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Promising green synthesis of silver nanoparticles from Arjuna, Baheda and cotton plant extracts and study of their comparative antimicrobial activity

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Abstract
Recently, plant mediated synthesis of silver nanoparticles is gaining huge and remarkable importance as compared to available and most widely used physical and chemical methods. In the present study, silver nanoparticles were synthesized using Arjuna (*Terminalia arjuna* Wight & Arn.), Baheda (*Terminalia belerica* Roxb.) and Cotton (*Gossypium herbaceum* L.) plant extracts. Phytochemicals present in plant extracts carry out bio-reduction of silver ions and results in change in the color of the solution. The Ag NPs formed were confirmed by UV-VIS spectroscopy and TEM, SEM and PSA analysis were used to determine the shape and size of the synthesized nanoparticles. Further, antibacterial and antifungal activities of biosynthesized Ag NPs were assessed by well diffusion method by measuring the zone of inhibition. Ag NPs synthesized using all three plant extracts showed significant antibacterial and antifungal activity against bacterial (*Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa*) and fungal (*Aspergillus niger, Candida albicans* and *Trichoderma sp.,*) strains. It was observed that, bacterial and fungal growth was inhibited with increase in volume of Ag NPs. Maximum inhibitory activity was exhibited to Ag NPs synthesized from cotton plant extract. This study highlights, preparation of plant extracts and their use in synthesis of silver NPs and its novel potential use as an antimicrobial agent.

Keywords: plant extracts, plant mediated synthesis, silver nanoparticles, antibacterial, antifungal activity

Introduction
Plant-mediated nanoparticle (NP) synthesis, is also known as green synthesis based on the utilization of plants in the synthesis of NPs. Plant parts are used for the preparation of plant extracts and then these plant extracts are employed for the synthesis of NPs. This method is particularly beneficial due to its various characteristics as it is safe, eco-friendly, cost-effective and free of using high pressure, energy, temperature and other factors that can be hazardous to living organisms [1]. The other very important aspect of this method is that, it is also simple to scale up for large-scale NP production [2]. Many phytochemicals, such as flavonoids, saponins, terpenoids, alkaloids, phenolics, macromolecules, quinones and others present in plants, aid in the biological synthesis of NPs without the use of harmful chemicals [3, 4]. These phytochemicals function as a bio reducing and stabilizing agent in the process of synthesis. NPs are found to exhibit high reactivity and chemical stability due to high surface area to volume ratio that is ultimately responsible to enhance their interaction with other molecules [5]. There are different uses of silver NPs (Ag NPs) as effective water purifying and food preservative agent [7]. Silver has been found to be toxic to micro-organisms compared to mammalian cells. This property enables silver NPs to exhibit strong antibacterial, anti-inflammatory, anticancer, antifungal and antioxidant activities [5, 6]. Therefore, employing plant mediated synthesis of AgNPs can aid in medical field to be used as antimicrobial agent by reducing the toxic effects caused by physical, chemical and irradiation methods. In the present study, plant extract mediated synthesis of silver NPs was carried out. Plant extracts from three plants viz., Arjuna (*Terminalia arjuna* Wight & Arn.), Baheda (*Terminalia belerica* Roxb.) and Cotton (*Gossypium herbaceum* L.) were used as bio reducing agents for Ag NP synthesis. The green synthesized silver NPs were further characterized by UV-VIS spectroscopy, scanning electron microscopy (SEM), transmission electron microscopy (TEM) and particle size analysis (PSA). Ag NPs synthesized using these three plants were further assessed for their antibacterial and antifungal activities. The results obtained from Ag NPs synthesized using all three plant extracts are compared for their antimicrobial property and found to be dose and size dependent.

Materials and Methods
Materials
The analytical reagents, culture media and chemicals used for the study were purchased from Himedia Laboratories Pvt. Ltd., Mumbai, India. Autoclaved distilled water was used to conduct all the experiments. Plant materials [Arjuna (*Terminalia arjuna* Wight & Arn.), Baheda (*Terminalia belerica* Roxb.) and Cotton (*Gossypium herbaceum* L.)] used for synthesis of silver nanoparticles were collected from Ahmednagar,

Maharashtra, India. Bacterial (*Escherichia coli, Pseudomonas aeruginosa* and*Staphylococcus aureus*) and fungal (*Aspergillus niger, Candida albicans* and *Trichoderma sp*.,) strains were procured from Ingenious Biosciences, Consultancy Contract Services, Pune.

