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Green Synthesis of Silver Nanoparticles and Antibacterial Studies with Leaf Aqueous Extract of *Pittosporum napaulense* (DC.) Rehder & E.H Wilson.

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Abstract

In the present study we report the green synthesis of stable silver nanoparticles (SNPs) from aqueous leaf extract of *P. napaulense*. The colour change from grey to brown is observed upon synthesis at 428 nm peaks obtained from UV-VIS spectroscopic analysis. Zeta potential at -13.2 mV, negative value indicates the high stability of nanoparticles. FTIR spectroscopic studies confirms that phenols and proteins of leaf extract is main responsible for capping and stabilization of these SNPs. Crystallographic studies from XRD indicated the SNPs are crystalline in nature. High resolution and magnification studies with TEM analysis revealed that the nanoparticles are spherical in shape having the size range from 8 to 18 nm. EDAX pattern of synthesized SNPs showed 78.86 weight percentage of Ag metal in the sample indicates the purity of sample. Further, the antimicrobial studies of SNPs show high toxicity towards different bacterial isolates as *Bacillus subtilis* with 18.5 mm diameter zone of inhibition followed by *staphylococcus aureus, Escherichia coli* and *Klebsiella pneumonia*. The results revealed that the selected medicinal plant possess the potentiality towards the synthesis of narrow range nanoparticles also combat with the pathogens.

Keywords

UV-Vis Spectroscopic, Zeta potential, Synthesized Nanoparticles, Crystallographic.

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INTRODUCTION

Nanotechnology is one of the most fascinating research areas in modern material science. Nanoparticles are gaining importance in the fields of biology, medicine and electronics owing to their unique physical and biological properties [1]. Recent studies are focused on synthesis of nanoparticles using plant materials like calcium, iron, zinc, palladium, gold, and silver. Silver nanoparticles (AgNPs) are one of the most vital nanomaterials among several metallic nanoparticles that are involved in biomedical applications [2]. Research based on advanced nanomaterials of noble metals like silver has conquered a lot of interest among scientists during the past decades for its physiochemical properties such as size, distribution and morphology, they have been studied for catalytic activity, optical properties, electronic properties, antibacterial properties and magnetic properties [3], [4], [5] and its application in various fields such as biomaterial production, biochemistry, medical and pharmaceutical products, toothpastes, optical receptors, bio sensing, etc. [6], [7] Nanoparticles of noble metals, such as gold, silver and platinum are broadly applied in many fields and also directly come in contact with the human body, such as shampoos, soaps, detergents, shoes, cosmetic products, and

tooth paste, besides medical and pharmaceutical applications [8].

The selected medicinal plant *Pittosporum napaulense* (Pittosporaceae) (Fig.1) is called 'Rakamuki' (Telugu),'Kattu sampangi', Najundai', 'Tammata' (Tamil) ,Tumari','Vikharl' 'Vekhali' (Marathi)**.** The plant parts are used against skin diseases, piles and itches**.** Bark is aromatic, bitter and greenish black with resinous oil glands. In Ayurveda bark in high doses acts as narcotic used as antidote to snake poison, general weakness and as a stimulant**.**

(a) Habitat

(b) Leaf

Fig.1 *Pittosporum napaulense*

The narcotic action of the bark is due to the presence of yellow oleoresins, and also contains saponins and Pittosporins **[9-13]**. Bark is bitter and aromatic; possess narcotic properties used as febrifuge, chronic bronchitis which acts as good expectorant. Oil used for rheumatism, skin diseases, sprains, leprosy, bruises, sciatica, chest infections, opthalmia, cutaneous diseases, secondary syphilis and chronic rheumatism, supports the presence of glycosides **[14]**. In New Zealand Mori people used the gum, leaves, flowers and oils of *P. eugenoids* to anoint their bodies. Flowers, roots, bark and leaves are used as anti-inflammatory, antiseptic and in rheumatic disorders. Bark consists of oleoresins, triterpenoids, saponins, stigmasterols **[15-18].**

MATERIAL AND METHODS

Plant material collection and identification

Pittosporum napaulense was collected from Tirumala forest, during the months of July and December. The plant was authenticated by Prof. N.Yasodamma and voucher specimens AU 01, AU 02 were prepared as per the standard method **[19]** and deposited in the herbarium, Department of Botany.

