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A Study on Silver Nano-Particle Production from *Aristolochia Bracteata* and Its Antimicrobial Activity

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ABSTRACT

Nanotechnology is the study of extremely small structures. The prefix "nano" is a Greek word which means "dwarf". The word "nano" means very small or miniature size. Nanotechnology encompasses the production and application of physical, chemical, and biological systems at scales ranging from individual atoms or molecules to submicron dimensions. Nanotechnology applications that could impact the global market in agricultural, pharmaceutical and other non-fuel commodities are being developed currently, nanotechnology is described as a revolutionary discipline in terms of its possible impact on industrial applications. Even today various disease like cancer, cardiovascular diseases, diabetes, Parkinson's disease, multiple sclerosis and Alzheimer's disease, as well as complex illnesses pose a major problem for mankind. Nanotechnology can deliver medicine or drugs into specific parts of the human body, thereby making them more effective and less harmful to other parts of the body. Once the Nano shells enter into tumor cells and radiation treatment is applied, they absorb the energy and heat up enough to kill the cancer cells. To synthesis silver nanoparticles using medicinal plants in and around Vellore district, Tamilnadu. The screening on the leaf extract of the selected medicinal plant to evaluate the optimal synthesis of silver nanoparticles revealed that leaves extract of the plant Aristolochia bracteata was found to be an effective reducing agents of Ag ions to produce silver nanoparticles of nanosized. The aqueous leaf extracts were employed as reducing agents for the development of silver nanoparticles from silver nitrate solution. The aim of the present study was to evaluate the antibacterial activity of synthesized silver nanoparticles from Aristalochia bracteata leaf extract. Three Bacterial pathogens Bacillus subtilis, Staphylococcus aureus, Salmonella typhi were used for experimental studyIt was found that the minimum inhibitory concentration (MIC) of silver nanoparticles from Aristalochia bracteata aqueous extract was 25µg in the six bacterial strains.

Keywords: *Silver Nanoparticles, Aristolochia Bracteata Bacillus Subtilis Staphylococcus Aureus Salmonella Typhi.*

1. INTRODUCTION

Nanotechnology is the study of extremely small structures. The prefix "nano" is a Greek word which means "dwarf". The word "nano" means very small or miniature size. Nanotechnology is the treatment of individual atoms, molecules, or compounds into structures to produce special properties. Science and technology research in nanotechnology promises breakthroughs in areas such as materials and manufacturing, medicine and healthcare. It is widely felt that nanotechnology will be the next Industrial research revolution. For examples include biological synthesis, spontaneous self-assembly of molecular clusters (molecular self-assembly) from simple reagents in solution and biological molecules (e.g., DNA) used as building blocks for the production of three-dimensional nanostructures.

Nanoparticles

Nanoparticles have a long list of applicability in improving human life and its environment. It has been found that a 5000 years old Indian system of medicine Ayurveda had some knowledge of Nano scale fabrication. Nanoparticles of noble metals, such as silver, gold, and platinum are widely applied in products that directly come in contact with the human body.

Classification of Nanoparticles

Nanoparticles can be divided into two types such as organic and inorganic nanoparticles. Organic nanoparticles may include carbon nanoparticles while some of the inorganics may include magnetic nanoparticles, noble nanoparticles like silver and gold and semiconductor nanoparticles like titanium dioxide and zinc oxide. There is a growing interest in nanoparticles as they provide superior material properties with functional versatility.

Nanosilver

One of the substances used in nanoformulation is silver (nanosilver). Due to its antimicrobial properties, silver has also been incorporated in filters to purify drinking water and to clean swimming pool water. . Due to the unique properties of silver at the nanoscale, nanosilver is nowadays used in an increasing number of consumer and medical products. Still, its remarkably strong antimicrobial activity is the driving force for the development of nano-silver products.