Plant extract preparation

The plant materials viz. leaves and peels of all the three plants namely Arjuna (*Terminalia arjuna Wight &Arn*.), Baheda (*Terminalia belerica Roxb.*) and Cotton (*Gossypium herbaceum L.*) were collected. These were rinsed thoroughly to remove the adhered pollutants and dust particles with running tap water many times followed by sterile distilled water [8]. The leaves and peels were then dried in an incubator (40°C, 48 hrs) before being ground into fine powder in a blender. A total of 500 mg of resultant fine powder was collected and blended with 20 ml ethanol (80% v/v). To dry the mixture, it was stirred occasionally for 3 hours and once the ethanol had evaporated, the dried powder was dissolved in 2 mL sterile distilled water [9]. This mixture was stored at 4°C for further experiments.

Synthesis and Characterization of AgNPs

Synthesis of AgNPs

1ml of the obtained mixture was diluted in 14 ml sterile distilled to obtain 15 ml of solution for each plant extract. Ag NPs were synthesized by reducing freshly prepared silver nitrate (1mM) using the above prepared plant extract. The ratio of silver nitrate to plant extract used in the reaction was maintained as 1:1. The obtained 30 ml solution was then incubated in incubator (60°C, 5 hrs). The initial color and change in color obtained after synthesis of Ag NPs was recorded [10].

Characterization of AgNPs

The AgNPs synthesized were characterized by UV-Visible spectroscopy, FESEM (Field Emission Scanning Electron Microscopy) and TEM (Transmission Electron Microscopy) and PSA (Particle Size Analysis). The absorption spectra of AgNPs synthesized by bioreducing silver ions from silver nitrate solution using plant extract was analyzed by UV-Visible spectrophotometer in the range of 300-700 nm (Double beam UV-1, thermo scientific) [8, 10, 11]. TEM and FESEM were used to determine the morphology, shape, elemental mapping and nanoparticle size, etc. of AgNPs. Libra 120 (Carl Zeiss make) was used to examine the silver nanoparticles synthesized using plant extracts [12]. FESEM analysis was carried on Zeiss EVO-18 scanning electron microscope (NOVA NanoSEM 450) [13]. Particle size analysis of synthesized Ag NPs was performed on NANOPHOX (NX0088) (Sympatec GMBH make) [14].

Antimicrobial activity of AgNPs

Well diffusion method was employed to study the antibacterial and antifungal activity of synthesized Ag NPs from three different plant extracts. Different volumes of Ag NPs in the range of 100 - 500 µl at a difference of 100 µl was used for studying both the activities.

Antibacterial activity of AgNPs

Antibacterial activity of different volumes of Ag NPs was checked against *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus*. To conduct antibacterial activity, 100 µl of bacterial inoculum (revived initially and grown until it reaches optical density 0.5-1.0) was spread on nutrient agar plates for each culture separately. Further varied volumes of Ag NPs synthesized using each plant extract were inoculated in the wells separately along with positive (ampicillin) and negative (sterile distilled water) controls. These plates were incubated in incubator (for 24 hrs, 37ºC) to record the diameter of zone of inhibition (mm). All the experiments were conducted in triplicates [15].

Antifungal activity of AgNPs

Antifungal activity of different volumes of Ag NPs was checked against *Aspergillus niger, Candida albicans* and *Trichoderma sp*. Initially uniform suspension of fungal cultures was prepared by scrapping spores from specific fungi grown in petri-plates and inoculating in 2 ml sterile phosphate buffer saline. Fluconazole and sterile distilled water were used as positive and negative controls. Using spread plate technique, 100 µl spore suspension of each fungal strain was spread aseptically on PDA petri plates separately and varied volumes of Ag NPs synthesized using each plant extract were inoculated in the wells separately along with positive and negative controls. These plates were incubated in incubator (for 48 hrs, 30ºC) to record the diameter of zone of inhibition (mm). All the experiments were conducted in triplicates [16].

