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Green synthesis of silver nanoparticles using *Aloe vera* extract and assessing their antimicrobial activity against skin infections

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Abstract— The synthesis of nanomaterials is currently one of the most active in nanoscience branches especially those help to improve the human quality life. Silver ions have the disadvantage of forming complexes and the effect of the ions remained only for short time. This disadvantage have been overcome by the use of silver nanoparticles (AgNPs) which are in inert form and also exhibit antimicrobial function by inducing the production of reactive oxygen species. In fish spas, clients may submerge their hands, feet or whole body in basins with *Garra rufa* fish, for dead skin removal. Skin infections may result from using these spas. We also focused on identification of some common pathological bacterial and fungal isolates responsible for causing foot cracks. In the present work we have made an attempt to evaluate antibacterial and antifungal activities against various pathogens isolated from fish spa water samples, fish gut and from human foot cracks. The fungal isolates identified were *Aspergillus sp., Penicillium sp*. and *Trichophyton sp.,* and bacterial isolates as Gram positive and Gram-negative organisms. In this work we report the synthesis of silver nanoparticles by green synthesis reduction method of silver nitrate ($AgNO_3$) solution of different concentrations like 1mM,5mM,10mM using *Aloevera* plant extract of various dilutions like 4:1,3:2,2:3,1:4 respectively. The results show that higher silver nanoparticles were synthesized by using plant extract and water in the ratio of 3:2 and optimal concentration of silver nitrate was found as 10mM. Green synthesized Silver nanoparticles obtained were characterized by UV−visible spectroscopy and Scanning Electron Microscopy (SEM). The zones of inhibition of synthesized silver nanoparticles against the bacterial and fungal pathogens were variable and comparatively the highest was shown by 3:2 diluted plant extract prepared by using 10mM silver nitrate solution of about 24mm and 20mm against Gram positive and Gram negative organisms respectively isolated from fish spa samples. From the present study, it is concluded that green synthesized silver nanoparticles have wide spectrum antibacterial activity in low concentration than antifungal activity and therefore may be used as an alternative approach in medicine and pharmaceutical field in future.

Keywords—*Green synthesis, silver nanoparticles, fish spa isolates, antimicrobial activity.*

I. INTRODUCTION

In the recent years, biologically synthesized nanoparticles are in great demand when compared to their chemical counterparts due to their applications in various areas like medicine, catalysis, energy and materials [1].Silver ions have the disadvantage of forming complexes and the effect of the ions remained only for short time. This disadvantage have been overcome by the use of silver nanoparticles (AgNPs) which are in inert form and also exhibit antimicrobial function by inducing the production of reactive oxygen species[2]. The AgNPs due to their large surface to volume ratios, high dispersion and crystallographic surface structure, display strong biomedical properties like antimicrobial, anticancer, antidiabetic, antioxidant and anti-inflammatory[3]. Apart from this, silver nanoparticles are extensively used in food processing industries, medical implants, in the fabrication of ointments and creams to inhibit burns and wound related infections. The physico-chemical, optical and electronic properties of nanomaterials depend on the size, shape, and surface morphology of nanoparticles[4].When compared to the other metal nanoparticles, silver nanoparticles (AgNPs) have gained much attention due to the surface plasmon resonance (SPR) (strong absorption in the visible region), which can be easily monitored by UV–visible spectrophotometry[5]. Silver nanoparticles can be synthesized by a variety of chemical and physical methods that involves chemical reduction, photochemical reduction, electrochemical reduction, heat vaporization, sol–gel method, microwave-assisted synthesis and ultra sonication method [6,7]. The chemical agents used in reduction are often toxic, expensive and non-ecofriendly in nature. Hence, there is a need to develop an eco-friendly process that breaks the scenario of using toxic chemicals for the purpose. In order to overcome toxicity in the synthesis and biological applications, bio-reduction methods based on

microorganisms, plant extracts are being used due to the ease of synthesis, environmentally benign nature and greater stability of nanoparticles [8].