Characterization of Nanoparticles

Characterization of nanoparticles is important to the understanding and control of nanoparticles synthesis and its applications this can be using develop the sophisticated techniques such as transmission and scanning electron microscopy (TEM, SEM), atomic force microscopy (AFM), dynamic light scattering (DLS), X-ray photoelectron spectroscopy (XPS), powder X-ray diffractometry (XRD), Fourier transform infrared spectroscopy (FTIR), and Uv–Vis spectroscopy can be used. (Petla et al., 2012), Selected Area Diffraction Pattern (SAED) and High-Resolution Transmission Electron Microscopy (HRTEM) were used to characterized silver nanoparticles.

Nanotechnology and its Applications

Nanotechnology is fundamental over the next 50 years to provide sufficient energy for a growing world and to protecting the environment in which we live. For all practical solutions, nanotechnology will play a critical role in any successful outcome. Because of the strong inter disciplinary character of nanotechnology there is scope for research in different fields and several potential applications that involve nanotechnology.

Nanotechnology in the Area of Medicine

Even today various disease like cancer, cardiovascular diseases, diabetes, Parkinson's disease, multiple sclerosis and Alzheimer's disease, as well as complex illnesses pose a major problem for mankind. Nanomedicine is an application of nanotechnology in the field of medicine. Gene sequencing has become more efficient with the invention of nano devices like nano particles, these nano particles when tagged with short segments of DNA can be used for detection of genetic sequence in a sample.

Nanotechnology in Cancer

Nanotechnology can deliver medicine or drugs into specific parts of the human body, thereby making them more effective and less harmful to other parts of the body. Anti-cancer property of nanoparticles has been found very effective. . Once the nanoshells enter in to tumor cells and radiation treatment is applied, they absorb the energy and heat up enough to kill the cancer cells.

Nanotechnology in the Agricultural sector

Nanotechnology can boost agricultural production, which can be done by nanoformulation of agrochemicals for applying pesticides and for produced on of fertilizers for crop improvement. In recent years, agricultural waste products have attracted attention as a source of renewable raw materials to be processed as a substitute for fossil resources for several different applications as well as a raw material for nanomaterials production. Many production processes are being developed nowadays to obtain useful nanocomposites from traditionally harvested materials

Nanotechnology in the Pharmaceutical industry

Nanotechnology is the science that deals with the processes that occur at molecular level and of nanolength scale size. There are several examples from nature like DNA, water molecules, virus, red blood corpuscles (RBC) etc., The term nanotechnology has been most commonly used in other fields of science like electronics, physics and engineering since many decades.

Silver nanoparticles

The properties of silver nanoparticles applicable to human treatments are under investigation in laboratory and animal studies, assessing potential efficacy, toxicity, and costs. Silver is widely distributed in human body fluids and tissues including heart, lungs, aorta, blood, erythrocytes, plasma, bones, brain, breast, caecum, oesophagus, colon, diaphragm, duodenum, hair, ileum, larynx, kidney, urinary bladder, urine, liver, pancreas, adrenal gland, thyroid gland, lymph nodes, muscles, nails ovary, prostate gland, rectum, serum, skin, spleen, testes, teeth (dentine and enamel), trachea, uterus etc ..,Such wide distribution in the human body suggests that this metal could have some specific functions.

Synthesis of silver nanoparticles

Nanotechnology is rapidly growing by producing Nano products and nanoparticles that can have novel and size-related physicochemical properties differing significantly from larger matter. Among them, silver nanoparticles have attracted increasing interest due to their unique biological properties compared to their macro-scaled counterparts. Because of these properties they have potential value in inks, microelectronics, and medical imaging. Besides, Ag-NPs exhibit broad spectrum bactericidal and fungicidal activity that has made them extremely popular in a diverse range of consumer products, including plastics, soaps, pastes, food and textiles, increasing their market value.

Applications of silver nanoparticles

It is important to note that despite decades of use, evidence regarding of toxicity of silver is still not clear. Products made with AgNPs have been approved by a range of accredited bodies, including the US FDA, US EPA, SIAA of Japan, Korea's Testing and Research Institute for Chemical Industry and FITI Testing and Research Institute. implantable devices showed significant antimicrobial efficiency. Researchers have also recommended the use of silver,gold and copper ions as superior disinfectants for wastewater generated from hospitals containing infectious microorganisms.

