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SCREENING OF MEDICINAL PLANTS FOR EFFECTIVE BIOGENESIS OF SILVER NANO PARTICLES AND EFFICIENT ANTI-MICROBIAL ACTIVITY

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ABSTRACT

Metal nanoparticles have been using as an ingredients in the preparation of complementary medicines to cure different diseases is an age old medicinal practices. The plant based Ayurvedic preparations are preferred by 80% of the world population and WHO is encouraging the green medicine due to its less side effects. Hence an attempt has been made to screen this important medicinal plant for secondary metabolites biogenesis of silver nanoparticles (SNPs) and their antimicrobial efficacy. The stem, roots, stembark and leaves were selected for synthesis of SNPs. The SNPs formation was confirmed with the help of UV-VIS spectroscopy and characterized by AFM. The phytosynthesized SNPs were tested for antibacterial and antifungal activities using paper disc diffusion method. The results indicate that the selected plant revealed that it is source for different secondary metabolites like anthocyanins, emodins, triterpenoids, tannins, saponins, luecoanthocyanins, reducing sugars, flavonoids, lignins, anthroquniones, phenols and glycosides. But steroids, coumarins, fatty acids and alkaloids are absent. The SNPs are having more anti microbial efficacy than that of the plant extracts. Moreover the SNPs of selected plant parts are having toxicity towards bacterial species than that of fungal species. It is concluded that the environmentally benign SNPs synthesized from different parts of *Shorea tumbuggaia* can be used effectively against bacterial and fungal strains. The SNPs may have important advantage over conventional antibiotics to which the bacteria got resistance.

INTRODUCTION: Recently the researchers from Indian Institute of Technology, Bombay have discovered that the age old complementary medicines of Homeopathic pills and Ayurvedic Bhasmas are having metal nanoparticles such as gold, silver, copper, platinum, tin and iron [1]. Nano-particles have found widespread use for biological assays. These have substantial advantages over classical organic dyes because of their superior photo physical properties, which overcome many of the spectral limitations as molecular fluorophones [2]. The silver nanoparticles have various and important applications. Historically, silver has been known to have a disinfecting effect and has been found in applications ranging from traditional medicines to culinary items. It has been reported that silver nanoparticles (SNPs) are non-toxic to humans and most effective against bacteria, virus and other eukaryotic microorganisms at low concentrations and without any side effects [3]. Moreover, several salts of silver and their derivatives are commercially manufactured as antimicrobial agents [4].

In small concentrations, silver is safe for human cells, but lethal for microorganisms[5]. Antimicrobial capability of SNPs allows them to be suitably employed in numerous household products such as textiles, food storage containers, home appliances and in medical devices [6]. The most important application of silver and SNPs is in medical industry such as tropical ointments to prevent infection against burn and open wounds [7]. Biological synthesis of nanoparticles by plant extracts is at present under exploitation as some researchers worked on it [8, 9], and testing for antimicrobial activities [10-12].

For the last two decades, extensive work has been done to develop new drugs from natural products because of the resistance of microorganisms to the existing drugs. Nature has been an important source of a products currently being used in medical practice [13]. The pathogens like *Klebsiella pneumoneae*, *E. coli* and *Bacillus* species developed resistance to number of antibiotics [14] empirical therapy is needed to overcome this problem. *Boswellia ovalifoliolata*, *Shorea tumbuggaia, Svensonia hyderobadensis Thespesia populnea* and *Vinca rosea* are important medicinal plants used in the preparation of number of Ayurvedic, Homeopathic and Allopathic drugs these medicinal plant species belonging to the families Burseraceae, Dipterocarpaceae, Verbenaceae, Malvaceae and Apocyanaceae respectively.

Tribals like Nakkala, Sugali and Chenchu used *B. ovalifoliolata* and *S. tumbuggaia* to treat number of ailments [15]. *Svensonia hyderobadensis* is used to cure hepatotoxic disease (Antihepatoxicity.blogspot.com). *Thespesia populnea* is used in skin diseases, hepatitis, jaundice, ulcers, wounds, psoriasis, scabies, urinary tract infections, diabetes, cholera, cough, asthma, swollen joints and guneaworm infections [16, 17]. *Vinca rosea* is a renowned medicinal herb used in the treatment of various diseases like diabetes, menstrual regulations, hypertension, cancer and antigalacto-gogue etc, in number of countries [18]. The present study is an attempt to screen the important medicinal plants for the synthesis of SNPs with high antimicrobial efficacy.

