for developing a biological process for large scale production .These silver nanoparticles may be used

in efficient treatment process for reducing the microbial load; Based on the presence of phytoconstituents supported its anthelmintic activity and antiseptic due to the Presence of Phenolic compounds, Glycosides, Alkaloids, Saponins and flavonoids. Nano particles synthesis of *G.lycopescifolia* was compared with other species of Asteracea like, *e.alba, T.procumbens , V.cinerea and X.strumarium* and noticed the smallest particle of size as 7-10 nm compare to that of *V.cinerea* as 5-50 nm; Antimicrobial activity on *K.pneumoniae* was reported effectively only in this species, than other species reported earlier.

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REFERENCES

1. Nair B and Pradeep T "Coalescence of nano clusters and the formation of sub-micron crystallites assisted by *Lactobacillus* strains," *Crystal Growth and Design*, vol. 2, no. 4, pp. 293–298, 2002.
2. Harbome JB. Phytochemical Methods. London: Chapman and Hall; 52.1973.
3. Abdel-Kader MS, Bahler BD. *DNA*-Damaging steroidal alkaloids from *Eclipta alba* from Surinam rainforest. J Nat Prod.; 61:1202–1208, 1998.
4. Mabry TJ, Markham KR, Thomas MB. The systematic identification of flavonoids. New York: Springer-Verlag Publication; 215–217, 1970.
5. Harborne JB. Functions of flavonoids in plants. In: Goodwin TW, editor. Chemistry and Biochemistry of Plant Pigments, Vol. I. *New York: Academic Press*; 736–778, 1976.
6. Pathak M, Sahai RP, Pathak R, Jha AK, Pandey RK, Chakravarty A, Kumar V, Sahay LK. Chemical investigation of *Eclipta alba* (family Asteraceae)*:* a study of flavonoids. J Haematol Ecotoxicol.; 2:41–44, 2007.
7. Kumari S, Govindaswamy S, Sukumar E. Lipid lowering activity of *Eclipta prostrsta* in extensive hyperlipidemia. J Ethanopharmacol. 105:332–335, 2006.
8. Singh B, Saxena AK, Chandan BK, Agarwal SG. In-vivo hepatoprotective activity of active fraction from ethanolic extract of *Eclipta alba leaves.* Ind J Physiol Pharmacol.; 45:435– 441. 2001.
9. Chandran SP, Choudhary M, Pasricha R, Ahmad A, Sastry M. Synthesis of gold nano triangles and silver nanoparticles using *Aloe vera* plant extract. *Biotechnol Prog*.; 22:577–583, 2006.
10. Shiv Shankar S, Rai A, Ahmad A, Sastry M. *Rapid synthesis of Au, Ag and bimetallic Au core-Ag shell nanoparticles using Neem (Azadirachta indica) leaf broth. J Colloid Interface Sci*. 275:496–502, 2004.
11. Ikewuchi C, Jude C, Ikewuchi Catherine M & Igboh Ngozi, Chemical Profile of *Tridax procumbens* Linn. *PakNut*,8(5) 548 2009.
12. Saraf S, Pathak A and Dixit V K, Hair growth promoting activity of *Tridax procumbens*, *Fitoterapia*, 62 495, 1991.
13. Taddei A and Rosas-Romero A J, *Bioactivity studies of extracts from Tridax procumbens*, *Phytomedicine*, 7235; 2000.
14. Tiwari U, Rastogi B, Singh P, Saraef D K and Vays S P, Immunomodulatory effects of aqueous extract of *Tridax procumbens* in experimental animals, *Ethnopharmacol*, 92 113, 2004.
15. Salahdeen H M, Yemitan O K and Alada A R A, Effect of aqueous leaf extract of *Tridax procumbens* on blood pressure and heart rate in rats, *African J Biomed Res,* 7 27 2004.
16. Ravikumar V, Shivashangari K S and Devaki T, Hepatoprotective activity of *Tridax procumbens* against d-galactosamine/lipopolysaccharide-induced hepatitis in rats, *Ethnopharmacol*, 101 55, 2005.
17. Ajitha B Reddy Y.A.K P.S. Reddy P.S Biogenic nano-scale silver particles *by Tephrosia purpurea* leaf extract and their inborn antimicrobial activity. *Spectrochim. Acta Part A Mol*. Biomol. Spectrosc. 128, 257–262, 2014.
18. T.N. Mishra, R.S. Singh, J. Upadhyay, R. Srivastava, J. Chemical Constituents of *Vernonia cinerea*, Part I. Isolation and Spectral Studies of TriterpenesNat. Prod. 47, 368–372 1984.
19. U.K. Mazumder, M. Gupta, L. Manikandan, P.K. Bhattacharya, P.K. Haldar, S. Roy, Phytomedicine 10, 185–188 2003.
20. YadavSS, Ganie S A, Gulia S S and Yadav N Phytochemical constituents and ethno pharmacological properties of *Ageratum conyzoides* L.Int. *J. Phytomed.* 6 471, 2014.
21. Rangacharyulu, D.1991.Floristic studies of Chittoor district, Ph.D thesis, S V. University, Tirupati.
22. Madhava chetty K, Sivaji K, Tulasi Rao K. Flowering Plants of Chittoor District Andhra Pradesh, India. 2015.
23. Sudarsanam G. Penchal Pratap G. Nagaraju V. Ethnobotany of Kuppam Chittoor District Andhra Pradesh, India, 2019.
24. Jain S.K., Rao R.R. A Handbook of Field and Herbarium Today and Tomorrow Printers and Publishers, New Delhi, 1997.
25. Shankar SS, Rai A, Ahmad A, Sastry M Rapid synthesis of Au, Ag, and bimetallic Au core Ag shell nanoparticles using Neem *(Azadirachta indica)* leaf broth Colloid Interface Sci 275:496–502.2004.
26. P. Mulvaney, Langmuir 12 788. 1996.
27. Chandran SP, Chaudhary M, Pasricha R, Ahmad A, Sastry M Synthesis of gold nanotriangles and silver nanoparticles using *Aloe vera* plant extract. Biotechnol Prog 22:577–583. 2006.
28. Green wood,D. Slack,R ,and Pantherer ,J. Medical microbiology- A guide to microbial infection .In

