Research Article

Antimicrobial Efficacy of Silver Nanoparticles Synthesized from *Withania somnifera* – An Important Ethnomedicinal Herb of Kurnool District, Andhra Pradesh, India

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ABSTRACT

Withania somnifera roots are extensively using by the ethnic groups of Kurnool district, Andhra Pradesh, India to cure leucoderma. Biological synthesis of silver nanoparticles was carried out from root aqueous extract of *Withania somnifera* 10 ml root extract was mixed to 90 ml of 1 mM aqueous of $Ag(NO_3)_2$ and was heated at 60-80^{0}C for 20 min. The colour change of aqueous solution into dark brown colour. For characterization using UV-Vis spectrophotometer and AFM. AFM, UV-Vis spectrophotometer showed the formation of silver nanoparticles with spherical shape and average size 25.02 nm. SNPs have good antimicrobial activity against different microorganisms.

Keywords: Anti microbial efficacy, Atomic Force Microscope (AFM), Inhibition zone, Medicinal plant, Secondary metabolites, Silver nanoparticles.

INTRODUCTION

Withania somnifera L. is an important medicinal plant, the roots of which have been employed in Indian traditional systems of medicine, Ayurveda and Unani. The plant has been found useful in the treatment of burns, wounds and dermatological disorders.[1] *W. somnifera* reduces tumor cell proliferation and enhances the effectiveness of radiation therapy while potentially mitigating undesirable side effects.[2] India is one of the twelth mega biodiversity hotspot in the world. Eastern Ghats are one among them, which are characterized by different wild medicinal flora. Nallamalai hills as a part of Eastern Ghats in Kurnool District of Andhra Pradesh, India. Mainly four ethnic groups (Chenchu, Sugali, Yerukala and Yanadi) are inhabited in this region.

Leucoderma is a skin disorder in humen for a number of reasons depigmentation occurs due to auto immune disorder[3] or lacking of pigments due to absence of melanocytes.[4] Skin diseases are commonly occurring among the rural masses due to poor hygienic conditions, poor sanitation facilities and contaminated water etc., the traditional healers of these ethnic groups are extensively using *W. somnifera* roots to treat leucoderma.

W. somnifera root contains a variety of important chemical compounds. Withaferin[5] and reported to have immunosuppressive.[6] Withanolid A, Withanone and Withanolid D are present in the medicinal plant are reported to have antioxidant, immunomodulatory and other activities.[7-9] The most important of these phytochemicals are alkaloids, tannins, flavonoids and phenolic compounds.[10]

Nanoparticles are gaining much importance especially in medicinal field. Synthesis of metal nanoparticles receives great attention due to their unusual optical, chemical, phytochemical and electronic properties.[11] Silver a nobel metal is known to improve the immunity since ancient times, $Ag(NO_3)_2$ was using for biosynthesis of nanoparticles by using root aqueous extract of *W. somnifera.* The possibility of using plant materials for the synthesis of nanoscale metals was initially reported by Gradea-Torresdey.[12-13] SNPs have particular properties that may perhaps have numerous applications in the field of dentistry, clothing, catalysis, mirrors, optics, phytography, electronics and food industry.[14]

At present extensive work has been done to develop new drug from natural products because of the resistance of microorganisms to the existing drug. The pathogens like *E. coli, Bacillus, Salmonella typhi* and *Staphylococcus aureus.*[15]

The present study is aimed to study the qualitative analysis of phytochemical constituents and biological synthesis of silver nanoparticles by using root extract of *W. somnifera* L. and also screening of SNPs for microbial efficacy.

MATERIALS AND METHODS

Plant material

The fresh roots of *W. somnifera* L. was collected in January 2012 from Srisailam reserve Forest, Kurnool District of Andhra Pradesh, India. The root was cleaned, cut into small pieces (1-2 cm) dried at room temperature and ground to fine powder.

Preparation of extract

25 g of root powder of *W. somnifera* L. was taken into 250 ml conical flask and added 100 ml of sterile distilled water and boiled for 10 min at 100°C on water bath. Then plant material extracts were collected in separate conical flask by standard filtration method and stored in refrigerator for further use.