Aloe vera is a natural product frequently used in the field of cosmetology and dermatology as it is used since centuries for its health, beauty, medicinal and skin care properties. It belongs to Asphodelaceae *(Liliaceae*) family and is a shrubby or arborescent, perennial, xerophytic, succulent, pea-green color plant. Inside the leaves an inner clear gel that contains 99% water and rest is made of glucomannans, amino acids, lipids, sterols and vitamins. *Aloe vera* contains 75 potentially active constituents: vitamins, enzymes, minerals, sugars, lignin, saponins, salicylic acids and amino acidsetc[9,10].The other beneficial effects includes immunomodulatory, wound and burn healing, hypoglycemic and anticancer, gastro-protective, antifungal and anti-inflammatory properties. These beneficial therapeutic properties of *Aloe vera* have been employed for a number of commercial applications [11].

II. RELATED WORK

Reports of the reduction of precious metals by inactivated plant materials date almost 100 years ago. *Rafique et al.2017* studied the reduction of Ag by plant chlorophyll and the reduction of Au by extract of fresh leaves respectively. About 66 years ago *Peralta Videa et al, 2016* reported the reduction of $AgNO_3$ by plant cells. These researchers reported that the solution pH is a key variable to control the size and to reduce the polydispersity. One of the main challenges using traditional methods to synthesize AgNPs is the high cost of equipment. Moreover, sometimes the addition of stabilizers is needed during the synthesis to avoid nanoparticle aggregation. In the present study we focused on stability of green synthesized AgNPs by using *Aloe vera* gel which can be used traditionally manifold ranging from applications to reduce perspiration to oral dosing for gastro intestinal ailments. Therefore we made an attempt to check the antimicrobial activity of synthesized AgNPs coated on fabric material that can be used as nano coated socks material to lower the infections commonly spread by fish spa and also against human foot crack pathogens.

III. MATERIALS AND METHODS

Preparation of *Aloe Vera* plant extract

Fresh leaves of *Aloe vera* were collected in and around the Bhavan's Vivekananda College campus. For preparation of aqueous leaf extract, 20 gm fresh leaves were washed with distilled water and then de-ionized water to remove dirt particles and air-dried. The dried leaves were crushed with mortar and pestle [12].The mashed sample of fresh leaves are then mixed with 100 ml of sterile double distilled water in a 250 ml beaker and boiled for 10-15 mins and then filtered using Whatmann No. 1 filter paper. The filtered leaf extract sample was stored at 4^0C for further use.

Green synthesis of silver nanoparticles

Silver nitrate solutions (1mM, 5mM, 10mM) were prepared in distilled water separately. Then 4:1, 3:2, 2:3, 1:4 dilutions of plant extracts were added to different concentrations of silver nitrate at room temperature. The transparent colorless solution was converted to the brown color indicating the formation of silver nanoparticles.

Characterization of AgNPs

UV-Vis spectroscopy

UV-Vis absorption spectrum of the sample was done in the wavelength range from 300 to 700 nm to determine absorption maxima where the reduction of pure Ag^+ ions takes place. The measurement of AgNPs synthesis under different conditions like variation of concentration of leaf extract, molarity of $AgNO_3$ solution and incubation time were taken at a particular wave length that gave absorption maxima at 430nm.

Scanning electron microscope (SEM)

Scanning electron microscope was used to observe the size, shape of green synthesized AgNPs which are prepared from 3:2 dilutions of plant extract and 10mM $AgNO_3$ solution

ISOLATION OF PATHOGENS AND ASSESSING ANTIMICROBIAL ACTIVITY OF SILVER NANOPARTICLES

Pathogens are isolated by using different Medias such as NA and PDA for bacteria and fungi respectively. Pieces of 1-2 cm size of sterile cotton fabrics were soaked in green synthesized AgNPs for required time period. The pieces were dried and washed repeatedly to remove any adsorbed particles. Finally the fabric pieces were dried at 60°C in a hot air oven for 10min.Then the antimicrobial activity of nano silver-treated fabrics was evaluated against test pathogens.