MATERIAL AND METHODS:

Plant material and synthesis of Silver Nanoparticle: Leaves of *B. ovalifolilata*, *S. tumbuggaia S. hyderobadensis*, *T. populnea* and *V. rosea* were collected from the forest of Seshachalam hill range of Andhra Pradesh, India. The leaves were air dried for 10 days and kept in the hot air oven at 60°C for 24-48 hours. The leaves were ground to a fine powder. 1 mM silver nitrate was added to the plant extracts separately to make up a final solution of 200 ml and centrifuged at 18,000 rpm for 25 min.

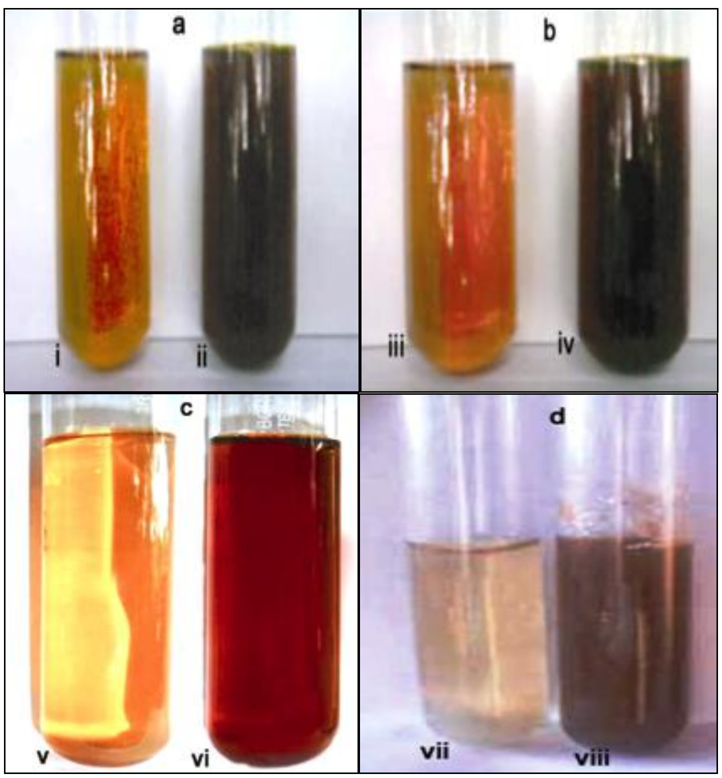
The supernatants were heated at 50 to 95°C. A change in the colour of the solution was observed in 10-15 min during heating process. The colour changes indicate the formation of silver nanoparticles (SNPs). The reduction of pure Ag^{2+} ions were monitored by measuring the UV-Vis spectrum of the reduction media at 5^{th} h after diluting a small aliquot of the sample in distilled water by using systronic 118 UV-Vis Spectrophotometer.

Microorganisms: Pure cultures of *Escherichia coli, Pseudomonas aeruginosa, Bacillus subtills, Proteus vulgaris* and *Klebsiella pneumoneae* species of bacteria and *Fusarium oxysporum, Curvularia lunata, Rhizopus arrhizus, Aspergillus niger* and *Aspergillus flavus* species of fungi were procured from the Department of Microbiology of Sri Venkateswara Institute of Medical Science (SVIMS). The experiments of antimicrobial activity were carried out in the Department of Applied Microbiology, Sri Padmavathi Mahila University (SPMU), Tirupati, Andhra Pradesh, India.

Antibacterial Activity: The antibacterial activities of SNPs were carried out by paper disc diffusion method [19]. Nutrient agar medium plates were prepared, sterilized and solidified. After solidification bacterial cultures were swabbed on these plates. The sterile discs were dipped in SNPs solution (10 μg/ml) and placed in the nutrient agar plate and kept for incubation at 37°C for 24 h. Zones of inhibition for control, SNPs and silver nitrate were measured. The experiments were repeated thrice and mean values of zone diameter were presented.

Antifungal Activity: Potato dextrose agar plates were prepared, sterilized and solidified, after solidification fungal cultures were swabbed on these plates. The sterile discs were dipped in SNPs solution (10 μg/ml) and placed in the agar plate and kept for incubation for 7 days. After 7 days the discs dipped in plant extracts and 5mm $Ag(NO_3)_2$ solution were also placed in the agar plates along with SNPs as control to compare the results.