Phytochemical screening

10 ml root extract was used for preliminary phytochemical screening. The qualitative analysis of secondary metabolites was carried out by using the methods for flavonoids[16]; steroids, alkaloids and phenols[17]; triterpenoids and glycosides[18]; for tannins, anthraquinons, leucoanthocyanins and emodins[19]; saponins[20] and reducing sugars and anthocyanins.[21]

Preparation of 1 mM Silver nitrate solution

1 molar silver nitrate stock solution was prepared by 1.7 g of $AgNO_3$ was dissolved in 10 ml distilled water. 1 mM solution was prepared by 1 ml of 1 M solution was made up to 100 ml with 99 ml of distilled water. This solution was stored in amber colored bottle for further use.

Synthesis of silver nanoparticles

SNPs were synthesized by using root powder extract of *Plumbago zeylanica*. The reduction of pure Ag^{2+} ions were monitored by measuring the UV-Vis spectrum of the reduction media at 5^{th} h after diluting a small aliquot of the sample in distilled water by using Systronic 118 UV-Vis Spectrophotometer. The size and shape of SNPs were confirmed with AFM.

UV-Vis spectra analysis

The reduction of pure silver ions was monitored by measuring the UV-Vis spectrum of the reaction medium at 3 hrs. after diluting a small aliquot of the sample into distilled water. UV-Vis spectral analysis was carried out by using UV-Vis spectrophotometer (Systronics type 118).

AFM analysis

The silver nanoparticles extracted by the above protocol were visualized with an Atomic Force Microscope (AFM). A thin film of the sample was prepared on a glass slide by dropping 100 µl of the sample on the slide and was allowed to dry for 5 min, the slides were then scanned with the AFM (Nano surf ® AG, Switzerland, Product: BTO2089, 3RO). Nanosurf ® Easyscan-2 software was used for the AFM Analysis (VIT, Vellore, Tamil Nadu).

Microorganisms

Pure cultures of *Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Salmonella typhi* species of bacteria and *Paecilomyces varioti, Pencillium rubrum* and *Aspergilus flavus* species of fungi were procured from the Department of Microbiology of Sri Venkateswara Institute of Medical Sciences (SVIMS), Tirupati, Andhra Pradesh, India.

Antimicrobial activity

The antimicrobial activities of SNPs were carried out with paper disc diffusion method using nutrient agar medium and potato dextrose agar medium for bacterial and fungal cultures respectively. Zones of inhibition for control, SNPs and silver nitrate were measured after 24 h and 7 days and compared with standard drugs Gentamycin and Nystatin for bacterial and fungal growth respectively. The experiments were repeated thrice and mean values of inhibition zone diameter were presented.

RESULTS AND DISCUSSION

The ethnic groups (Chenchu, Sugali, Yanadi and Yerukala) of Kurnool District, Andhra Pradesh, India. The traditional healers of these ethnic groups have staunch confidence to treat leucoderma. The fresh roots of *W. somnifera* L. ground in to paste is applied to depigmented parts of skin to treat leucoderma. This information was cross checked with Ayurvedic Physicians Sri Venkateswara Ayurvedic College, Tirupati, Andhra Pradesh, India for authentication. In this regard *W. somnifera* L. roots are extensively used in Ayurveda to treat leucoderma.

The phytochemical study of *W. somnifera* L. showed that the root is rich in alkaloids, antraquinones, flavonoids, glycosides, phenols, reducing sugars, saponins, steroids, tannis and triterpenoids and lacking antrocyanins, coumarins, emodins, lignins and laucoanthocyanins (Table 1). The secondary metabolites like flavonoids and phenolic compounds are medically used as antistomatic, diarrhoea, anti-inflammatory, anticancer and antioxidative. The biological function of flavonoids include protection against allergies; inflammations, platelets aggregation, microbes, ulcer and tumors.[22-23] It also known to possess antiviral and anti fungal[24-25] and antimicrobial properties.[26] The presence of bioactive compounds indicates the medicinal values of the plant. Steroids and triterpenoids possess anti bacterial activity.[27] Tannins possess astringent, anti-inflammatory, antidiarrhoeal, antioxidant and antimicrobial activities.[28] Similar chemical constituents were also found in *Shorea tumbuggaia*[29], *Thespesia populnea*[30] and in *Curcuma longa*.[31]

