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Research Paper

GREEN SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES BY USING *Aloe barbadensis* AND ITS ANTIBACTERIAL ACTIVITY

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Abstract

Nanotechnology is a field that is making a mark in research field day by day and making an impact in all sphere of human life. Nano particles possess unique, electrical, optical as well as biological properties and are thus applied in catalysis, cosmetics, bio sensing and antimicrobial activity by using plant extract of *Aloe barbadensis* under different experimental condition eco-friendly silver nanoparticles was synthesized. *Aloe barbadensis* has been reported to possess immunodulatory, anti-inflammatory, and UV protective, antiprotozoal, wound and burn healing promoting properties. Synthesis of silver nano particles can be observed qualitatively by colour differentiation pattern (colour changed from pale yellow to reddish brown). Biosynthesized silver nanoparticles were characterized by double beam UV-Vis spectrophotometer and FT-IR. Green synthesized nanoparticles was found to be 194.2 nm determined by Zeta potential. Antibacterial activity shown by synthesized silver nanoparticles against pathogen such as *E-coli, S.typhi, S.aureus, B.thuringiensis, A.tumefaciens* by agar well diffusion method. Bacteriocidal efficiency of synthesized silver nanoparticles was also analysed by viable counts. The Haemolysis percentage was found to be 3.8 by green synthesized silver nanoparticles which were prepared by using Aloe Vera extract which was relatively less toxic. Thus the AgNP's biosynthesis using *Aloe barbadensis* would prove a novel tool in the study of nanotechnology.

Key words: *Aloe barbadensis*, Silver nanoparticles, FT-IR, Zeta potential, antibacterial activity.

INTRODUCTION

Nanotechnology is the study of manipulating matter on an atomic and molecular scale. The term "Nanotechnology" was defined by Tokyo science university Professor Norio Taniguchi in 1974 as "Nanotechnology mainly consists of the processing of separation, consolidation and deformation of materials by one atom or by one molecule". [1]. One nanometre (nm) is one billionth of meter. Nanoparticles posses unique electrical, optical as well as biological properties and are thus applied in catalysis, biosensing, imaging, drug delivery, nanodevice fabrication and in medicine [2].

Nanoparticles can be synthesized using various approaches including chemical, physical, and biological. Although chemical method of synthesis requires short period of time for synthesis of large quantity of nanoparticles, this method requires capping agents for size stabilization of the nanoparticles. Chemicals used for nanoparticles synthesis and stabilization are toxic and lead to non-ecofriendly by-products. The need for environmental non-toxic synthetic protocols for nanoparticles 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"[3]. Biologically active compounds present in the plant extracts such as proteins, polysaccharides and vitamins play a major role in the reduction of silver nitrate into silver nanoparticles. [4a].

Aloe barbandensis gel contains vitamins A (beta-carotene), C, and E, which are known antioxidants. It also contains salicylic acid that possesses anti-inflammatory and antibacterial properties. Extracts from plants usually contains various polyphenol, such as flavanoids, which are excellent reducing agent useful in the synthesis of silver nanoparticles. *Aloe barbandensis* is used for the synthesis of silver nanoparticles because of the presence of natural phytochemicals such as AloinA and AloinB and emodin which provide natural capping and reducing property [5]. The bacteriocidal properties of silver nanoparticles are due to the release of silver ions from the particles, which confers the antimicrobial activity With respect to the microbes. The nanoparticles may also penetrate deep inside the cell wall, thus causing cellular damage by interacting with phosphorus and sulphur containing compounds, such as DNA and protein, present inside the cell. [6].Our main objective is to synthesize silver nanoparticles and use it as antibacterial activity against various bacterial strains.

MATERIALS AND METHODS:

Materials:-

Nutrient broth (M002-100G) and Silver nitrate was of Himedia and all reagents used are of analytical grade.