IV. RESULTS AND DISCUSSION

Aloe vera leaf extract used for green synthesis of silver nanoparticles acts as both reducing and stabilizing agent. The reduction of silver ions into silver nanoparticles during exposure to the leaf extract is indicated by color change that could be read spectrophotometrically. Silver nanoparticles exhibit brown color in aqueous solution due to the excitation of surface plasma vibrations (Figure. 1). Silver nanoparticles prepared from leaf extract and silver nitrate solution exhibit a sharp emission peak at 430nm with in 24 hr of incubation time (Figure. 2). The SPR occurred at 430 nm in the beginning of the reaction and it was stabilized in the same wavelength even after the completion of the reaction.

Various concentration of silver nitrate was optimized for the maximum synthesis of silver nanoparticles. Interestingly, 10

mM concentration of silver nitrate supported higher amount of AgNPs synthesis[13,14].Similarly, different concentration ratios of leaf extracts and silver nitrate solution were also optimized for maximum production of silver nanoparticles. Interestingly 3:2 reaction medium solution was turned to brown colour within 30 min of incubation period, indicating rapid formation of silver nanoparticles (Figure 3). Thus the optimized medium supported the maximum formation of silver nanoparticles and the reaction was occurred very rapidly. To access the stability of silver nanoparticles formed in the reaction solution at pH 7, UV–Vis spectroscopic analysis was carried out. This study clearly showed no alteration in the peak at 430 nm even after 1 month of incubation period, indicating strong stability of biosynthesized silver nanoparticles (Figure. 4). Therefore, it is clear that the optimization process played a pivotal role in the particles stability and aggregation.

Recently, there is an enormous interest in understanding the possible reaction mechanism for the plant mediated nanoparticles synthesis. Some studies have indicated that biomolecules present in the plant extract play a crucial role in reducing the ions to the nano size[15]. Although the reduction of Ag+ ions is environmentally benign, it is chemically a complex phenomenon involving an array of biomolecules such as enzymes/proteins, flavonoids, phenols, vitamins, organic acids such as citrates, amino acids, and polysaccharides [16].

The size and morphology of synthesized nanoparticles (prepared from 10mM$AgNO_3$ and 3:2 dilution of *Aloevera* leaf extract) was determined by scanning electron microscope (Figure-7). Synthesized AgNPs were spherical in shape with well dispersion and less aggregated. The particle size of AgNPs was found to be 49-82 nm. The formation of large size particles may occurs due to increase in concentration of silver nitrate solution [17].

The morphological characteristics of bacteria and fungi isolated from fish spa and foot crack samples were shown in table 1 &figure 6, table 2 & figure 7 respectively. To these isolates i.e Gram positive and Gram negative bactetia (*Bacillus sp*.) and fungi (*Penicillium sp.,Aspergillus sp.,Trichophyton sp.)* the antimicrobial activity of AgNPs coated on fabric pieces (socks material) were checked. The AgNPs synthesized by *Aloe vera* extract showed excellent antibacterial activity against tested pathogens as shown in figure 8.The highest antibacterial activity of AgNPs from *Aloe vera* extract was found against *Bacillus sp*. as 25mm and 20mm (zone of inhibition) respectively and comparatively lowest antifungal was found against *Aspergillus sp.* as 11mm as shown in figure 8.Therefore green synthesized AgNPs showed significant antibacterial activity against test pathogens indicating their potential use as alternate for antibiotics.