Importance and scope of medicinal plants

Medicinal plants therefore have made important contribution in the primary healthcare systems of local communities as the main source of medicines for the majority of the rural population (WHO, 2014). The World Health Organisation (WHO) estimates that up to 80% of the world's population in developing countries depend on locally available plant resources for their primary healthcare, since western pharmaceuticals are often expensive or inaccessible.

A number of modern drugs have been isolated or derived from natural sources based on their use in traditional medicine as herbal remedies as purified compounds approved by various regulatory agencies**. Pharmacological activity of selected medicinal plants** *Aristolochia bracteata* Aristolochia is a genus of evergreen and deciduous lianas (woody vines) and herbaceous perennials. The smooth stem is erect or somewhat twining. The simple leaves are alternate and cordate, membranous, growing on leaf stalks. There are no stipules. The flowers grow in the leaf axils. They are inflated and globose at the base, continuing as a long perianth tube, ending in a tongue-shaped, brightly colored lobe. There is no corolla. Thecalyx is one to three whorled, and three to six toothed. The sepals are united (gamosepalous). There are six to 40 stamens in one whorl. They are united with the style, forming a gynostemium. The ovary is inferior and is four to six locular. . The common names "Dutchman's pipe" and "pipevine" (e.g. common pipevine, A. durior) are an allusion to old-fashionedmeerschaum pipes at one time common in the Netherlands and northern Germany. "Birthwort" (e.g. European birthwort A. clematitis) refers to these species' flower shape, resembling a birth canal. The scientific name Aristolochia was developed from Ancient Greek "childbirth" or "childbed,", relating to its known ancient use in childbirth.

Plant Mediated Green Synthesis of Silver Nanoparticles

Green synthesis of nanoparticles is the field of nanoparticle synthesis and assembly by utilization of biological systems in medicinal plants (Singh *et al*., 2016). The development of silver nanoparticles using green synthesis method has revolutionized the whole world of nanoparticles synthesis. Nowadays this method is very popular because of vast reserves of medicinal plants that are easily available, commonly distributed, safe to handle, availability of wide range of metabolites and also because it minimizes the waste and energy costs. As these nanoparticles result in significantly low toxicity on adoption of this method, it could be used for encapsulation of drug molecules. Further research in the field of nanomedicine with respect to Silver nanoparticles is going on worldwide.

Green synthesis mechanism of silver nanoparticles

Medicinal plants have a complex network of antioxidant metabolites and enzymes that work together to prevent oxidative damage to cellular components. It was reported that plants extracts contain biomolecules including polyphenols, ascorbic acid, flavonoids, sterols, triterpenes, alkaloids, alcoholic compounds, polysaccharides, saponins, β-phenylethylamines, glucose and fructose etc. Specifically flavonoids are strong reducing agents which contribute to the reduction of $Ag+$ ions to nanoparticles.

Antibacterial activity of silver nanoparticles and its mechanism

More recently the metal is finding use in the form of silver nanoparticles. In ancient Indian medical system (called Ayurveda), silver has been described as therapeutic agent for many diseases. Out of all the metals with antimicrobial properties, it was found that silver has the most effective antibacterial action and is least toxic to animal cells. Among noble metal nanoparticles, silver nanoparticles have received considerable attention owing to their attractive physicochemical properties. Ag-nanoparticles have already been tested in various field of biological science, drug delivery, water treatment and as antibacterial compound against both Gram (+) and Gram (-) bacteria by various researchers.

Effect of nanoparticles size on antibacterial activity

The size of the nanoparticles implies that it has a larger surface area to come in contact with the bacterial cell and hence will have a higher percentage of interaction than a bigger particle. It is basically difficult to disperse metallic nanoparticles in a solvent, as nanoparticles tend to aggregate due to their high surface energy.

Objective of the Present Study

- To synthesis silver nanoparticles using medicinal plants in and around Vellore district, Tamilnadu.
- To characterize the synthesized nanoparticles by using UV-Vis spectroscopy, XRD, Fourier transform infrared spectroscopy (FTIR), Scanning electron microscopy (SEM), Transmission electron microscopy (TEM) and EDX were and to confirm the biosynthesis of silver nanoparticles.
- To study the antibacterial activity green synthesized silver nanoparticles.