Methods:-

Preparation of Plant Extract:-

Aqueous Extract of *Aloe barbedensis* was prepared as described by Jenila Rani Duraraj with some modification .Fresh *Aloe barbadensis* leaves were collected. The gel was extracted from the leaves. 25g of gel was chopped into pieces and grinded using mortar and pestle. The gel was mixed with equal volume of distilled water and heated at 85°C for 10 minutes. The mixture was filtered by using Whattman No.1 filter paper & the extract was stored at 4°C. [7]

Synthesis of Silver nanoparticles:-

0.1M Silver nitrate solution was prepared in distilled water. 10ml, 15ml& 20 ml of the $AgNO_3$ solution was taken in a glass beaker and kept in magnetic stirrer for 15 minutes at 65°C. 1ml of plant extract was added drop wise in different volumes of AgNO3 solution with continuous stirring. The mixture was kept on magnetic stirrer for 15 minutes to observe colour change to reddish brown, change in colour indicates synthesis of silver nanoparticles. [8] .All experiments were carried out in triplicates.

Characterization of silver nanoparticles:-

The synthesized silver nanoparticles were characterized with the help of UV-Visible spectrophotometer. (Double beam ELICO BL198). To determine the functional groups of aqueous extract of *Aloe barbadensis* and their possible involvement in the synthesis and stabilization of silver nanoparticles, Fourier Transform Infra Red (FTIR) analysis was carried out of the control samples (Plant extract) and the test sample (plant extract after reaction with silver nitrate).A particle size analyzer (zeta potential) was used to find out the size distribution of AgNP's. In order to find out the particle size of AgNP's, sample was prepared in distilled water i.e. 1ml of sample was diluted with 9ml of distilled water and proceed for analysis. The analysis was carried out in computer controlled particle size analyzer [(ZETA seizers Nanoseries (Malvern Instrument Nano ZS) [9].

Antibacterial activity of green synthesized AgNP's:-

Five bacterial strains were acquisized from NCIM, Pune, Maharashtra and MICC, Chandigarh, Punjab. Viz. S. *typhi* (2501), *S.aureus(*2078*), E.coli(1692), B.thurengenisis* (2514) *and Agarobacterium tumefaciens(2146)*. Determination of antibacterial activity of green synthesized silver nanoparticles was done using two method viz., agar well diffusion method and colony forming unit as per the protocol elaborated by Mittal [10a]

A) Agar well diffusion method:-

Escherichia coli, Salmonella typhi, S.aureus, A.tumefaciens, and *B.thurengenesis,* was inoculated in nutrient broth and incubated overnight at 37^0C. 0.1ml of culture was added in soft agar and mixed well. This was poured on basal agar plate. After 30 min. of incubation at 4˚C, plates were punched using 6mm cork borer. Control plate was prepared by adding 10µl of silver nitrate solution & 10µl of plant extract in wells. Variable volumes of different concentration synthesized AgNP's (10µl, 20µl, 30 µl) were added in respective wells. All plates were incubated at 37^0C for 48hrs. After incubation, zone of inhibition was measured. [10b]. By using disc diffusion method different standard antibiotic discs (AX^{10}, AK^{30}, NX^{10}, NF^{300} and streptomycin) were kept against given bacterial strains as a positive control [7].

B) Colony forming unit method:-

Same strains were inoculated in 5ml of nutrient broth containing various volumes (0.1ml, 0.2 ml, 0.3ml, 0.4ml, 0.5ml and 0.6ml) of Green synthesized silver nanoparticles After overnight incubation, each tube was diluted up to 10^{-5} dilution using sterile saline (0.8% W/V), as described by Sondi and Sondi [11].

Haemolysis test:-

Silver nanoparticles cytotoxic effect was studied by performing haemolysis test. As per explained by Jenila Rani Durairaj [7] with slight modifications.