RESULTS AND DISCUSSION: Yellowish brown colour was developed by addition of Ag $(NO_3)_2$ to the leaf extracts within 10 to 20 min in all samples except *V. rosea* where 12 hrs required to change the colour of the extract (**Fig. 1 a-e**). The appearances of yellowish-brown colour in the reaction vessels suggest the formation of silver nanoparticles (SNPs) [20]. The time duration of change in colour and thickness of the colour varies from plant to plant. The reason could be that the quantitative variation in the formation of SNPs (or) availability of H^+ ions to reduce the silver.



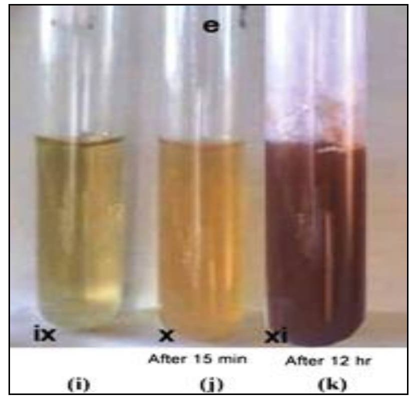


FIG. 1: THE COLOR CHANGE OF EXTRACTS AFTER ADDITION OF SILVER NITRATE (a) BOSWELLIA (b) SHOREA (c) SVENSONIA (d) THESPESIA (e) VINCA; (i, iii, v, vii, ix) PLANT EXTRACTS; (ii, iv, vi, viii, x, xi) SILVER NANOPARTICLES

It is well known that SNPs exhibit yellowish brown colour in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles [12]. Silver nitrate is used as reducing agent as silver has distinctive properties such as good conductivity, catalytic and chemical stability. The aqueous silver ions when exposed to herbal extracts were reduced in solution, there by leading to the formation of silver hydrosol. 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 *B. ovaliofoliolata*, *S. tumbuggaia*, *S. hyderobadensis*, *T. populnea* and *V. rosea* have absorbance peaks at 400 nm, 350 nm and 360 nm 340 nm and 335 nm respectively (**Graph 1-5**); and the broadening of peak indicated that the particles are poly-dispersed [21]. The weak absorption peak at shorter wavelengths due to the presence of several organic compounds which are known to interact with silver ions.