2. REVIEW OF LITERATURE

Selection and screening of medicinal plants used for biosynthesis of silver nanoparticles and their characterization: It closely related to the biological science and medicine. It has been widely used in various field for the development of nanoparticles based drug delivery system nanomedicine based cancer diagnosis and therapy.

UV- vis Spectroscopy

The photograph of sample solutions containing silver nitrate and silver nitrate in the presence of optimal amounts of aqueous leaf extract after completion of the reaction shows appearance of a yellowish-brown color which confirms the existence of silver nanoparticles. The UV-Vis spectrum of the synthesized Ag nanoparticles was carried out by using UV-VIS spectrophotometer UV-2450 (Shimadzu). The formation of silver nanoparticle in the reaction mixture was confirmed by plasmon resonance of silver nanoparticles at 425nm, indicating the presence of spherical silver nanoparticles.

Anti-Microbial Activity

The size, shape and surface morphology of nanoparticles plays an important role in controlling the physical and chemical properties. The chemical synthesis of nanomaterials uses organic solvents and toxic reducing agents. Green synthesis of nanoparticles has attracted considerable attention in recent years. In this regard, plants extracts and natural resources such as microorganisms and enzymes have been found to be good alternative reagents in nanoparticles synthesis.

3. MATERIALS AND METHODS

Chemicals and reagents: The chemicals used in all experiments were obtained from Sigma (Bangalore, India) and Merck (Mumbai, India). MEM (Hi Media), FBS (Cistron), Trypsin, methylthiazol diphenyl -tetrazolium bromide (MTT), and dimethyl sulfoxide (DMSO) (Sisco, Mumbai). All the other chemicals and reagents were obtained from Sigma Aldrich, Mumbai.

Selection of medicinal plants

Aristolochia bracteata plants were obtained from natural source. 5g of plant leaf powder was milled using an ordinary grained plant. It was mixed with 50ml of deionized distilled water in a 250 ml beaker and was kept overnight. The extract was filtered using whatman No.1 filter paper three times and stored at 4^0C. After filtration, clear leaf extract was obtained for further using silver nitrate ($AgNO_3$) Merck analytical grade was purchased from Sigma-Aldrich. All the aqueous solutions were prepared using deionized distilled water.

Synthesis of silver nanoparticles

In a typical reaction procedure, 5ml of leaf extract was added to 20 ml of 10^{-3} (M) aqueous silver nitrate solution which was heated up to 80^0 C The resulting solution became brown in colour after 15 min of heating. 5mL, 10ml and 15ml of the leaf extract was added to 25 mL of the aqueous solution of AgNO3 (10−3 M) and stirred vigorously for 5 min (Harekrishna *et al*., 2009) reduction takes place slowly at 300 K and gets completed in 30 min by stable light brown colour formation, depending on the intensity of colour formation, respectively to the volume of the extract added besides, at 373 K, silver nanoparticle was obtained by adding 25 mL of the extract to 100 mL AgNO3 (10−3 M).Also, by adding 5 mL of the extract to 25 mL of AgNO3 solution,the silver nanoparticles were synthesized by rapid reduction at 300 K at a pH of 8,which as indicated by an be intense brown colour.

4. RESULTS AND DISCUSSION

The screening on the leaf extract of the selected medicinal plant to evaluate the optimal synthesis of silver nanoparticles revealed that leaves extract of the plant *Aristolochia bracteata* was found to be an effective reducing agents of Ag ions to produce silver nanoparticles of nanosized. The formation and stability of silver nanoparticles in aqueous leaf extract solution was conformed using Ultraviolet/Visible (UV) spectral analysis. Extinction spectra of silver synthesized from different concentrations of AgNO3 (Figure:1) and characteristic surface plasmon absorption bands are observed 425 nm for the yellow to brown coloured silver nanoparticles synthesized from 10-3 (M) AgNO3 and the fixed volume fraction (f =0.2) aqueous leaf extract. The red with increasing concentration of silver nitrate from 10-2 to 10-3 (M) and the corresponding colour changes were observed from yellow to deep brown.