RESULT AND DISCUSSION:

Silver Nanoparticle synthesis:

In present study, when we added the plant extract of *Aloe Barbadensis* to the 0.1M of silver nitrate aqueous solution , colour of reaction mixture rapidly change yellowish to reddish brown due to the reduction of silver ions Ag^+ to silver nanoparticles and excitation of surface plasmon vibrations, which indicates the formation of silver nanoparticles (figure 1). Colour intensity was increased while increasing time incubation revealed that increased silver nanoparticle synthesis. Similar results were reported by Shankar SS, Ahmed A, Akkamwar B, Sastry M, Rai A, Singh A. [4b] using leaves of *Catharanthus roseus.* Here our plant extract of *Aloe Barbadensis* have wide capability to synthesized silver nanoparticles till reported.

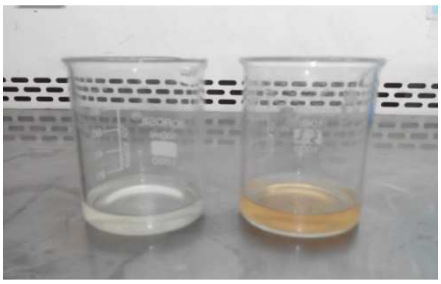


Fig.1 **Silver Nanoparticle synthesis**

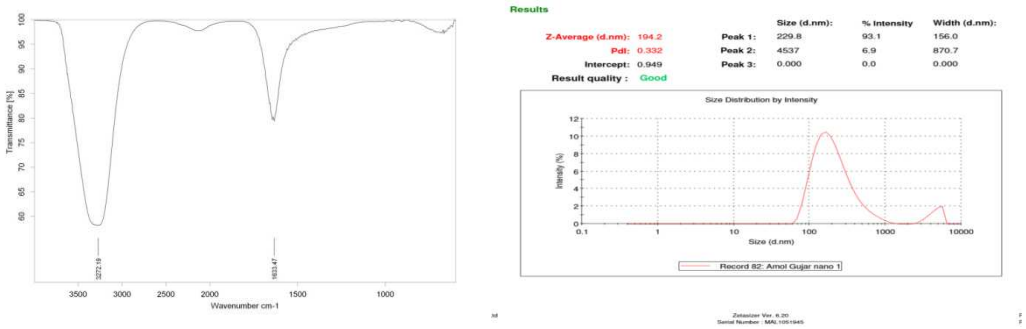
Characterization of silver nanoparticles:

The synthesized nanoparticles were primarily characterized by UV-Vis spectroscopy for the analysis of nanoparticles. In our work, no absorbance peak was observed in control and synthesized AgNPs shows highest absorption peak at 420nm ,Near about same result was observed by Jenila Rani Durairaj [7], their absorbance peak was at 410 nm. In FT-IR analysis, the strong peak was observed at 3271.91 showed C-H stretching, indicates the presence of alkynes group and peak at 1633.49 showed N-H bonds stretching indicates the presences of primary amines group (figure 2). The presence of this functional group is responsible for the stabilization of synthesized silver nanoparticles and also acts as reducing agent. Exactly similar results reported by Firdhouse [12] that the peak at 3371cm^{-1} and 1635cm^{-1} by using pomegranate fruit peel extract.

Efficiency of nanoparticle depends on their size, smaller the size of nanoparticles higher their efficiency .In present study we got nanoparticles of size 194.2 d. nm which was determined by

zeta potential(figure 2). Whereas Kirthika P. and Satoshi kokura, et al, [9] reported the size of created nanoparticles 581nm & 316nm respectively, from five different herbal plants such as Terminalia chebula, Mimusops elengi, Myristica fragrans, Centella asiatica and Hemidesmus indicus. Hence *Aloe barbadensis* proved to be the best reducing agent for green synthesis of silver nanoparticles.