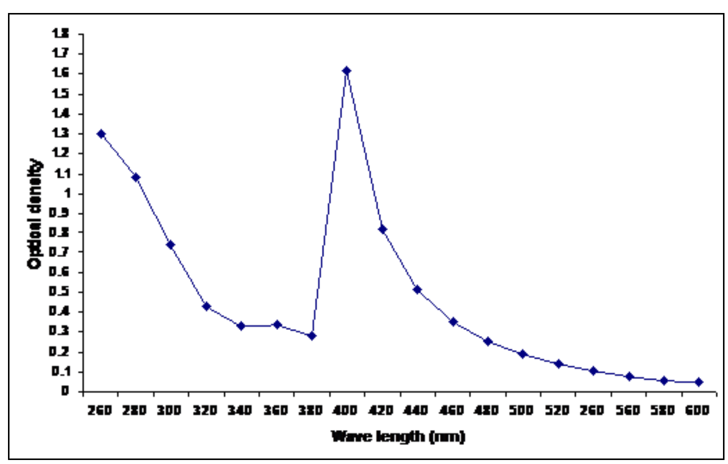


FIG. 1: UV-VIS SPECTROSCOPY OF SILVER NANOPARTICLES SYNTHESIZED FROM LEAVES OF *BOSWELLIA OVALIFOLIALATA*

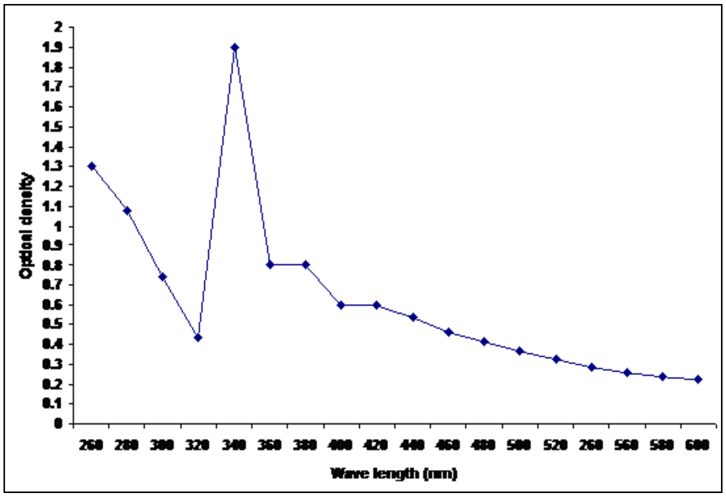


FIG. 2: UV-VIS SPECTROSCOPY OF SILVER NANOPARTICLES SYNTHESIZED FROM LEAVES OF *SHOREA TUMBUGGAIA*

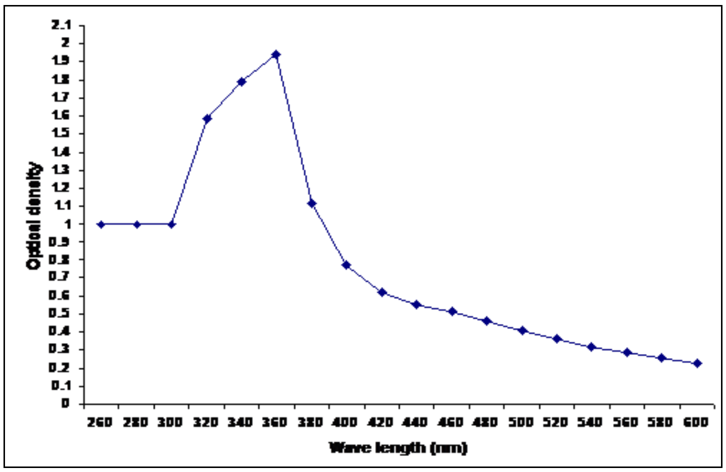


FIG. 3: UV-VIS SPECTROSCOPY OF SILVER NANOPARTICLES SYNTHESIZED FROM LEAVES OF *SVENSONIA HYDEROBADENSIS*

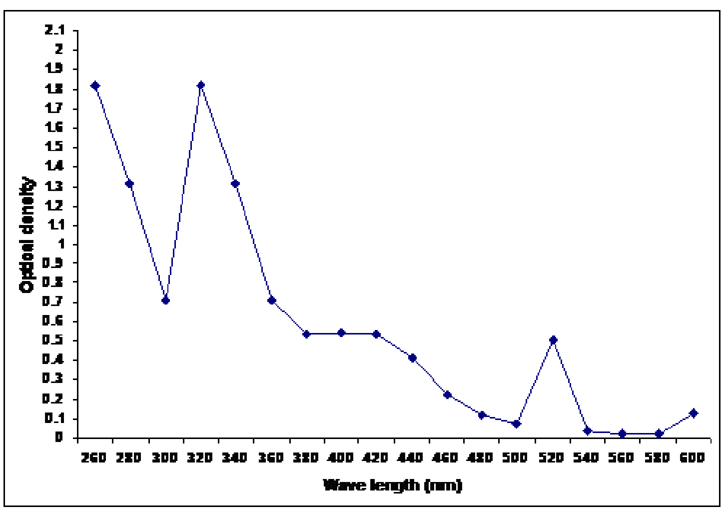


FIG. 4: UV-VIS SPECTROSCOPY OF SILVER NANOPARTICLES SYNTHESIZED FROM LEAVES OF *THESPESIA POPULNEA*

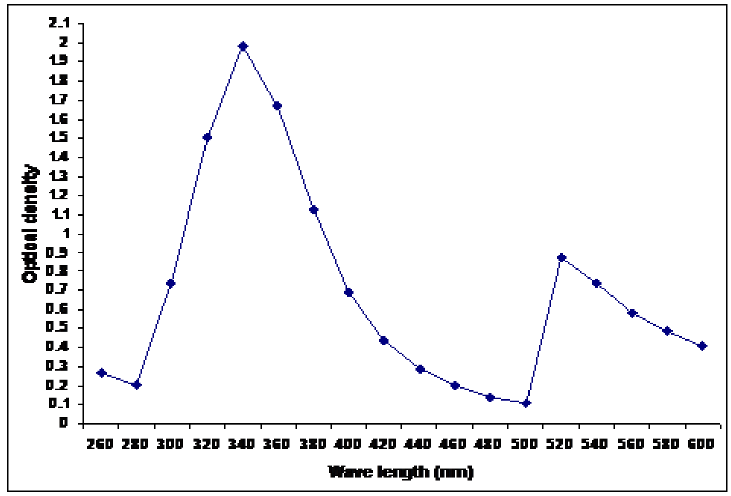


FIG. 5: UV-VIS SPECTROSCOPY OF SILVER NANOPARTICLES SYNTHESIZED FROM LEAVES OF *VINCA ROSEA*

The green leaves were selected for synthesis of SNPs because they are the site of photosynthesis and availability of more H^+ ions to reduce the silver nitrate into silver nanoparticles. The molecular basis for the biosynthesis of these silver crystals is speculated that the organic matrix contain silver binding proteins that provide amino acid moieties that serve as the nucleation sites [22]. The efficiency of various silver based antimicrobial fillers in polyamide toward their silver ion release characteristics in an aqueous medium was also investigated and discussed in number of plants including algae, yeast and fungi [23].

Ahmad *et al.,* mentioned three different routes for the reduction of silver in plant extracts [24]. The secondary metabolites present in plant systems may be responsible for the reduction of silver and synthesis of nanoparticles. The second biogenic route is the energy (or) electron released during Glycolysis of photosynthesis for conversion of NAD to NADH led to transformation of $Ag(NO_3)_2$ to form nanoparticles and the another mechanism is releasing of an electron when formation of ascorbate radicals from ascorbate reduces the silver ions. Similar results were observed in *Cleodendrum inerme* [25], *Euphorbia hirta* [26] and *Argimone mexicana* [11].

Toxicity studies on pathogen opens a door for nanotechnology applications in medicine. Biological synthesis of metal NPs is a traditional method and the use of plant extracts has a new awareness for the control of disease, besides being safe and no phytotoxic effects [27]. The biologically synthesized SNPs using medicinal plants were found to be highly toxic against different pathogenic bacteria and fungi of selected species. The SNPs of *Boswellia ovalifoliolata* showed highest antibacterial activity against *E. coli* and *Proteus* followed by other three bacterial strains (**fig. 2 a-e**) and has no effect on selected fungal species.

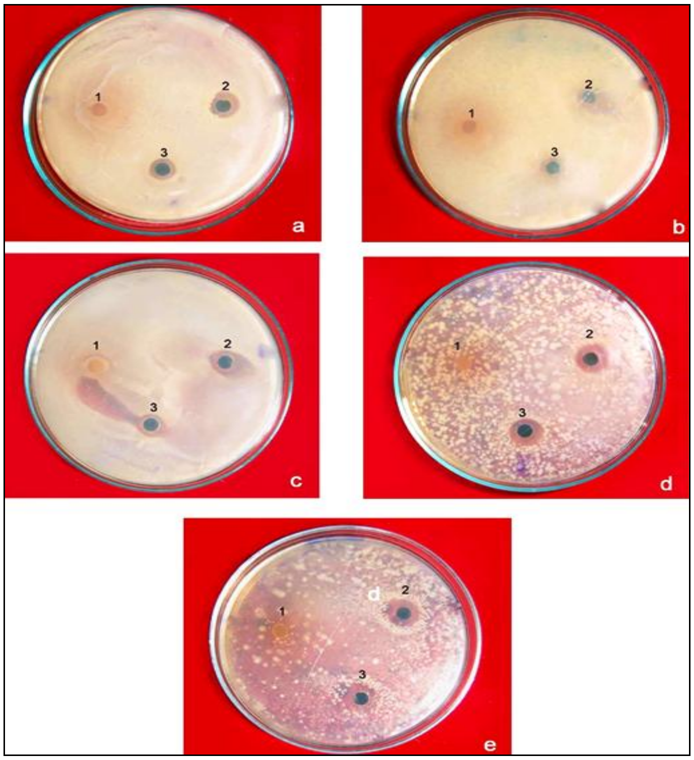


FIG. 2: ANTIBACTERIAL ACTIVITY OF *BOSWELLIA OVALIFOLIOLATA*
(a) *Bacillus* (b) *E. coli* (c) *Klebsiella* (d) *Proteus* (e) *Pseudomonas*
1. Control, 2. Silver Nitrate, 3. Silver Nanoparticles

Shorea tumbuggaia showed highest antibacterial activity against *E. coli* followed by *Klebsiella, Pseudomonas, Proteus* and *Bacillus*; and antifungal activity against *Curvularia* and *Rhizopus* followed by *A. flavus* and *A. niger.* The SNPs of *Svensonia hyderobadensis* have highest toxicity towards fungal species than bacterial strains. Maximum inhibition zone was observed against *Pseudomonas* followed by *Klebsiella, E.coli, Bacillus* and *Proteus* species; and antifungal activity against *Rhizopus* followed by *A. flavus, Curvularia, A. niger* and *Fusarium* (**fig. 3 a-e**).