Colour Change

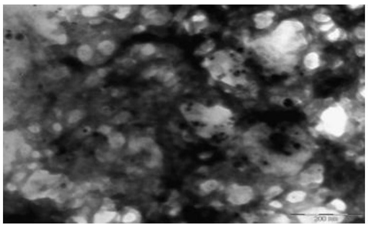
During phytoreduction from AgNO3 to AgNPs over for *Aristolochia bracteata*

4.2 Colour change during phytoreduction from AgNO3 to AgNPs over for *Aristolochia bracteata*

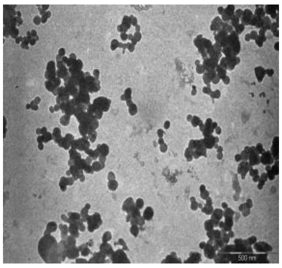
Discussions

The aqueous leaf extracts were employed as reducing agents for the development of silver nanoparticles from silver nitrate solution. The appearance of the colour was due to the excitation of the surface plasmon vibrations, typical of Ag NPs having λmax values which are reported in the visible range of 400-450 nm (Sastry *et al*., 1997; Bhattacharjee, 2015). As the *Aristolochia bracteata* extract was mixed in the aqueous solution of the silver ion complex, it started to change the color from watery to yellowish brown due to reduction of silver ion which indicated formation of silver nanoparticles. Reduction of Ag ions to AgNPs could be followed by a color change and UV-Vis spectroscopy.

The above results envisaged that the optimum aqueous leaf extract and concentration of silver nitrate were recorded has 45^0c and 1mM silver nitrate respectively. Pai *et al*., (2015) reported approximately similar result in their study absorption spectra (at 425nm) of silver nanoparticle formed by reaction. Kaushik and Joshi (2015) reported the synthesis of silver nanoparticles and characterization of UV-vis spectrophotometer was given the absorbance peak at 425 nm which was showing exactly similar result.



4.3 SEM micrographic of synthesized silver nanoparticles using aqueous leaf extract of *Aristolochia bracteata*



4.4 TEM micrograph of the synthesized silver nanoparticles aqueous leaf extract of *Aristolochia bracteata*

In Scanning Electron Microscope (SEM) analysis, high resolution images are generated by focusing a high energy beam of electrons on the surface of the specimen. These electrons interact with the specimen to produce signals that provides information about the sample such as the surface morphology, elemental or chemical composition, crystal structure and position of atoms or materials that makes up the sample (Lin *et al*., 2014). Silver nanoparticles exhibited unique and tunable optical properties on account of their SPR, shape, size and size distribution of the nanoparticles (Muthukumar *et al*.,

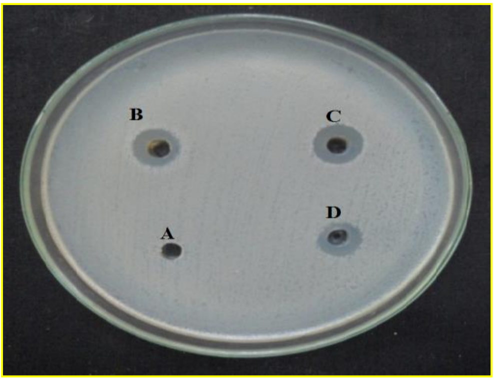
Biosynthesis of silver nanoparticles by using *Aristalochia bracteata* leaf extract and their antibacterial activity.

In this work the synthesis of AgNPs from Aristalochia *bracteata* leaf extract and the antibacterial activity of these synthesized silver nanoparticles against six bacterial pathogens was evaluated.