Fig.2: FT-IR spectra and zeta analysis graph of green synthesized silver nanoparticles from aqueous extract of *Aloe barbadensis.*



Antibacterial activity determination of biosynthesized silver nanoparticles:

Medicinal plants mediated synthesized silver nanoparticles showed good inhibitory activity against *Escherichia coli, Micrococcus sp., Staphylococcus aureus, Corynebacterium diptheriae* and *Candida albicans*[13]. In this study the silver nanoparticle synthesized using aqueous extract of *Aloe Barbadensis* as reducing agent has exhibited a fairly significant antibacterial activity against *B.thureingenesis , S.aureus ,E.coli, Agarobacterim tumefaciens* and *S.typhi* with distinct zone of inhibition as per table no.1 which is more as compare to reported one by [14]against only two pathogen here in our present work we detect antibacterial activity against five pathogens. The zone of inhibition was found to be increased by increased in concentration of Green synthesized silver nanoparticles from 10ul to 30ul. It was also observed that there was no zone of inhibition in the control (Aloe Vera plant extract and silver nitrate solution) figure no. (3-8) & antibiotic discs viz. streptomycin, Ampicillin/Cloxacillin, Amikacin, Norfloxacin & Nitrofurantoin showed standard zone of inhibition against the same bacteria.

Bactericidal efficiency of silver nanoparticles also analyzed by viable counts determined for the control of *E.coli* and *S.typhi*, and the silver nanocompsite containing samples (as shown in figure 9-12), was found to be more efficient bactericidal agent, as the concentration increases efficiency found to be increased, similar results were reported by Mittal [10c].The Haemolysis percentage was found to be 3.8 by green synthesized silver nanoparticles which were prepared by using Aloe barbadensis extract which was relatively less toxic.

Table no.1: Zone of inhibition (in diameter in mm) obtained by silver nanoparticles produced by *Aloe barbadensis* extract.

Sr. no.	Name of micro-organism(culture No)	Zone of inhibition in mm			NX	NF	AX
		10ul	20ul	30ul	10mcg	300mcg	10mcg
1	*B.thuringiensis* (2514)	5	11	11	11		-
2	*S.aureus*(2078*)*	10	11	11	-		10
3	*E.coli* (1692)	13	13	15	14		-
4	*S.typh*(2501)	3	6	10		12	
5	*A.tumefaciens*(2146)	11	11	14	12		-

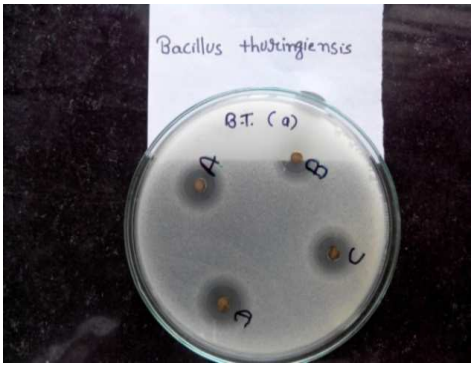


Fig **3: Zone of inhibition of *B.thurengenesis***

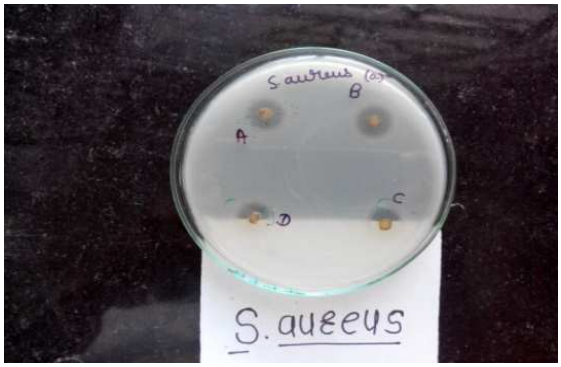


Fig: 4: Zone of inhibition of *S. aureus*

Fig: 5: Zone of inhibition of *E.coli*.