Biosynthesized SNPs via *T. populnea* leaf extract shows toxicity towards all selected bacterial strains but weak inhibitionary zones are formed against *Aspergillus* species only. SNPs of *V. rosea* responded almost all similarly against all selected bacterial strains. Among the selected medicinal plants *B. ovalifoliolata* showed highest antibacterial efficacy and *S. hyderobadensis* have maximum antifungal activity (**Table 1**). This may be due to the SNPs synthesized via green route are highly toxic towards bacterial strains when compared to fungal strains. Silver ions have been demonstrated to interact with the protein and possibly phospholipids associated with the proton pump of bacterial membranes.

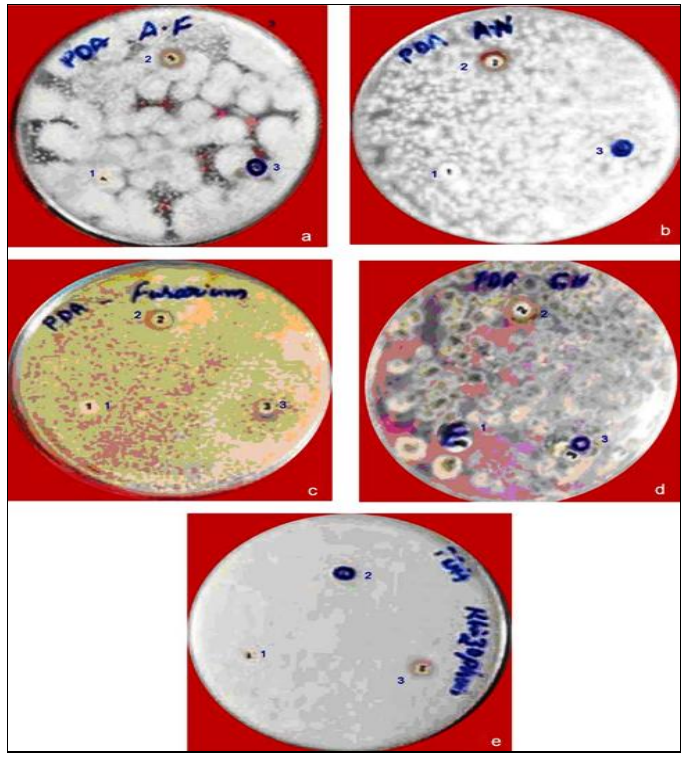


FIG. 3: ANTIBACTERIAL ACTIVITY OF *SVENSONIA HYDEROBADENSIS*
(a) *Aspergilus flavus* (b) *Aspergilus niger* (c) *Fusarium* (d) *Curvularia* (e) *Rhizopus;* 1. Control, 2. Silver Nitrate, 3. Silver Nanoparticles

This results in a collapse of membrane proton gradient causing a disruption of many of the mechanisms of cellular metabolism and hence cell death [28]. Among the five plants tested for antibacterial effect the silver nanoparticles of *B. ovalifoliolata* inhibited bacterial growth at maximum level silver ions do not possess a single mode of action they interact with a wide range of molecular processes with in micro organisms resulting in a range of effects from inhibition of growth and loss of infectivites. The mechanism depends on both the concentration of silver ions present and the sensitivity of the microbial species to silver. The spectrum of activity is very wide and the development of resistance relatively low [29]. The use of plant extracts is effective against various microorganisms including plant pathogens [30].