Pathogenesis immunity,Laboratory Diognosis and control.15 th edn.Addison Wesley Longman Chinna Ltd.1998.
29. Curran. J.P., Al-salihi, F. L Neonatal Staphylococcal Scalded skin syndrome: massive outbreak due to an unusual phage type.J. Pediatrics66 (2). P: 285-2901980.
30. Hudault, S.,Guignot,Servin,A. *Escherichia coli* strains colonising the gastro intestinal tract protect germ free mice against *Salmonella typhimurium* infection .J.Gut 49(1).P:47-55 2001.
31. Riley, R. R., J. W. Savell, C. E. Murphey, G. C. Smith, D. M. Stiffler, and H. R. Cross. a. Effects of electrical stimulation, subcutaneous fat thickness and masculinity traits on palatability of beef from young bull carcasses. J. Anim. Sci. 56:584, 1983.
32. Cruickshank R. Medical microbiology: a guide to diagnosis and control of infection. Livingston publishers, Edinburghand London, 1986.
33. Jacobs, C.; Müller, R.H. Production and characterization of a budesonide nanosuspension for pulmonary administration. *Pharm. Res.*, *19*,189–194 2002.
34. Khaja peer Mulla and Yasodamma Nimmanapalli Green synthesis of silver Nanoparticles and antimicrobial studies in Leaf aqueous extract of *Sophora interrupta* Bedd. International journal of Pharmacy and Biological Sciences.8: 954-961, 2018.
35. Jain N Bhargava a Majumdar S et al., Extracellular biosynthesis and characterization of silver nanoparticles using *Aspergillus flavus* NJP08: A mechanism perspective. *Nanoscale*.3:635-641. 2011.
36. Anal K.Jha, Kamlesh Prasad. Biosynthesis of Silver Nanoparticles using *Eclipta* Leaf AIChE: 1476–1479, 2009.
37. Himakshi Bhati Kushwaha and CP Malik. Biosynthesis of Silver Nanoparticles using fresh extract of *Tridax Procumbens* Linn IJEB: 359-368, 2014.
38. Sahayaraj K, Roobadevi M Rajesh,S, S Aziz.Vernonia cinerea(L.) Less.*silver nanocomposite and antibacterial activity against a cotton pathogen* RCI 41:5495-5507, 2015.
39. Jitendra Mittal, Rohit jain and Madanmohan sharma, Phyto *fabrication of Silver nanoparticles using aqueous leaf extract of Xanthium Strumarium L. their bacterial efficacy* Adv. Nat. Sci.; Nanosci. Nanotechnol. 8 025011 ,2017.
40. Pulipaka Shankaraiah, Anasuri Santhosh Phytochemical investigation and *in-vitro* anthelmintic activity of the leaves of *Gynura lycopersicifolia* DC. 6:125:969-978. 2017.
41. Gurucharan Singh Plant systematics theory and Practice. Published by raju primalans for oxford &IBH Publishing Co.Pvt, 66 Janpath, New delhi.P:181 183, 2000.
42. Irfan khan A. and Atiya Khanum.Role of biotechnology in medicinal and aromatic plants. Ukaaz Publications 16/11-511/D408. Shalivashana nagar Moosarambagh, Hyderabad.1998.
43. Fransworth, N.R., Biological and phytochemical screening of Plant. Journal Pharmaceutical sciences. Vol 55.P: 225 -276.1985.
44. Desai,U.R. Cardiac glycosides. Virginia Commonwealth University, School of Pharmacy, Retrived,2000.
45. Nigam, S.K. and Misra,G. Phytochemistry: basic Principle in relation to Ethnobotany .In S.K Jain (Ed), Methods and Approaches in Ethnobotany. Sociaty of Ethnobotanist, Lucknow (India). P;125-140.1989.
46. Tripathi, V.D.and Rastogi,R.P. Flavonoids in Biology and medicine.*J.sci.Ind.Res* 40.P116-124.1981