Green Synthesis, And Antimicrobial Activity Of Silver Nanoparticles From The Medicinal Plant *Vernonia Amygdalina*

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Abstract

Silver nanoparticles of different concentrations were synthesized from the medicinal plant Vernonia amygdalina biologically, as this technique is cost effective and environment friendly. The characterization of the silver nanoparticles was analysed by the UV-Vis Spectrophotometer, Transmission Electron Microscopy (TEM), Scanning Electron Microscopy, X-Ray Diffraction (XRD), and Fourier Transform Infra Red (FTIR). The absorption spectra of silver nanoparticles studied using the UV-Vis spectroscopy, had an absorbance peak at 475nm, in all the three different concentrations (1mM, 3mM and 5mM) of silver nanoparticles. The silver nanoparticles have a 50-70nm size range and appeared to be dominantly spherical and were occasionally triangular. The XRD pattern revealed that these silver nanoparticles have a crystalline nature. The antimicrobial activity of these nanoparticles was studied against E.coli, S.aureus, P.aeuruginosa, and C.albicans. They appeared to have satisfactory inhibitions against the four mentioned microorganisms. Among the different concentrations used in the study, the 3mM (20mg/500µl distilled water) appeared to have the highest sensitivity.

Keywords: Amygdalina vernonia, Bioreduction, Silver nanoparticles, Microorganisms

1. Introduction

Among the noble metals, silver(Ag) is the metal of choice in the field of biological system, living organisms and medicine[1]. Green synthesis of nanoparticles is an emerging branch of nanotechnology[2]. The use of environmentally benign materials like plant leaf extract, bacteria and fungi for the synthesis of silver nanoparticles offers numerous benefits of eco-friendliness and compatibility for pharmaceutical and biomedical applications as they do not use toxic chemicals in the synthesis protocols.[3]. Bio-inspired synthesis of nanoparticles provides advancement over chemical and physical methods as it is a cost effective and environment friendly and in this method there is no need to use high pressure, energy, temperature and toxic chemicals[4].

In the present study an attempt was made to prepare a silver nanoparticles of various concentrations as these particles are fast and specific in their target towards the applications where they are used [2] and evaluate their antimicrobial activity. Medicinal plants are used by a large proportion of developing nation. The reason for this may be a true improvement of disease conditions after herbal treatment. In these countries, the search for new drugs is centered upon the investigation of medicinal plants [5]. The plant used for the synthesis of nanoparticles in the present investigation is Vernonia amygdalina (literally called Grawa in Tigrigna) grown in large amounts in Eritrea, North East Africa. The bacterial strains and the fungal strain used to study the antimicrobial activity were Escherichia coli, Staphylococcus aureus, Pseudomonas aeuruginosa and Candida albicans respectively.

2. Materials and Methods

Homogenate was prepared by weighing 20grams of fresh leaves of Vernonia amygdalina, collected from GEZA BANDA, Asmara. Washed thoroughly (thrice) in distilled water and homogenized using a mortar and pestle. The homogenate was then filtered using a sterile gauze cloth. This homogenate extract prepared was then transferred to a sterile container and used for the study.

2.1 Preparation of Silver Nitrate Solution

Commercially purchased silver nitrate (molecular weight 169.87) was used to prepare three different concentrations 1mM, 3mM, and 5mM. Appropriate amount of silver nitrate was weighed and dissolved in distilled water.

2.1.1 Preparation of Silver Nanoparticles

To 750ml of each millimolar concentration of silver nitrate, 7.5ml of the plant homogenate was added, respectively into a clean conical flask. The conical flasks were then exposed to the sunlight (while being continuously shaken) for the synthesis of the nanoparticles to begin. The colors of the mixture turns from green to brown when exposed to sunlight and once it turns to colorless the particles were settled at the bottom of the flasks. The particles were then centrifuged (high speed centrifuge) and the supernatant was removed. To the particles now settled at the bottom of the centrifuge tubes, about 1ml acetone was added for the removal of the moisture content from the nanoparticles. The nanoparticle suspension were transferred to a watch glass, air dried, collected, weighed and stored in a sterile container.

2.1.2 Preparation of Silver nanoparticles with Different Concentrations

Silver nanoparticle weighing 1mg, 2mg, 3mg, 4mg, 5mg, 10mg, 15mg, 20mg & 25mg from each of the three different concentrations of silver nanoparticles [1mM, 3mM and 5mM] were prepared by suspending them in 500microlitres of sterile distilled water.