TABLE 1: ANTIMICROBIAL EFFICACY OF SNPS SYNTHESIZED FROM THE LEAVES OF IMPORTANT SELECTED MEDICINAL PLANTS

Bacterial species	$Ag(NO_3)_2$ Average inhibition zones (mm)	*B. ovalifoliolata* Inhibition zones (mm)		*S. tumbuggaia* Inhibition zones (mm)		*S. hyderobadensis* Inhibition zones (mm)		*T. populnea* Inhibition zones (mm)		*V. rosea* Inhibition zones (mm)	
		Control	SNPs	Control	SNPs	Control	SNPs	Control	SNPs	Control	SNPs
Bacillus	13.26	6	20.1	6	9	6	8	9	10.2	8	10.6
E. coli	12.3	6	20.3	7	15.2	8	10	7	10	10.2	20
Klebsiella	12.62	6	20.1	8	14	6	12	9	10.6	7	10.4
Proteus	17.88	6	20.3	6	10	7	7	7	10.6	7	20.3
Pseudomonas	14.1	6	20.1	6	13	6	15	6	10.6	7	10.7
Fungal species											
A. flavus	-	-	-	6	8	-	14	wz	wz	wz	wz
A. niger	-	-	-	8	-	6	10	wz	wz	wz	wz
Curvularia	-	-	-	6	10	-	12	-	-	-	-
Fusarium	-	-	-	6	7	6	8	-	-	-	-
Rhizopus	-	-	-	6	10	6	15	-	-	-	-

NOTE: (-) indicates no effect (wz) weak zones

Oligodynamic silver antimicrobial efficacy extends well beyond its virotoxicity [31]. The ionic silver strongly interacts with thiol group of vital enzymes and inactivate the enzyme activity [32]. Experimental evidence indicates that DNA loses its replication ability once the bacteria have been treated with silver ions [33]. Ahmad *et al.,* mentioned that the pathogenic effect of nanoparticles can be attributed to their stability in the medium as a colloid, which modulates the phosphotyrosine profile of the pathogen proteins and arrests its growth [24].

The growth of microorganisms was inhibited by the green synthesized SNPs showed variation in the inhibition of growth of micro-organisms may be due to the presence of peptidoglycan, which is a complex structure and after contains teichoic acids or lipoteichoic acids which have a strong negative charge. This charge may contribute to the sequestration of free silver ions.

The SNPs synthesized from plant species are toxic to multi-drug resistant microorganisms. It shows that they have great potential in biomedical applications. Similar observation was found in *Allium cepa* [10], *Argimone mexicana* [11], *Artocarpus heterophyllus* [12]. Warisnocicharoen *et al.,* found that silver nanoparticles have an ability to interfere with metabolic pathways [34].

The findings of Sereemaspun suggested that the inhibition of oxidation based biological process by penetration of metallic nano sized particles across the microsomal membrane [35]. SNPs would interfere with the bacterial growth signalling pathway by modulating tyrosine phosphorylation of putative peptide substrates critical for cell viability and division [36]. When compare with $Ag(NO_3)_2$ the SNPs have more toxicity towards selected microorganisms (Table 1) the reason could be that the silver has more microbial efficacy and more effective in the presence of proteinaceous material.

SNPs synthesized from *S. hyderobadensis* have antifungal activity apart from antibacterial efficacy (Table 1). The mechanism of inhibitory action of SNPs is believed that they are able to penetrate inside the cell and cause further damage by possibly interacting with sulphur and phosphorus containing compounds such as DNA. DNA loses its replication ability expression of ribosomal subunit and cellular proteins and enzymes essential to ATP production become inactivated and SNPs binds the functional group of proteins resulting in the protein denaturation [37].

Silver primarily affects the function of membrane bound enzymes such as those in the respiratory chain. The disruption of membrane morphology may cause a significant increase in permeability, leading to uncontrolled transport through the plasma membrane and finally cell death [33].

The selected five plant species have been used in traditional medicine, so for the SNPs of these plants have not been tested to antimicrobial activity. The present work supports the medicinal values of these plants were confirmed; and also revealed that a simple, rapid and economical route to synthesis of silver nanoparticles and their capability of rendering the antimicrobial efficacy. Moreover the synthesized SNPs enhance the therapeutic efficacy and strengthen the medicinal values of these plants.

CONCLUSION: The present study included the bio-reduction of silver ions through medicinal plants extracts and testing for their antimicrobial activity. The aqueous silver ions exposed to the extracts, the synthesis of silver nanoparticles were confirmed by the change of colour of plant extracts. These environmentally benign silver nanoparticles were further confirmed by using UV-Vis spectroscopy. The results indicated that silver nanoparticles have good antimicrobial activity against different microorganisms. It is confirmed that silver nanoparticles of some medicinal plants are capable of rendering antifungal efficacy and hence has a great potential in the preparation of drugs used against fungal diseases.

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