Synthesis of SNPs

Dry powder 5 gms of the plant material was extracted 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 $Ag(NO_3)^2$ 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, the synthesized nanoparticles were used for characterization and antimicrobial studies.

Characterization of Silver Nanoparticles (SNPs)

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 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.

Antimicrobial studies of SNPs

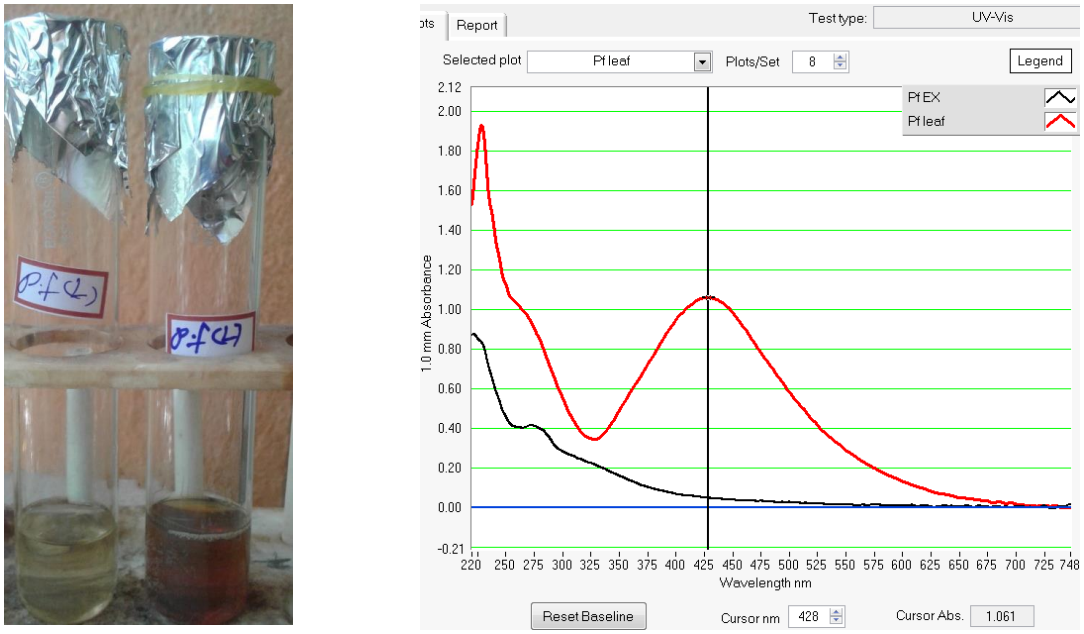
The antimicrobial activity of green synthesized silver nanoparticles from leaf extract was analyzed against two Gram positive bacterial strains like *Bacillus subtilis*, *Staphylococcus aureus* and Two Gram negative bacterial strains like *Escherichia coli*, and *Klebsiella pneumonia* using Disc diffusion method

[20]. Comparative studies were made with plant leaf extract as a positive control, 1mM Ag $(NO_3)^2$ 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 under 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 hrs then the zone of inhibition was measured.

RESULTS AND DISCUSSION

UV–visible spectral analysis:

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 428 nm, it is due to surface Plasmon resonance of silver nanoparticles in the reaction Mixture (fig.2 a,b).