Results and Discussion

Synthesis and Characterization of AgNPs

Synthesis of Ag NPs

Formation of Ag NPs using plant extracts and silver nitrate solution was determined based on color change of the solution. Silver ions from silver nitrate solution were reduced to silver nanoparticles with the aid of bioreducing agents present in plant extracts. This reduction led to change in color of the solution that was monitored and subjected to further characterization of these particles. On adding Arjuna plant extract to silver

nitrate solution, the color of solution changed from transparent, colorless to dark orange with progress in reaction, whereas in case of cotton plant extract color changed to dark reddish brown and in case of Baheda plant extract, colorless solution was changed to dark brownish color. There are numerous reports that suggest presence of various phytochemicals (tannins, flavonoids, macromolecules, resins, terpenoids, etc.) in plant extract that act as bio-reductant in synthesis of Ag NPs by reducing silver ions [3, 4, 17].

UV-VIS spectroscopy

The progress in reaction between silver nitrate and plant extracts was observed by UV-VIS spectroscopy to determine the formation and stability of synthesized Ag NPs. The green synthesized Ag NPs were scanned in the range of 300-700 nm and surface plasmon resonance (SPR) band was determined that occur due to excitation of surface vibration of NPs. Maximum absorbance of Ag NPs synthesized using Arjuna, Baheda and Cotton, plant extract was found to be at 440 nm, 415-440 nm and 450 nm respectively.

PSA, TEM and SEM

Table 1 illustrates the morphology, shape, size and polydispersity index of Ag NPs synthesized using three different plant extracts. Figure 1 shows the SEM image of Ag NPs synthesized from all the three plant sources.

An organic layer was seen on the produced Ag NPs synthesized using all three plant extracts. Various polyphenolic compounds found in plant extracts, such as terpenoids, flavonoids, etc. could be implicated in coating the NPs. These compounds serve as capping agents, which help to keep the surface of produced NPs stable [18].

Table 1: Characterization of Ag NPs using PSA, TEM and SEM analysis.

Technique	Characters	Cotton	Arjuna	Baheda
PSA	Size	13.74 nm	85.87 nm	55.52 nm
	Polydispersity index	0.18	0.3	0.31
TEM	Ag NPs synthesized were polydispersed and coated with an organic layer			
	Shape	Cuboidal	Spherical, few cuboidal	Trigonal
SEM	Shape and nature	Oval, spherical, most were aggregated, few individual particles also seen	Spherical, multiple faces show cuboidal or rhombus shaped particles present inside	Superficially spherical

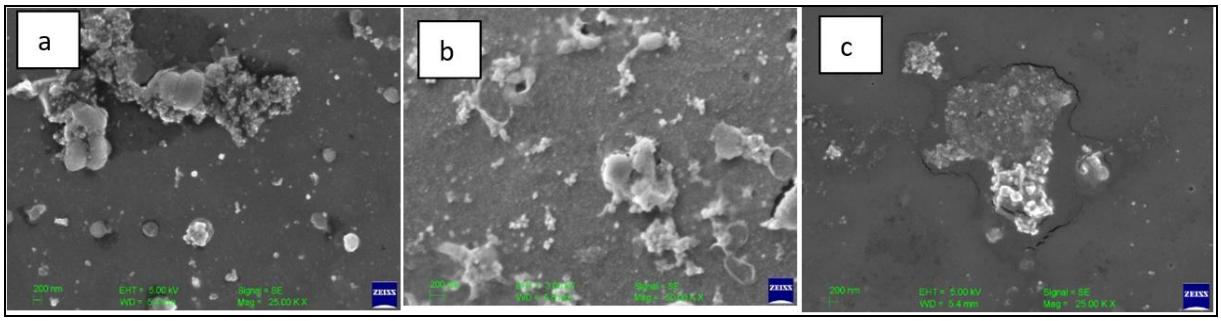

Fig 1: SEM image of synthesized Ag NPs a. Cotton b. Arjuna c. Baheda plant extract