V. CONCLUSION

In the present study a simple, one-step, cost effective and eco-friendly method of green synthesis of AgNPs using *Aloe vera* leaf extract was developed without the involvement of any hazardous chemical and also the synthesized AgNPs remained stable for 30 days without agglomeration as these plant extracts have high reducing potential and acts as capping agents too. The synthesized AgNPs comparatively showed significant antibacterial activity against bacterial isolates of various tested samples. The synthesis of AgNPs and their conjugation with the biomolecules present in *Aloe vera* gel extract can prove to be of much importance and advantageous in healing foot cracks without using antibiotics.

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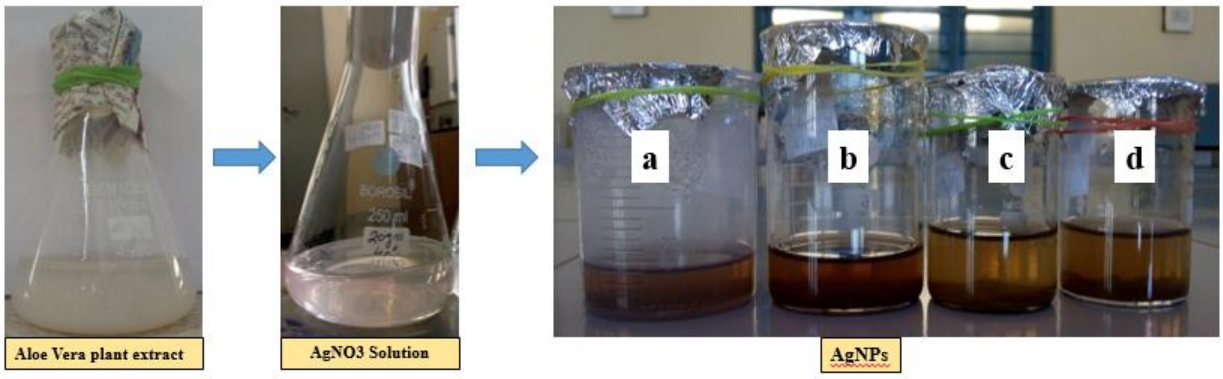

Figure 1: Formation of silver nanoparticles by using *Aloe vera* plant extract and silver nitrate solution indicated by formation of brown color (a. 4:1, b. 3:2, c. 2:3, d. 1:4 dilutions).

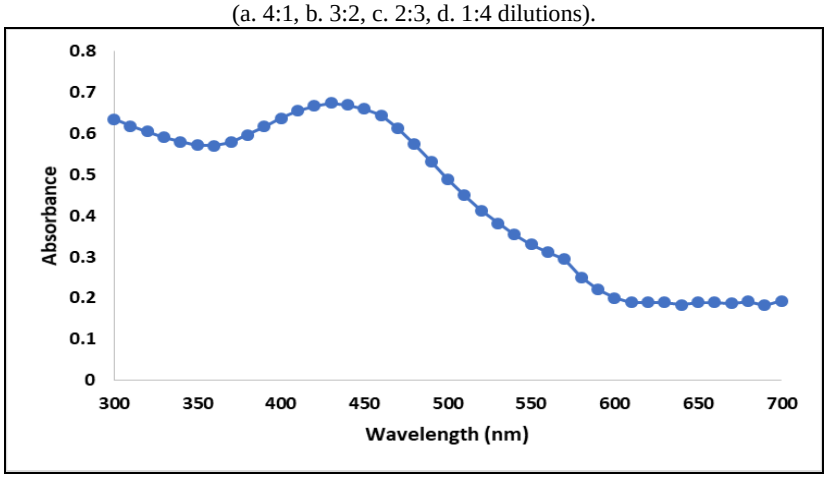

Figure 2: UV- visible spectroscopy of AgNPs synthesized using *Aloe vera* extract