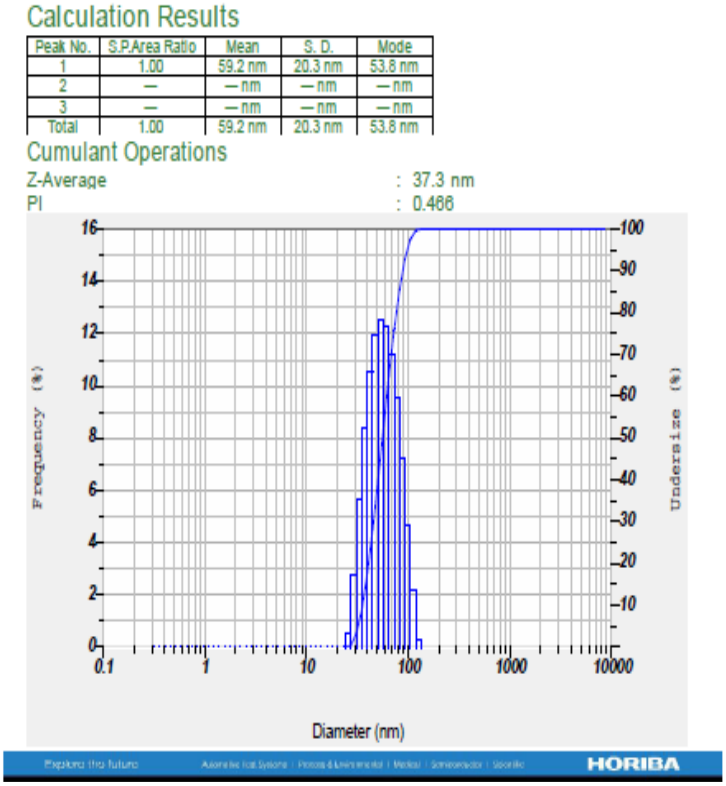


(a) (b)

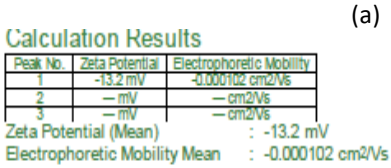
Fig.2 (a) Colour change grey to dark brown. (b) UV-VIS analysis of synthesized SNPs shows peak at 428 nm.

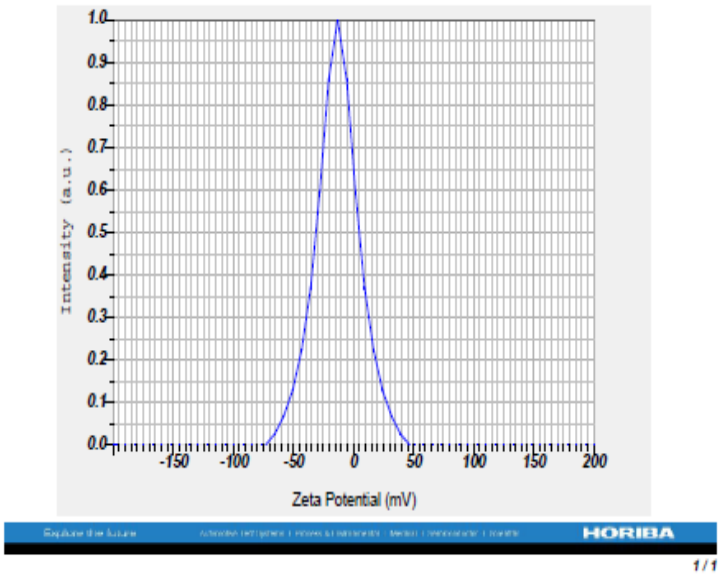
Particle size and Zeta potential analysis:

The particle size of the AgNPs is 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 found 59.2nm with an average size. (Fig. 3 a & b) and PI value 0.466 (poly disperse index). Further the zeta potential analysis of AgNPs was detected to be -13.2 mV, due to its high negative zeta potential it prevents the AgNPs from agglomeration in the medium, leading to long term stability, because of the electrostatic repulsive force between the AgNPs. Zeta potential is an essential parameter for the characterization of stability in aqueous nanosuspensions minimum of ± 30 mV Zeta potential values is required for indication of stable nanosuspension [21]. Zeta potential at-13.2mV, negative 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 green synthesized SNPs from leaf extract of *Pittosporum napaulense.*

Fourier Transform infra-Red (FTIR) analysis:
FTIR spectrum of synthesized SNPs was carried out to know the possible biomolecules responsible for capping and stabilization of nanoparticles. For this the FTIR spectrum was analysed between the scan ranges from 4000 to 500 (Fig.4).

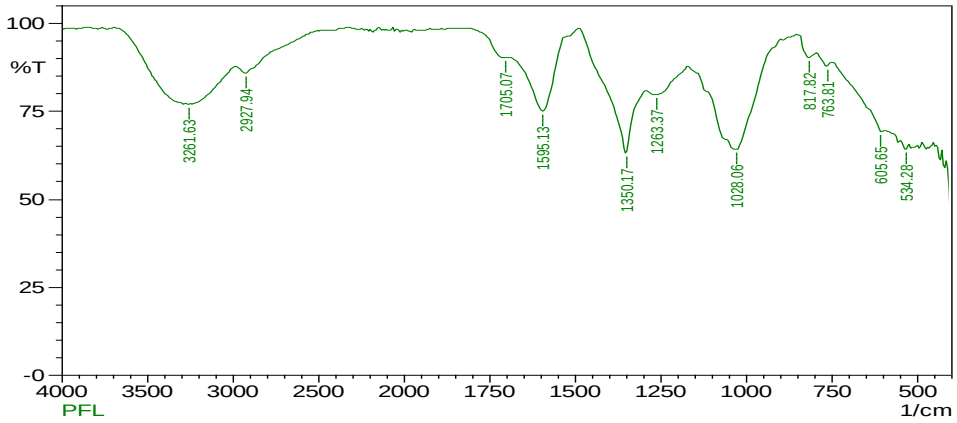


Fig.4 FTIR spectra of green synthesized SNPs from leaf extract of *P. napaulense*