Results

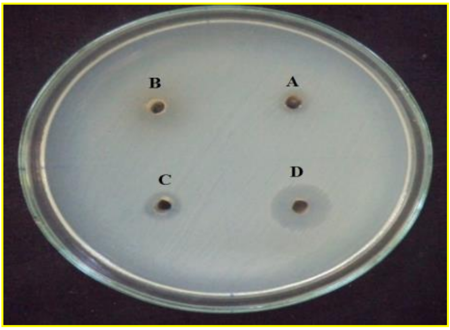
The aim of the present study was to evaluate the antibacterial activity of synthesized silver nanoparticles from *Aristalochia bracteata* leaf extract. Three Bacterial pathogens *Bacillus subtilis, Staphylococcus aureus, Salmonella typhi* were used for experimental study .It was found that the minimum inhibitory concentration (MIC) of silver nanoparticles from *Aristalochia bracteata* aqueous extract was 25µg in the six bacterial strains. Syntheses of Ag nanoparticles were considered for antibacterial activity against pathogenic microorganisms by using standard zone of inhibition. *Streptomycin*, *Gentamycin*, *Ampicillin*, *Erythromycin* of 10 mg/mL concentration were used as an antibacterial agents. The synthesized Ag nanoparticles showed inhibition zone against all the test organisms. Maximum zone of inhibition was found due to the presence of *Bacillus subtilis, Salmonella typhi* and *Staphylococcus aureus* in all the tested bacterial organisms.

Plate 1: Antibacterial activity of 1mM silver nanoparticles synthesized from *Aristalochia bracteata* against *Bacillus subtilis* by agar well diffusion method



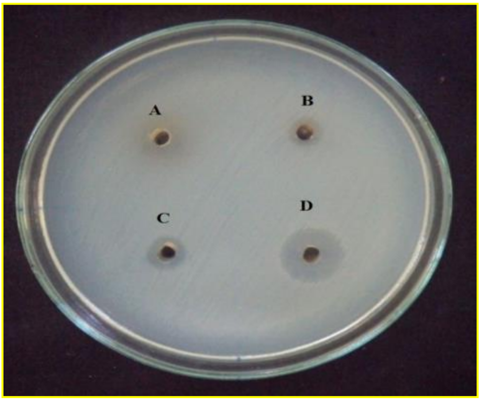
A- Crude AgNPs, B- AgNo3, C-Reference Drug, D-Optimized Ag NPs.

Plate 2: Antibacterial activity of 1mM silver nanoparticles synthesized from *Aristalochia bracteata against Staphylococcus aureus* by agar well diffusion method



A- Crude AgNPs, B- AgNo3, C-Reference Drug, D-Optimized Ag NPs.

Plate 3: Antibacterial activity of 1mM silver nanoparticles synthesized from *Aristalochia bracteata against Salmonella typhi* by agar well diffusion method



A- Crude AgNPs, B- AgNo3, C-Reference Drug, D-Optimized Ag NPs.

Table 1: Antibacterial activity of silver nanoparticles by reacting 1mM AgNO3 aqueous solution of *Aristalochia bracteata*

S No	**Bacterial name**	Zone of inhibition in mm			
		Crude AgNPs	Optimized AgNPs	$AgNO_3$	Reference Drug
1.	*Bacillus subtilis*	11±0.03	13±0.43	9±0.16	14±0.42[a]
2.	*Staphylococcus aureus*	9±0.36	13±0.56	8±0.24	16±0.49[b]
5.	*Salmonella typhi*	10±0.17	16±0.61	8±0.06	16±0.72[a]

Keys: AgNPs: Silver nanoparticle; Reference drugs: a-Streptomycin, b-Gentamycin.