2.1.3 Preparation of Silver Nanoparticle Crude Extract Discs

Sterile Whatman No.1 paper was punched into 5mm diameter disc sizes. The Whatman discs were placed in MacCartney bottles and sterilized in an autoclave at 120°C for 15 min. The bottle was transferred into a hot air oven at 60°C to dry for 30minutes. 30 microlitres of 3mM & 5mM silver nanoparticles prepared from Vernonia amygdalina, [20mg in 500microlitres of sterile distilled water] by using a mixer and suspended on the punch prepared discs by applying10µl inoculation each time followed by air drying and stored in sterile containers.

2.2 Antimicrobial activity

The microorganisms used for the study were Escherichia coli (ATCC 25922), Staphylococcus aureus (ATCC 25923), Pseudomonas aeuruginosa (ATCC 27853), and Candida albicans (ATCC 10231). Mueller Hinton agar (HI media) was used for the performance of the antimicrobial assay. Gentamycin (10µg), Tetracycline (30µg), Ciprofloxacin (5µg) and Ampicillin (10µg) were used as controls for the bacteria's. Wells were made (6mm diameter) by using an autoclave sterilized metallic borer. Well isolated fresh colonies of the microorganisms were used to prepare inoculum suspension equivalent to 0.5 Standard McFarland Turbidity (which is 1.5×10^8 Colony Forming Units per ml); microbes were inoculated and incubated at 37°C for 24 hours. After 24 hours the media were examined for inhibition zones and results were recorded in millimeter.

3. Results and Discussions

The inhibitory activity of the silver nanoparticles on microorganisms at different concentrations are presented in Table formats (Table 1to 5).

Table 1. *Different concentrations and milligrams of Nanoparticles.*

Name of organisms	Amount	1mM									3mM									5mM								
		mg/500µl distilled water									mg/500µl distilled water									mg/500µl distilled water								
		1	2	3	4	5	10	15	20	25	1	2	3	4	5	10	15	20	25	1	2	3	4	5	10	15	20	25
E.coli	50µl	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	+	+	+	+	+
	100µl	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	+	+	+	+	+
S.aureus	50µl	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	+	+	+	+	+
	100 µl	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	+	+	+	+	+
P.aeuruginosa	50µl	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	+	+	+	+	+
	100 µl	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	+	+	+	+	+
C.albicans	50µl	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	+	+	+	+	+
	100 µl	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	+	+	+	+	+
Key: "-" no inhibition zone "+" measurable inhibition zone																												

Table 2. *Antimicrobial effects of different concentrations of Silver Nanoparticles*

Name of microbes	1mM In 500µl distilled water					3mM In 500µl distilled water					5mM In 500µl distilled water				
	1mg	2mg	3mg	4mg	5mg	1mg	2mg	3mg	4mg	5mg	1mg	2mg	3mg	4mg	5mg
E.coli	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+
S.aureus	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+
P.aeuruginosa	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+
C.albicans	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+
Key: ''-'' no zone of inhibition ''+'' little zones of inhibition - 50µl SNP was applied/well															

The range of 5mg, 10mg, 15mg, 20mg & 25 mg of each of 3mM and 5mM concentrations of silver nanoparticles proved to have inhibitory effect against Escherichia coli, S. aureus, P. aeuruginosa and C.albicans, compared to that of the rest of the volumes in both 3mM and 5mM concentrations of silver nitrate respectively (Table 3).

Antimicrobial activity of 30µl (impregnated in discs) of the 3mM and 5mM concentration of silver nanoparticles synthesized from Vernonia amygdalina were placed over the media. The results are on Table 4.

Table 3. *Bioassay of 3mM and 5mM concentrations of Silver Nanoparticles against the microorganisms.*

Name of microbe	3mM In 500µl distilled water										5mM In 500µl distilled water									
	5mg		10mg		15mg		20mg		25mg		5mg		10mg		15mg		20mg		25mg	
	50µl	100µl	50ul	100ul	50ul	100ul	50ul	100ul	50ul	100ul	50ul	100ul	50ul	100ul	50ul	100ul	50ul	100ul	50ul	100ul
E.coli	6	7	6	8	7	8	13	15	12	13	6	7	6	7	7	8	12	13	11	13
S.aureus	6	6	6	7	6	7	13	15	11	12	7	7	6	7	7	8	12	13	11	12
P.aeuruginosa	8	9	9	1	11	11	15	16	11	13	8	9	9	10	11	11	13	14	11	12
C.albicans	8	9	9	11	11	12	16	18	11	13	8	8	10	11	11	11	14	15	12	13
Key:- Every inhibition is in millimeter																				

Table 4. *Bioassay of 3mM and 5mM concentration of Silver nanoparticles into the Crude Discs*