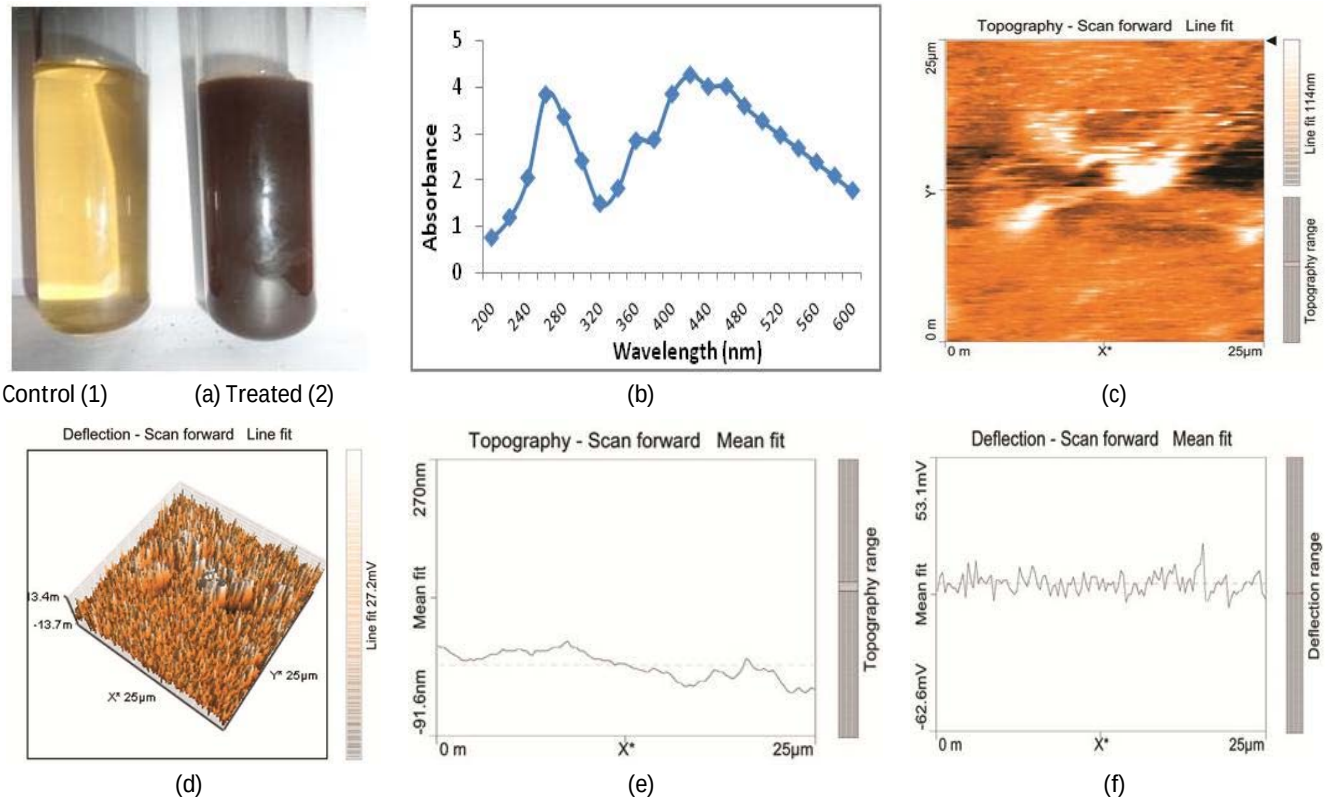
Table 1: Secondary metabolites of root extract of *W. somnifera* L.

Secondary metabolites	Root
Alkaloids	++
Anthocyanins	-
Anthraquinones	+
Coumarins	-
Emodins	-
Fatty acids	-
Flavonoids	++
Glycosides	++
Lignins	-
Leuco anthocyanins	-
Phenols	++
Reducing sugars	+
Saponins	+
Steroids	++
Tannins	+
Triterpenoids	++

Note: '+' indicates presence, '++' indicates presence of more amounts, '-' indicates absence

In the present study SNPs were synthesized by using root aqueous extract of *W. somnifera* rapidly within 10 min of incubation period dark brown color was developed by addition of $Ag(NO_3)_2$ (Figure 1a). The appearance of dark-brown color in the reaction vessels indicates the formation of SNPs.[32] The colour change in aqueous solution is due to the surface- Plasmon resonance (SPR) phenomina. The reason could be that the quantitative variation in the formation of SNPs (or) availability of H^+ ions to reduce the silver. It is well known that SNPs exhibit dark brown color in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles.[33] By using medicinal plants $Ag(NO_3)_2$ can be reduced into SNPs at the fast rate. The biomolecules found in plants induce in reduction of Ag^+ ions into silver nanoparticles.