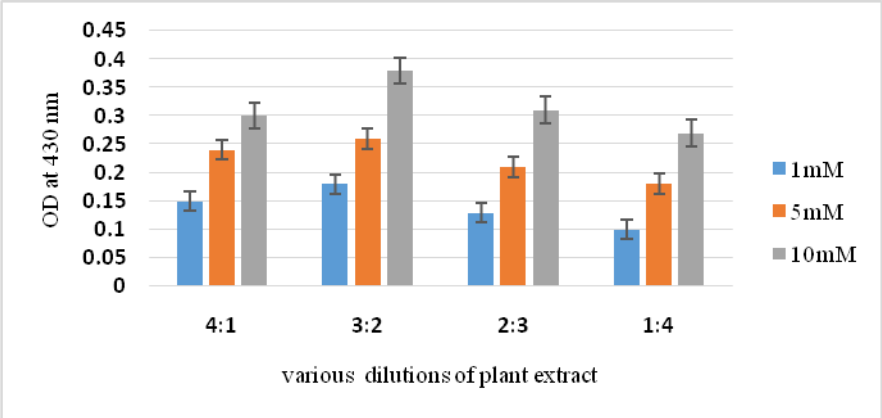

Figure 3: Absorbance of silver nanoparticles prepared using various dilutions of aqueous *Aloe vera* extract incubated for 24hrs

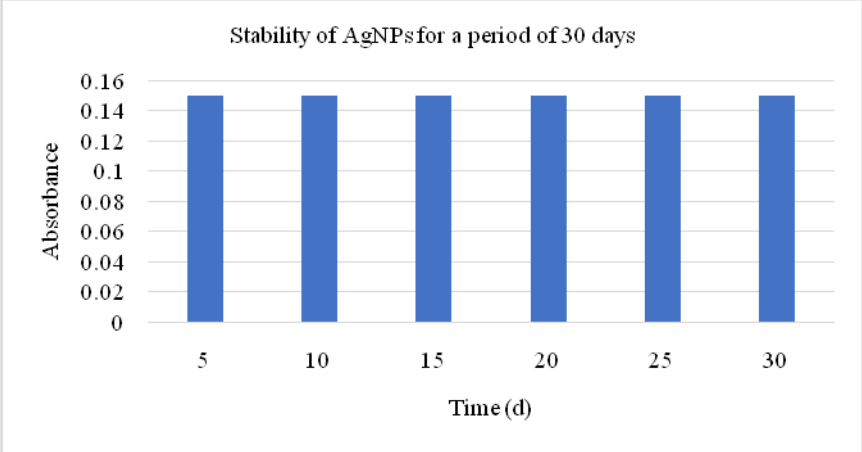

Figure 4: Stability of green synthesized silver nanoparticles for a period of 30 days using 3:2 dilution and 10mM silver nitrate solution

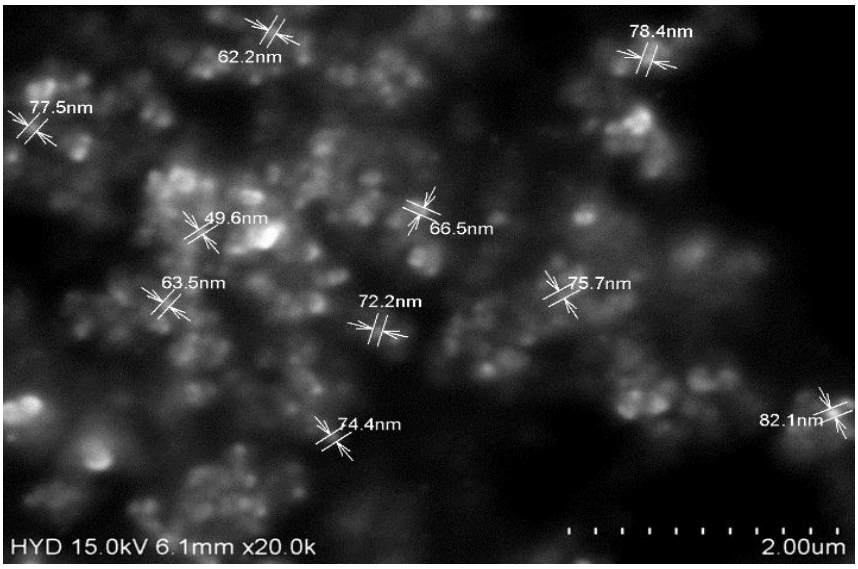

Figure. 5: SEM image of silver nanoparticles synthesized from 10mM silver nitrate with 3:2 dilution of leaf extract.