Name of microbes	3mM (in disc) 30ul	5mM (in disc) 30ul
E.coli	11mm	8mm
S.aureus	10mm	9mm
P.aeuruginosa	11mm	10mm
C.albicans	12mm	11mm

When the antimicrobial activity of the 20mg/500µl of the 3mM and 5mM concentration of silver nanoparticles were compared it was significantly proved that the 3mM concentration of the silver nanoparticles were more sensitive towards the microorganisms than that of 5mM concentration of the silver nanoparticles. Additionally, when the concentration of the nanoparticles was increased (i.e. from 50µl to 100µl) the sensitivity towards the microorganisms was also increased [6]. It was also found out that the higher the concentration of V.amygdalina extract (i.e. aqueous & ethanol extract), the higher the diameter of the inhibition zone.

Table 5 reveals that the 20mg (suspended in 500µl distilled water) of the 3mM concentration of the silver nanoparticle gave the highest sensitivity.

Table 5. *Comparison between the 20mg/500µl of 3mM and 5mM concentration of Silver nanoparticles*

Name of microbes	3mM (in well) 20mg in 500µl distilled water		5mM (in well) 20mg in 500µl distilled water	
	50µl	100µl	50µl	100µl
E.coli	13mm	15mm	12mm	13mm
S.aureus	13mm	15mm	12mm	13mm
P.aeuruginosa	15mm	16mm	13mm	14mm
C.albicans	16mm	18mm	14mm	15mm

However, when the silver nanoparticles were impregnated on the discs, the sensitivity recorded was poor as compared (see table-4 above) to that of the ones inserted into the well. This could be due to the proper distribution of the particles throughout the media when inoculated in the medium well. And also, the diameter of the discs was 5mm, as a 6mm puncher was not available, now since the diameter of the disc is reduced by 1mm; it is likely to have affected the results.

Additionally, when the effect of the particles against the bacteria's (i.e. Escherichia coli, Staphylococcus aureus, Pseudomonas aeuruginosa) and the fungus (Candida albicans), was evaluated, they seem to have a relatively higher zone of inhibition towards the fungus than the bacteria's. Study of [6] revealed that the fungus Candida albicans was found to be resistant when tested by the Vernonia amygdalina leaf 60% methanol extract.

The mechanism of the antimicrobial action of silver ions is closely related to their interaction with thiol (sulfhydryl) groups, although other target sites remain a possibility. Silver was also proposed to act by binding to key functional groups of enzymes [7]. Silver ions cause the release of K^+ ions from bacteria; thus, the bacterial plasma or cytoplasmic membrane, which is associated with many important enzymes, is an important target site for silver ions. In addition to their effects on bacterial enzymes, silver ions caused marked inhibition of bacterial growth and were deposited in the vacuole and cell wall as granules. They inhibited cell division and damaged the cell envelope and contents of bacteria. Bacterial cells increased in size, and the cytoplasmic membrane, cytoplasmic contents, and outer cell layers all exhibited structural abnormalities. Finally, silver ions interact with nucleic acids; they interact preferentially with the bases in DNA rather than with the phosphate groups, although the significance of this in terms of their lethal action is unclear [7]. It was shown in the present study that the antimicrobial activity of the silver nanoparticles synthesized from Vernonia amygdalina had sensitivity against the microbial strains Escherichia coli, Staphylococcus aureus, Pseudomonas aeuruginosa and Candida albicans. This was also evidenced by the work of [4] which used Pleurotus sajor caju silver nanoparticles. Microbes are unlikely to develop resistance against silver, as they do against conventional and narrow target antibiotics because the metal attacks a broad range of targets in the organisms which means that they would have to develop host mutations simultaneously to protect themselves [4].

4. Conclusion

It is concluded that the homogenate extract of Vernonia amygdalina are capable of producing silver nanoparticles and are

quite stable in solution. Achievements of such a rapid time scales for the synthesis of nanoparticles by various methods of analysis increases the efficiency of synthetic procedures using environmentally benign materials. The present study also concluded that the silver nanoparticles prepared biologically from the plant Vernonia amygdalina developed sensitivity against the microbial strains Escherichia coli, Staphylococcus aureus, Pseudomonas aeuruginosa and Candida albicans. It was proved that among the various concentrations of silver nanoparticles i.e. 1mM, 3mM and 5mM, the 3mM concentration proved to have the most effective antimicrobial activity compared with the other concentrations. The 1mM did not show any kind of sensitivity with all the concentrations used. Also the 100µl of 20mg/500µl of the 3mM silver nanoparticle showed the highest sensitivity among the different concentrations used.

5. References

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