The synthesis of SNPs had been confirmed by measuring the UV-Vis spectrum of the reaction media. The UV-Vis spectrum of colloidal solutions of SNPs synthesized from root extract of *W. somnifera* have the characteristic absorbance peaks at 260 nm and 440 nm (Figure 1b) and the broadening of peak indicated that the particles are poly-dispersed. This peak illustrates that the presence of a homogenous distribution of hydrosol silver nanoparticles after stirring.[34] It is generally recognized that UV-Vis spectroscopy could be used to examine size and shape of nanoparticles in aqueous suspension.[35] The weak absorption peak at shorter wavelengths due to the presence of several organic compounds which are known to interact with silver ions same results observed in *Boswellia ovalifoliolata* stem bark.[36] Silver nanoparticles have free electrons, which give rise to an SPR absorption bonds[37], due to the combined vibration of electrons of metal nanoparticles in resonance with the light waves.[38-39] The secondary metabolites present in plant systems may be responsible for the reduction of silver and synthesis of nanoparticles.



Control (1) (a) Treated (2) (b) (c)

(d) (e) (f)

Figure 1: The color change of root extract of *Withania somnifera* (1) blank extract without silver nitrate (2) root extract with 1 mM silver nitrate; b) UV-Vis spectroscopy of synthesized of silver nanoparticles, (c, e & f) AFM of Topography of SNPs (d) Three dimensional structure of SNPs.

The size and shape of SNPs was detected by using AFM (Atomic Force Microscope). Size of SNPs was 25.02 nm spherical in shape (Figure 1d). The results obtained in this study are interesting because it can serve as a foundation in terms of identification of potential medicinal plants for the synthesis of SNPs. The plant species pay a vital role to cure skin diseases. Biological synthesis of metal nanoparticles in a traditional method and the use of plants extract have a new awareness for the control of diseases, besides being safe, eco-friendly and no phytotoxic effects.

The SNPs of root extract of *w. somnifera* showed highest percentage of bacterial inhibition towards gram-negative bacterias *Salmonella typhi* (11.6±0.23) and *E. coli* (11.2±0.38) followed by gram-positive bacterias *Staphyllococcus aureus* (10.68±0.31) and *Bacillus* (10.61±0.83) and antifungal activity against *Pencillium rubrum* (8.8±0.66) followed by *Aspergillus flavus* (8.5±0.50) and *Paecilomyces varioti* (7.3±0.49) (Table 2), (Figure 3) and (Figure 2). The maximum toxicity was observed in SNPs than $Ag(NO_3)_2$ and plant extract. The reason could be that the smaller size of the particles

which leads to increased membrane permeability and cell destruction. The results were compared to that of standard antibiotics Gentamycin / Nystatin anti bacterial and antifungal respectively. Standard drugs (Gentamycin / Nystatin), showed higher inhibition zones, because these are highly purified forms which may be cost and leads to side effects in high dosage, whereas the SNPs are biologically synthesized form with less in cost, eco-friendly, safe and pollutant free with less or no side effects.

In general, gram-positive bacteria appeared to be more tolerant to silver than gram-negative cells. The cell wall of gram-positive bacteria contains multiple layers of peptidoglycon compared to the cell wall of gram-negative bacteria. Thus, gram-positive bacteria may allow less Ag^+ to reach the cytoplasmic membrane than gram-negative bacteria[40] and may therefore be less susceptible. The SNPs are also reported to be nontoxic to human and most effective against bacteria, viruses and other eukaryotic micro-organisms at very low concentrations and without any side effects Jeong *et al*.,[41] SNPs synthesized from *Svensonia hyderobadensis*[42], *Shorea tumbuggaia* and *Boswellia ovalifoliolata*[43, 29] and *Thespesia populnea*[30], *Curcuma longa* exhibit the antibacterial activity.[31]