Table 1: Bacterial pathogens isolated from fish spa samples

Bacterial Isolate	**Colony morphology**	**Staining reaction**
Gram positive	Small round or flat shaped and white pigmented colony	Gram staining
Gram negative	Large, convex, smooth surfaced, entire marginal colony	Gram staining

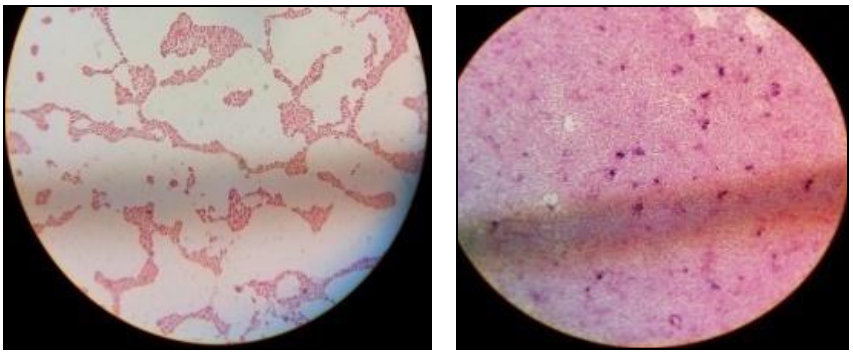

Figure 6: Microscopic observation of Bacterial isolates

Table 2: Fungal pathogens isolated from foot cracks samples of humans

Fungal Isolate	**Colony morphology**	**Staining reaction**
Penicillium sp.	Large ellipsoidal, cylindrical or fusiform, and greenish, smooth or rough-walled	Lacto phenol cotton blue
Aspergillus sp.	White surface, texture velvety, cottony, yellow,green,brown or black color colony	Lacto phenol cotton blue
Trichophyton sp.	Thin, smooth walled, fusiform or cylindrical,elongated,grape like clusters or cigar shaped	Lacto phenol cotton blue

Figure 7: Microscopic observation of fungal isolatesa. *Penicillium sp.*, b. *Aspergillus sp.*c. *Trichophyton sp.*

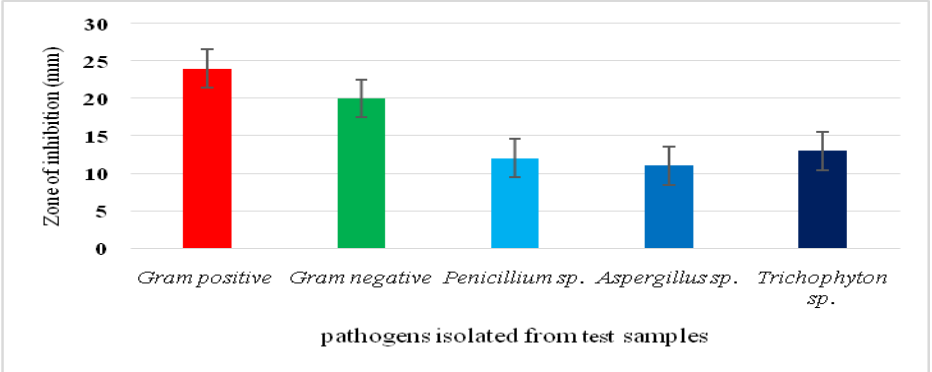

Figure 8: Antimicrobial activity of green synthesized AgNPs coated on fabric