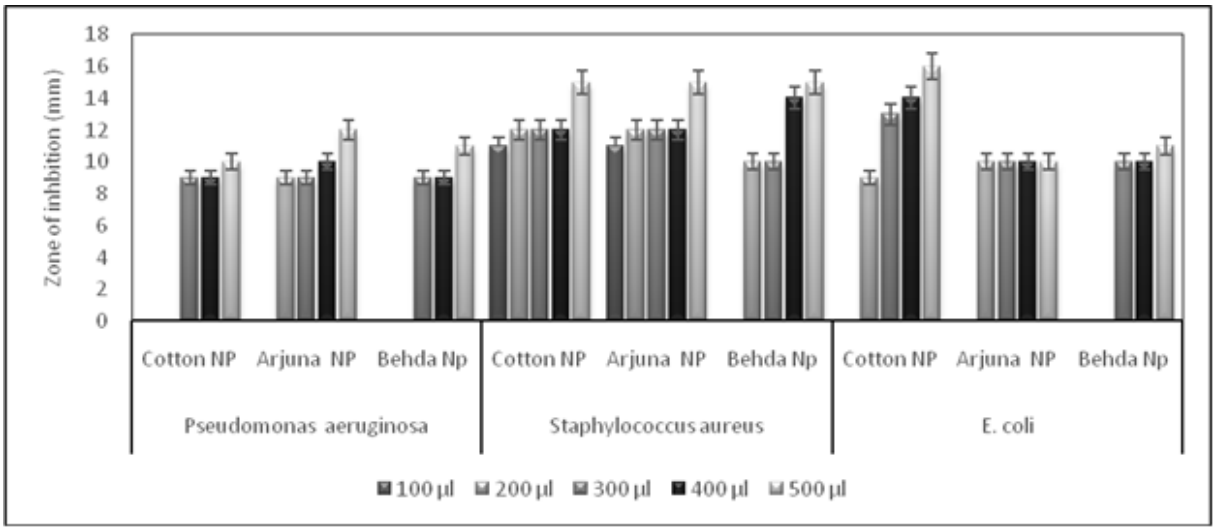

Fig 2: Antibacterial activity of Ag NPs against different pathogenic bacteria (Data expressed as median activities with standard deviation)

Table 2: Antibacterial activity of AgNPs against bacterial strains.

M.Os.	*Pseudomonas aeruginosa*			*Staphylococcus aureus*			*E. coli*		
NPs	**Cotton (mm)**	**Arjuna (mm)**	**Baheda (mm)**	**Cotton (mm)**	**Arjuna (mm)**	**Baheda (mm)**	**Cotton (mm)**	**Arjuna (mm)**	**Baheda (mm)**
100 µl	0	0	0	11	11	0	0	0	0
200 µl	0	9	0	12	12	10	9	10	0
300 µl	9	9	9	12	12	10	13	10	10
400 µl	9	10	9	12	12	14	14	10	10
500 µl	10	12	11	15	15	15	16	10	11
M.Os. – Micro-organisms, NPs- Nanoparticles Data is expressed as mean values.									

Table 3: Antifungal activity of Ag NPs against fungal strains.

M.Os.	*Aspergillus niger*			*Candida albicans*			*Trichoderma sp.*		
NPs	**Cotton (mm)**	**Arjuna (mm)**	**Baheda (mm)**	**Cotton (mm)**	**Arjuna (mm)**	**Baheda (mm)**	**Cotton (mm)**	**Arjuna (mm)**	**Baheda (mm)**
100 µl	18	16	13	11	11	9	10	13	12
200 µl	20	19	16	11	10	10	15	15	18
300 µl	21	20	19	12	12	10	18	18	20
400 µl	25	21	23	14	14	11	22	20	28
500 µl	31	27	27	15	15	16	25	22	30
M.Os. – Micro-organisms, NPs- Nanoparticles Data is expressed as mean values.									

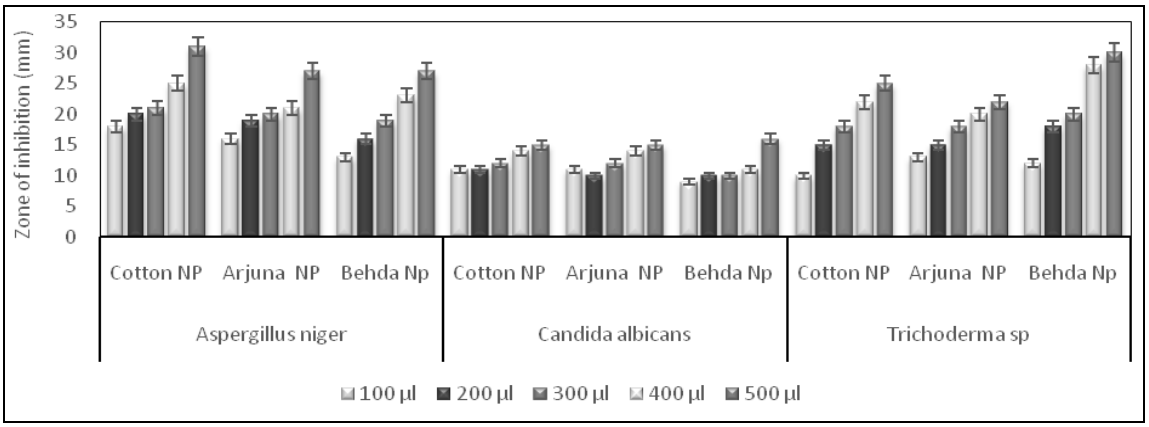

Fig 3: Antifungal activity of Ag NPs against different fungal strains (Data expressed as median activities with standard deviation)