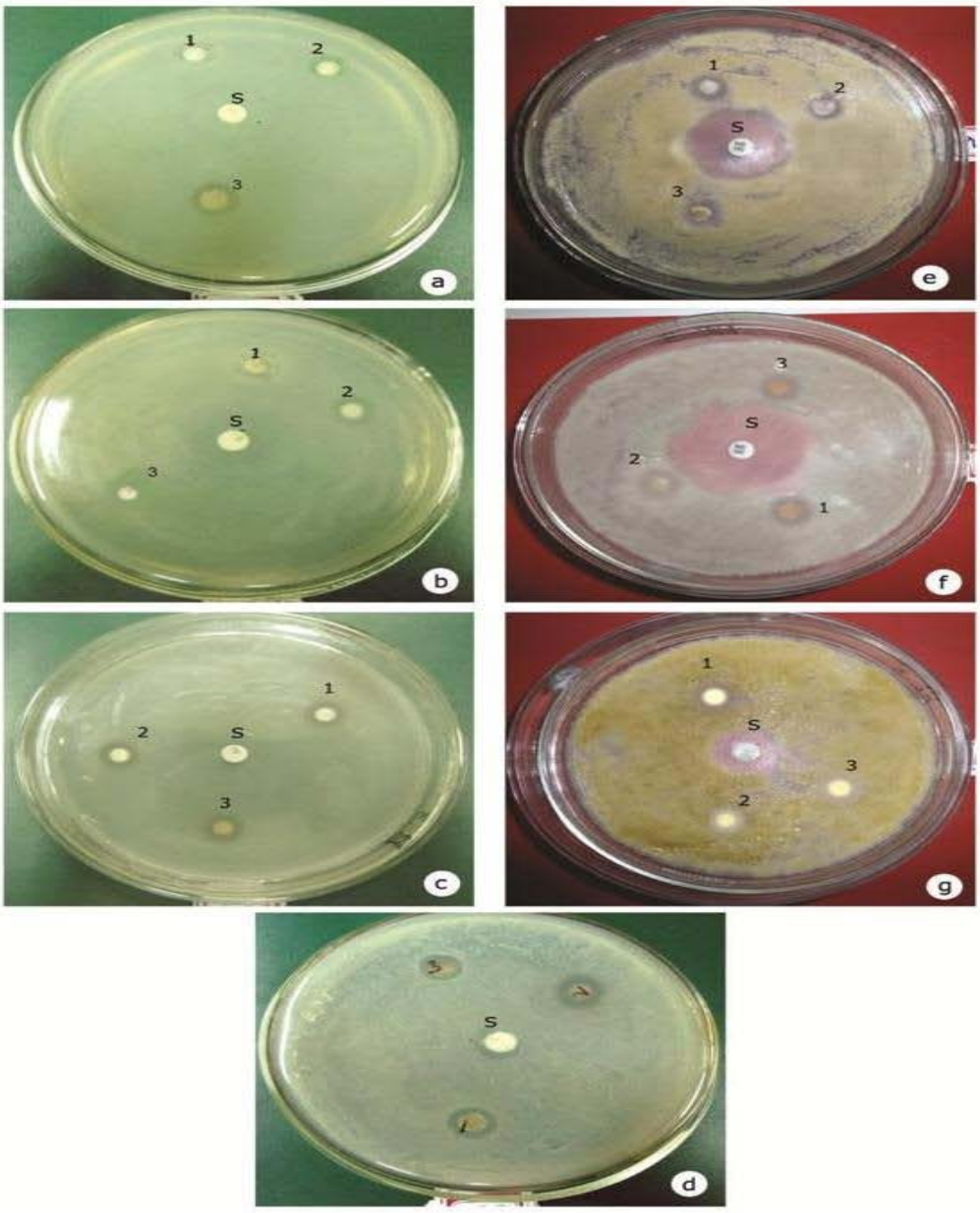


Figure 2: Antimicrobial activity of root extract of *Withania somnifera*

a) *Staphylococcus aureus,* b) *Salmonella typhi,* c) *E.coli,* d) *Bacillus,* e) *Paecilomyces varioti,* f) *Pencillium rubrum* and g) *Aspergillus flavus.* 1) $Ag(No_3)_2$, 2) Plant extract control, 3) SNPs and S) Standard(Gentamycin/Nystanin)

Table 2: Antimicrobial activity of SNPs isolated from root extract of *Withania somnifera*