3261.63 cm^{-1} assigned for O-H (Stretch) bond of Alcohol/phenols; 2927.94 cm^{-1} for C-H (Stretch) bond of primary amines; 1705.07 cm^{-1} for O-O (Stretch) bond of Ketones; 1595.13 cm^{-1} for N-H (Bend) bond of Amines.; 1360.17 cm^{-1} for C-N (Stretch) bond of aromatic amines; 1263.37 $cm^{-1;}$ for C-H (Wag) bond of alkyl halides; 1028.06 cm^{-1} for C-H (Bend) bond of alkynes; 817.82 cm^{-1} for C-H (Oop) bond of aromatic; 763.81 cm^{-1} for C-cl (Stretch) bond of alkyl halide; 605.65 cm^{-1} for C-H (Bend) bond of alkynes. 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 leaf extract was analysed by X-ray diffraction analysis. The XRD Shows four plant derived SNPs. An intensive peak at 38.19 44.51 64.68 and 77.19 of 2θ degrees of X-axis corresponds to 111, 200, 220 and 311 Bragg Reflections of Y-axis (JCPDDS No: 89-3722) (fig5). These Bragg reflections confirm that the nanoparticles are crystalline in nature.

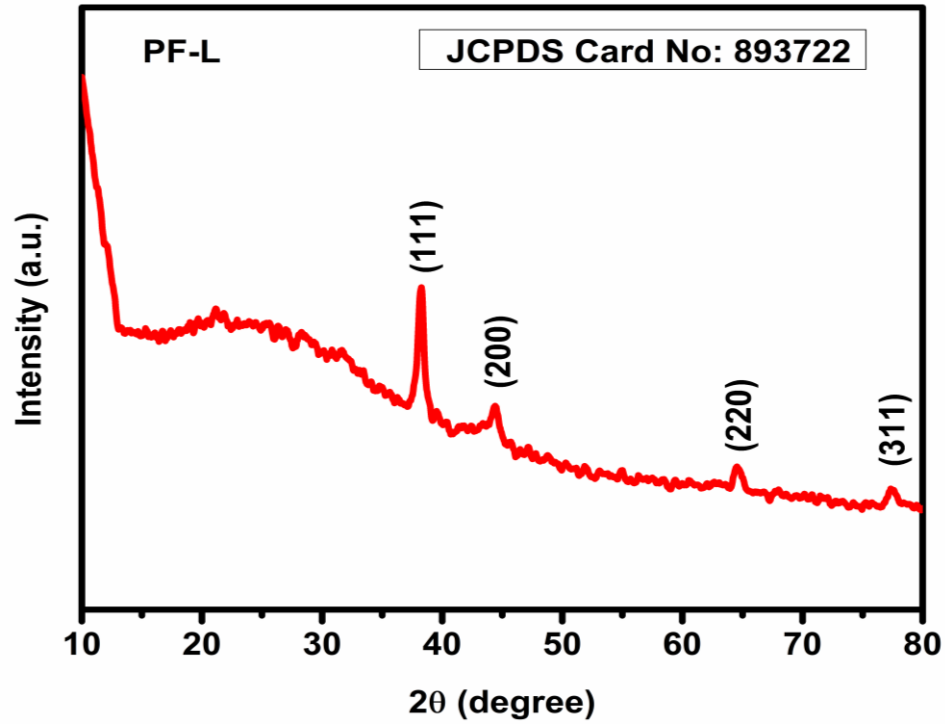
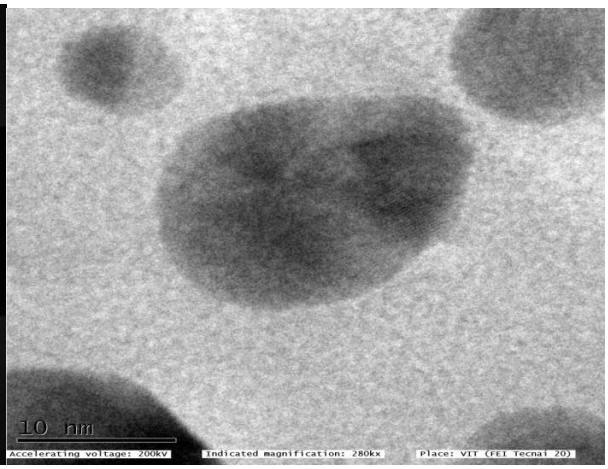