Antimicrobial activity of AgNPs

Different bacterial and fungal strains exhibit zone of inhibition as studied by well diffusion method. Different patterns of zone of inhibition were observed as depicted in table and figure (2 and 3).

Antibacterial activity of AgNPs

Antibacterial activity of Ag NPs synthesized from three different plant extracts was studied against *E. coli, P. aeruginosa,* and *S. aureus*. Table and figure 2 depict the results of antibacterial activity. It was observed that with increase in volume of Ag NPs, zone of inhibition was increased for all bacterial strains. Maximum inhibitory activity was observed at higher volumes compared to lower volumes of Ag NPs. Similar results were observed by [19] that indicated antibacterial activity of NPs to be dose dependent. Comparing the antibacterial activity of Ag NPs synthesized from three plant extracts, Ag NPs synthesized using Cotton plant extract displayed highest inhibitory activity against bacterial strains. The inhibition of growth of bacteria may be the result of high surface area of small sized Ag NPs synthesized using cotton plant extract. Nano size of these particles aid them to remain maximum in contact with bacteria and inhibit growth of bacteria. When nanoparticles adhere to the cell membrane, silver ions are released, resulting in reactive oxygen species (ROS). The ROS produced damages the cell membrane, causing cell leakage and slowing down cell metabolism. This consequently leads to death of cells [19, 20].

Antifungal activity of AgNPs

Antifungal activity of Ag NPs synthesized from three different plant extracts was studied against *A. niger, C. albicans* and *Trichoderma sp.* Table and figure 3 exhibits the results of antifungal activity. From the data obtained, it is clear that Ag NPs synthesized using all the three plant extracts inhibited growth of all fungal strains. As the volume of Ag NPs increased from 100 to 500 µl, there was increase in zone of inhibition against

fungal strains. Similarly, [6] also observed inhibitory activity of Ag NPs to be concentration dependent. Likewise, inhibitory action of Ag NPs against pathogenic fungi was observed by [21]. Ag NPs bind to the cell wall of fungi, reacts with sulfhydryl groups and form insoluble compounds. These changes form pores in the cell wall that leads to leakage of cell components. This hampers cell metabolism ultimately leading to cell damage. In this study, it was observed that Ag NPs synthesized using all three plant extracts exhibited highest inhibition of all fungal strains. However, Ag NPs synthesized using Cotton plant extract showed more promising results compared to other plant extracts.

Conclusion

The present study elaborated biosynthesis of silver nanoparticles using three plant extracts (Arjuna, Baheda and Cotton) and assessing its antimicrobial activity. The phytochemicals present in plant extracts reduced silver ions to form Ag NPs that resulted in color change. The resultant Ag NPs formed were confirmed by UV-VIS spectroscopy and characterized using SEM, TEM and PSA. Ag NPs synthesized using plant extracts were small in size and coated with organic layer making it more stable. These NPs exhibited strong antibacterial and antifungal activity against different micro-organisms in dose dependent manner. Also, comparing Ag NPs synthesized from three plants extracts, Ag NPs synthesized using Cotton plant extract showed maximum inhibition pattern. Thus, giving insight on use of Cotton agricultural waste to synthesize Ag NPs and applying in drug preparations. Considering antimicrobial property of Ag NPs, it can be employed in drug preparation against bacterial and fungal diseases. Moreover, it is also suitable for biological and biomedical applications. This property enables use of Ag NPs in water filtration and medicine delivery systems. These green synthesized Ag NPs provides new paths in anti-cancer, anti-diabetic, antioxidant, anti-inflammatory and anti-plasmodial studies. They can also be utilized as biosensors and in bio-imaging. Moreover, they can be used to enhance tensile strength and increase antimicrobial efficacy of Cotton fabric samples. As green synthesized Ag NPs are produced by eco-friendly way, they pose less threat to the environment and hence, can also be applied in agricultural field. These NPs can be employed to enhance the growth of plants.

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