Microorganisms	Inhibition zone in mm			
	$Ag(NO_3)_2$	Plant extract control	SNPs	Standard: Gentamycin/ Nystatin
Bacterial species				
Staphylococcus aureus	8.0±0.40	9.3±0.77	10.68±0.31	15.8±0.22
Salmonella typhi	9.6±0.16	8.9±0.23	11.60±0.23	13.7±0.16
E.coli	8.1±0.24	5.6±0.21	11.2±0.31	11.8±0.35
Bacillus	8.2±0.33	9.2±0.38	10.70±0.38	12.6±0.26
Fungal species				
Paecilomyces varioti	7.0±0.40	6.8±0.55	7.3±0.49	11.7±0.43
Pencillium rubrum	7.5±0.47	7.2±0.68	8.8±0.66	13.7±0.54
Aspergillus flavus	7.6±0.47	6.4±0.54	8.5±0.50	11.8±0.72

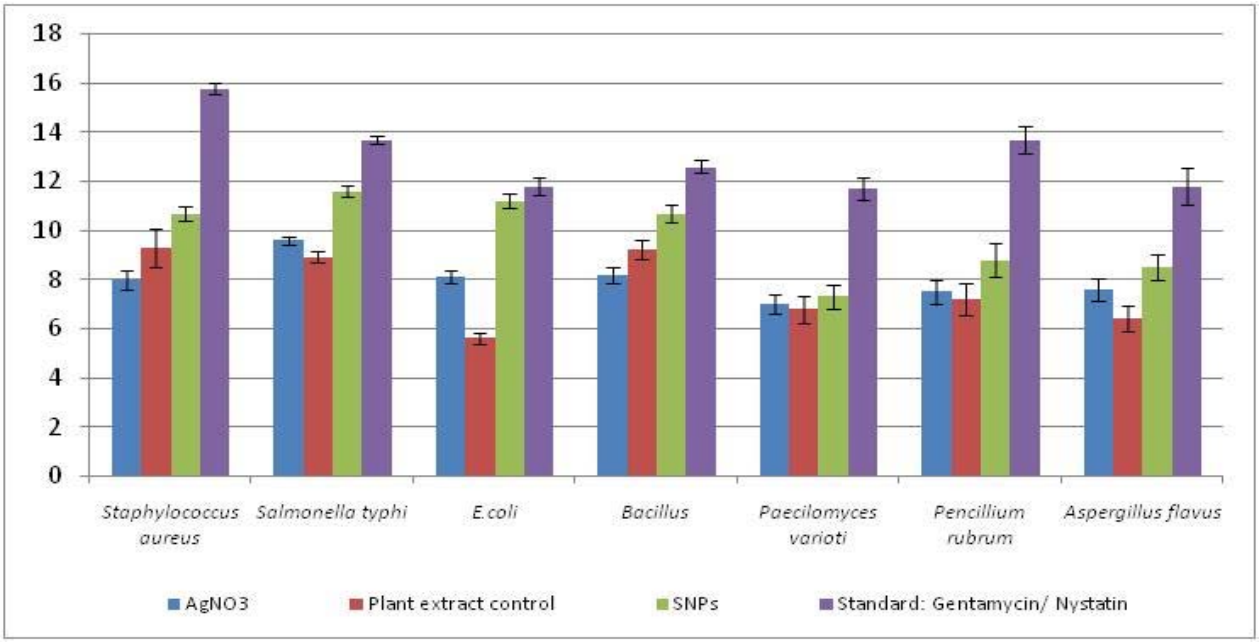


Figure 3: Antimicrobial activity of SNPs isolated from root extract of *Withania somnifera*

CONCLUSION

The present study includes the treatment of leucoderma using w. *somnifera* by the ethnic groups. Phytochemical screening indicates that the plant part is a good source for bio active principle for pharmacognostic and pharmaceutical industries. The SNPs prepared by using the aqueous root extract of *w. somnifera*. The aqueous silver ions exposed to the extracts, the synthesis of SNPs were confirmed by the change of color of plant extracts. These environmentally benign SNPs were further confirmed by using UV-Vis spectroscopy finally the size and shape of the SNPs was characterized by AFM analysis. The results indicated that SNPs have good antimicrobial activity against different microorganisms due to the cumulative effect of secondary metabolites or active molecules present in the plant extract of selected medicinal plant used by ethnic groups of Kurnool district of Andhra Pradesh, India to cure skin diseases. It is confirmed that SNPs of *w. somnifera* are capable of rendering antimicrobial efficacy and hence has a great potential in the preparation of drugs used against bacterial and fungal diseases.

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