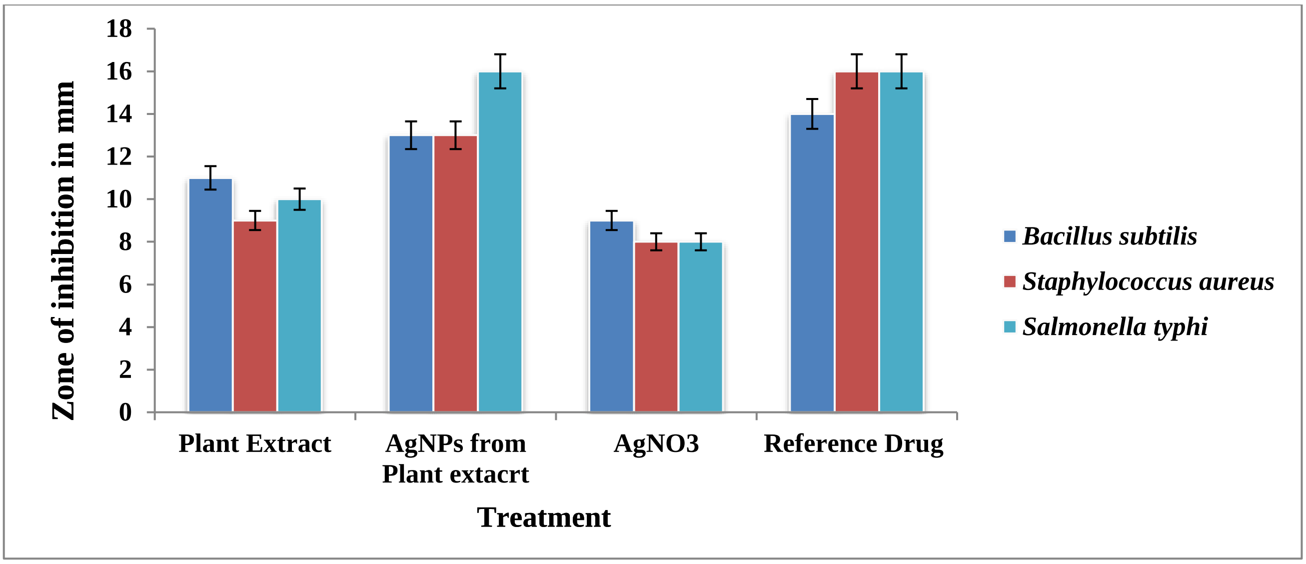
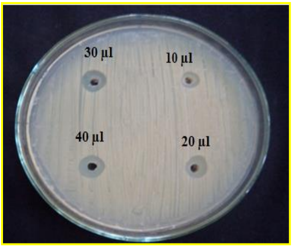
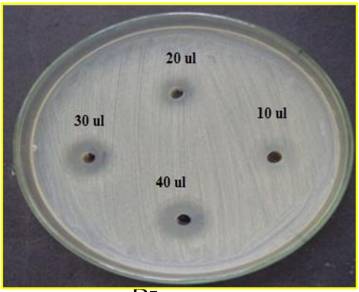


Figure 8: Antibacterial activity of silver nanoparticles by reacting 1mM AgNO3 aqueous solution of *Aristalochia bracteata* silver nanoparticles from *Aristalochia bracteata* against *Bacillus subtilis*



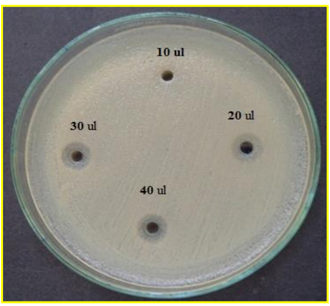
A-10µl, B- 20µl, C- 30µl, D-4

B- Plate 5: Minimum inhibitory concentration (MIC) test for green synthesized 1mM silver nanoparticles from *Aristalochia bracteata* against *Staphylococcus aureus*



B-
A-10µl, B- 20µl, C- 30µl, D-40µl

Plate 6: Minimum inhibitory concentration (MIC) test for green synthesized 1mM silver nanoparticles from *Aristalochia bracteata* against *Salmonella typhi*



A-10µI, B- 20µI, C- 30µI, D-40µI

Determination of minimum inhibitory concentration (MIC) for 1mM AgNPs synthesized from *Aristalochia bracteata*

S.No.	Bacterial name	Zone of inhibition (mm)			
		10 µl	**20 µl**	**30 µl**	**40µl**
1.	**Bacillus subtilis**	8±0.16	10±0.20	12±0.05	12±0.26
2	***Staphylococcus aureus***	10±0.11	12±0.10	14±0.43	15±0.43
5	***Salmonella typhi***	7±0.06	10±0.063	12±0.26	14±0.30