Fig 5 XRD pattern of green synthesized SNPs from leaf extract of *Pittosporum napaulense.*

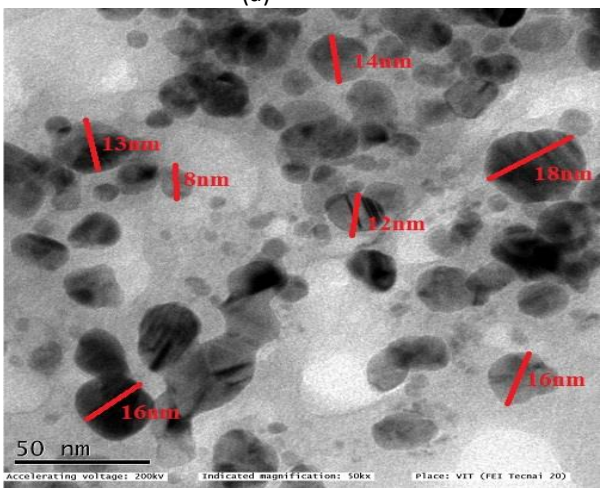
Transmission Electron Microscopy (TEM) and Energy Dispersive X-ray (EDAX) Analysis:

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. EDAX analysis was performed to know the percentage of Ag present in the sample. The EDAX spectra shows strong silver 78.86 % absorption peak along with different elements with their weight percentage like Carbon Copper (21.14. %) (fig.7) 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 [22]. Higher resolution studies with TEM analysis, to know the size, morphology, and agglomeration pattern of nanoparticles. 50 nm resolution studies of nanoparticles on TEM analysis reveals the nanoparticles are 8-18 nm in size owing spherical shape without any agglomeration observed between the particles (Fig. 6).

(a)

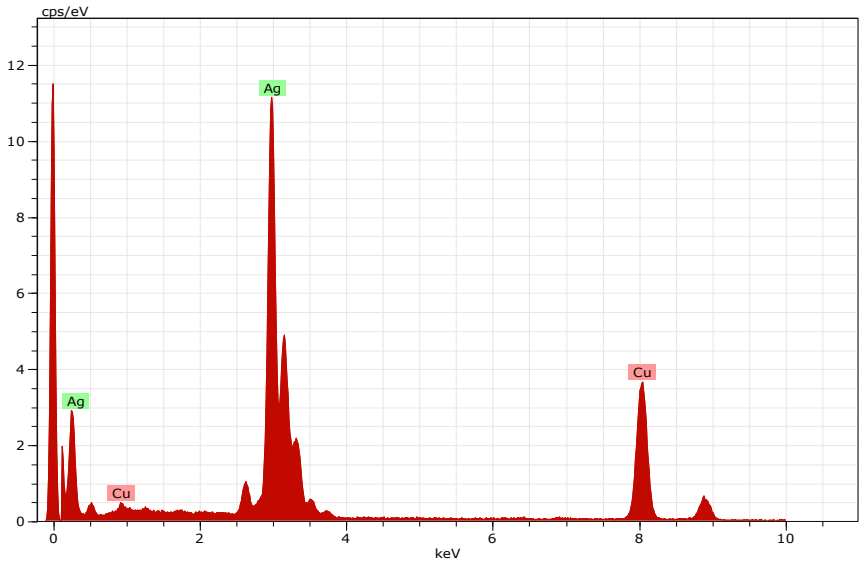


(b)



(C)

Fig.6 (a) Selected area electron diffraction (SAED) of green synthesized SNPs, (b) 10 nm resolution studies of green synthesized SNPs. (C) 50 nm resolution studies of green synthesized SNPs shows mostly spherical shaped with size between 8-18 nm .