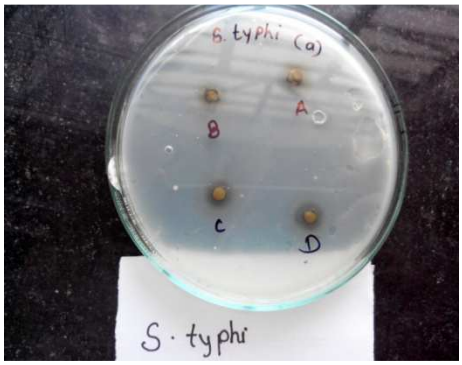


Fig: 6: zone of inhibition of *S.typhi.*

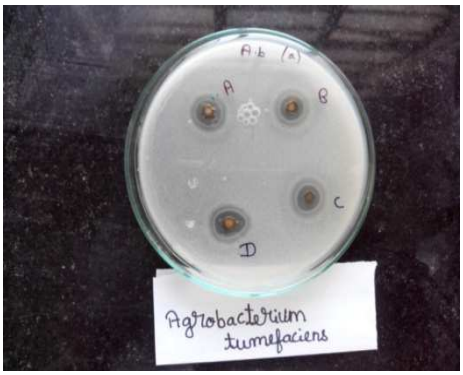


Fig: 7: Zone of inhibition of *Agarobacterium tumefaciens*.

Fig: 8 Control plate.

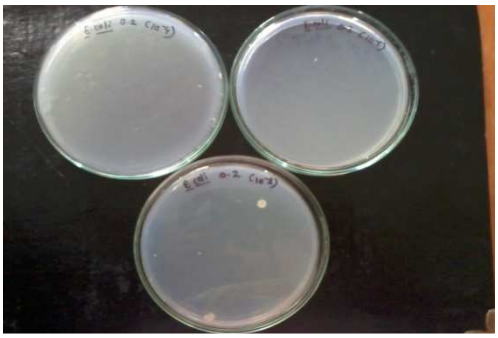


Fig no.9: Viable count of *E.coli* with nanoparticles.

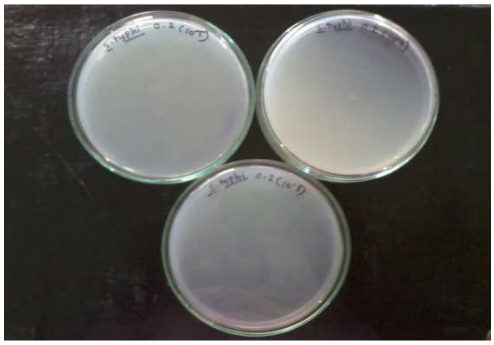


Fig.no.10: Viable count of *S.typhi* with nanoparticles.

Fig.no.11: Viable count of *E.coli* without nanoparticles.

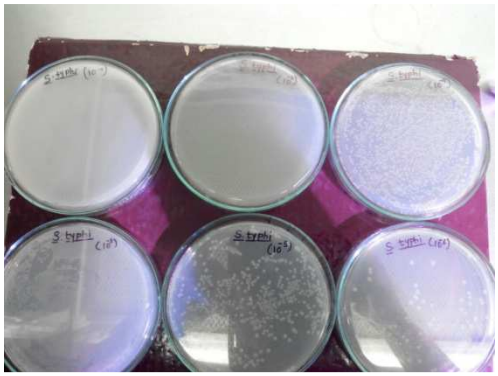


Fig. No12: Viable count of *S.typhi* without nanoparticles.

Conclusion:

Silver nanoparticles were synthesized by using *Aloe barbandensis.* Characterization of synthesized nanoparticles was studied by using UV visible spectra, FT-IR and Zeta potential. By the FT-IR,the functional group was found to be amide and maximum wavelength was at 1633.49. The maximum absorbance of silver nanoparticles by UV visible spectra was found to be at 420nm and the size was found to be 194 dnm by zeta potential. Synthesized AgNPs shows antibacterial activity against bacterial i.e., *E.coli, S.typhi, B.thuringenisis* and *A.tumeficiens* by agar well diffusion method.

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