Spectrum: Spectrum 442-PfL

Element	Series	Net unn. C [wt.%]	norm. C [wt.%]	Atom. C [at. %]	Error (3 Sigma) [wt.%]
Copper	K-series	36645	21.14	21.14	31.27	2.01
Silver	L-series	115242	78.86	78.86	68.73	23.75
Total:			100.00	100.00	100.00	

Fig.7 EDX analysis of green Synthesized **AgNPs** of ***P.napaulense***

From the TEM analysis reveals the green synthesized silver nanoparticles of *P. floribundum* shows the size range between 8 to 18 nm having spherical shape without any agglomeration between the particles.

Antimicrobial Activity of AgNPs :

These green synthesized silver nanoparticles were assessed for antimicrobial activities against two gram positive and Two-gram negative bacteria. Among the bacteria the highest inhibition zones were observed against *Bacillus subtilis* 18.5mm followed by *Staphylococcus aureus* 13.25mm (Fig 8, 9 and Table 1)

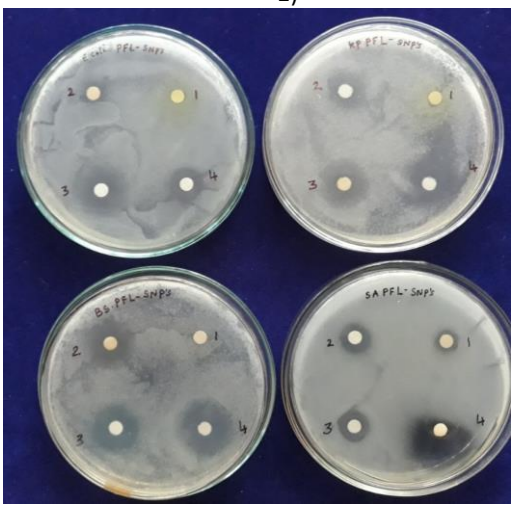


Fig.8 Antimicrobial activities of Synthesized SNPs from leaf extract of *P.napaulense*
E. coli, K.pneumonia, B.subtilis, S.aureus. (1) Ag $(NO3)^2$ (2) Plant extract (3) SNPs (4)*Streptomycin.*

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

Name of Organism	$Ag(NO_3)_2$	Plant Extracts	SNPs	Streptomycin
E.Coli	6.75 ± 0.25	7.5 ± 0.29	10 ± 0.41	14.25 ± 0.48
KP	6.25 ± 0.25	7.25 ± 0.25	9.25 ± 0.48	11 ± 0.41
BS	6.5 ± 0.29	9.25 ± 0.25	18.5 ± 0.29	21.75 ± 0.48
SA	9.25 ± 0.25	12.5 ± 0.29	13.25 ± 0.48	22.75 ± 0.48

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)

E.coli; *Escherichia coli,* **KP;** *Klebsiella pneumonia,* **BS;** *Bacillus subtilis* and **SA;** *Staphylococcus aureus.*

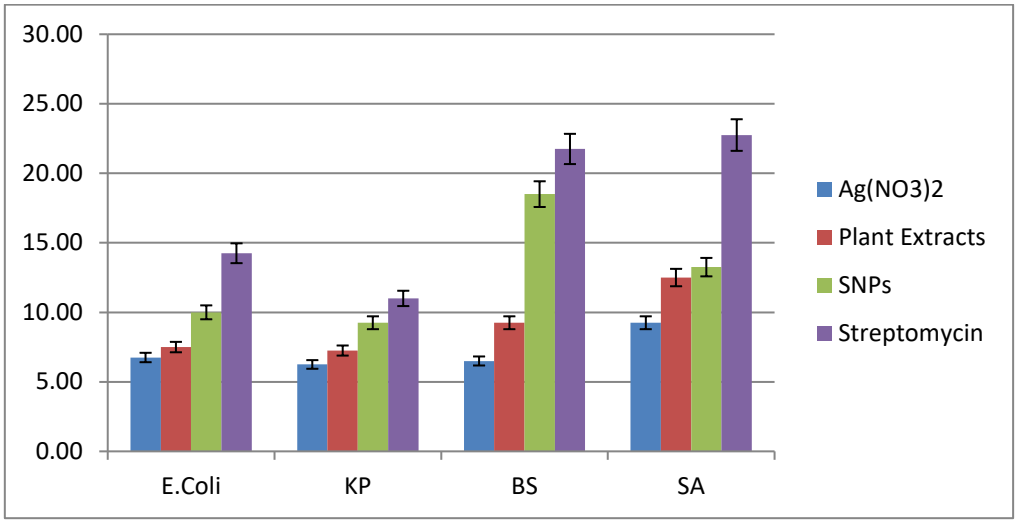


Fig 9 Zone of inhibition of different extracts on clinically isolated bacteria

(***E. coli;*** *Escherichia coli,* **KP;** *Klebsiella pneumonia,* **BS;** *Bacillus subtilis* and **SA;** *Staphylococcus aureus*.)

The SNPs shows less significant effect on Gram negative bacteria 20-25 nm sized, spherical shaped silver nanoparticles synthesized from *Olea europaea* leaf extract shows good antibacterial activity Against *S. aureus*, *P. aeruginosa* and *E. coli* [23]. Some of the scientists state that the SNPs penetrate inside the bacteria and fungi causing damage by interacting with electrons phosphorous and sulphur containing Compounds such as DNA and proteins, resulting in cell Death [24]

DISCUSSION

Leaf oil of *P. senacia* contains sesquiterpenes δ-cadinene 11.3% α murolol 15.9% and α- cadinol 19.0% **[25]**. *P. viridiflorum* leaf oil contains sesquiterpene δ-cadinene 10.6% and α-cadinol 18.3%. The major fruit oils sabinene 13.2%, decanal 10.3% β- elemene 9.5%, β-pinene 8.7%, α-pinene 8.0% and α-cadinol 8.1%. The leaf and fruit oils had similar inhibitory effects on all bacterial strains except fruit oil against *Pseudomonas aeruginosa*, with less activity **[26]**. The leaves of *P. viridiflorum* consists of 15 components and yield 85.4% of oils whereas the mature fruits contain 26 components and yield 94.5% of oils which showed effective antimicrobial activity against gram negative bacterial strains S. *aureus* and *Salmonella typhi*. The leaves and fruits of *P. neilgherrensis* contain 21 components of 97.6% of oils; fruits consist 20 components of 81.3% of oils. Undecane 62.2% as the major component in the leaf followed by caryophyllene oxide 9.0%, β- caryophyllene 8.7%. β- Selinene 11.9%, fruit oil consists of Undecane 11.3%, nonane 8.8% and α-pinene 8.4%. Oils show moderate activity against most of the tested gram-positive and gram-negative bacteria **[27]**. Essential oils from the bark of *P. dasycaulon* consists of dodencanal 53.43%, undecane 20.84%, hexadecanal 9.95% dodecanoic acid 3.6 and 1-tridecanol 2.15%. These oils also shows effective antimicrobial activity against all gram positive and gram negative bacteria except on *Bacillus subtilis*.With the Minimum Inhibitory Concentration ranges from 25-100 µl/ml **[28].** *P. undulatum* contain monoterpinoids, diterpionoids, sesquiterpinoids and alkanes, showed effective antimicrobial activity against *P. aureus, S. epidermis* and *S.aeruginosa* **[29].** Essential oils antifungal activity against *A. flavus* found the inhibition of the

aflotoxin B1 production **[30]**. Crude saponin mixture of *P. tetrasporum* leaves showed effective antifungal activity with 13.3mm Dz than that of the control *Nystatin* 12mm Dz **[31]**. Water, Ethyl acetate and chloroform extracts of *P. phylliraeoides* with major phytoconstituents as alkaloids, flavonoids, phenols, saponins and pro anthocyanidins, shows effective antimicrobial against 14 bacterial strains and 1fungal strain. But no activity against *Candida albicans.* **[32-33]**. phytochemical screening, antibacterial and antifungal studies of *Pittosporum floribundum* supports the herbal and traditional uses against skin diseases, arthritis, inflammatory, spasmodic, sciatica, sprains, bronchitis, chest pains, antidote to snake bite, narcotic and also in curing leprosy may be due to the presence of the major secondary metabolites in the crude extracts and the effective activity with lowest concentrations on all bacterial and fungal strains which may cause the health disorders to that of the herbal uses of *Pittosporum floribundum* **[34]**.

CONCLUSIONS

The biosynthesized silver nanoparticles using *Pittosporum napauelnse* whole leaf extract proved excellent antimicrobial activity against *Bacillus subtilis* with 18.5mm diameter zone of inhibition 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.

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