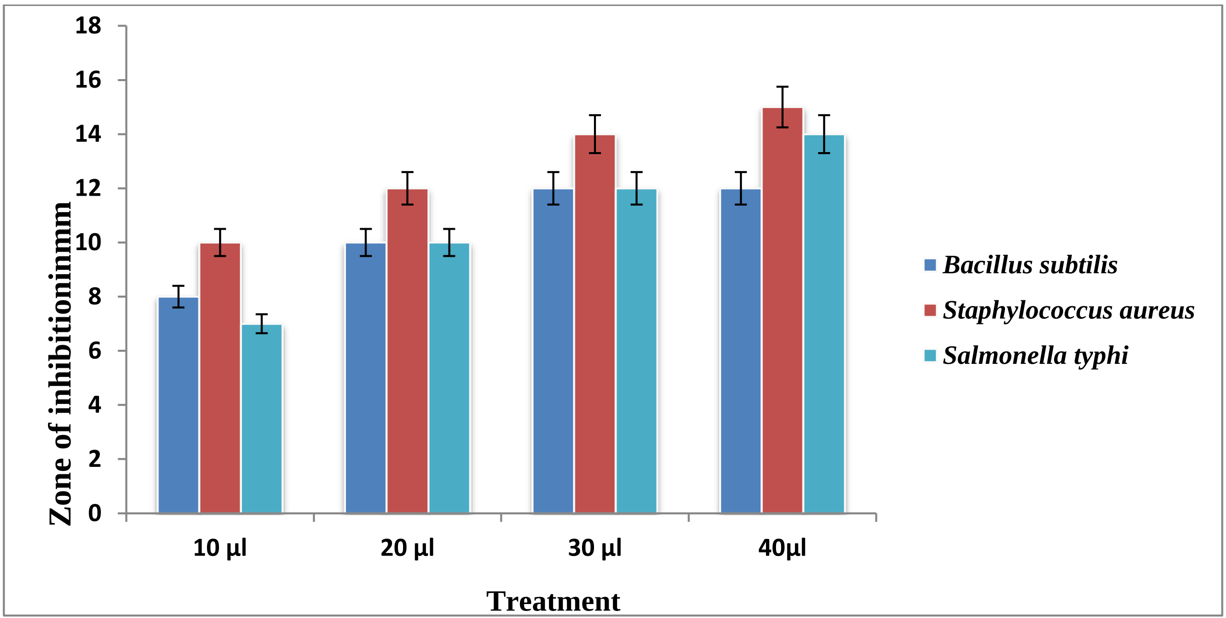


Figure 9: Determination of minimum inhibitory concentration (MIC) for 1mM AgNPs synthesized from *Aristalochia bracteata*

Discussions

It was found that the minimum inhibitory concentration (MIC) of silver nanoparticles from *Aristalochia bracteata* aqueous extract was 25µg in the six bacterial strains. Syntheses of Ag nanoparticles were considered for antibacterial activity against pathogenic microorganisms by using standard zone of inhibition. *Streptomycin, Gentamycin* of 10 mg/mL concentration were used as an antibacterial agent.

The synthesized Ag nanoparticles showed inhibition zone against all the test organisms. Maximum zone of inhibition was found due to the presence of *Bacillus subtilis, Salmonella typhi* and *Staphylococcus aureus* in all the tested bacterial organisms (Table:2; Figure: 8; Plates: 4- 6).

The mechanism of inhibitory action of silver nanoparticles on microorganisms is not very well known. However, several mechanisms have been proposed to explain the inhibitory effect of silver nanoparticles on bacteria it is assumed that silver ion has high affinity towards sulfur and phosphorus molecules containing amino acids inside or outside of bacterial cell membrane protein are the key element of the antimicrobial effect (Rai and Bai, 2011) this in turn affects the osmotic stability leads to bactericidal activity.

5. CONCLUSION

In conclusion, the study of the green synthesis of silver nanoparticles (AgNPs) has been carried out by various research institutes and including medicinal institutes. The study on the green synthesis of silver nanoparticle using the leaf extracts of six medicinal plants and the characterization of the synthesized silver nanoparticles showed that leaf extracts of Aristalochia *bracteata* of 1mM AgNO3 yielded stable silver nanoparticles in solution *Aristalochia bracteata a* plant extract solution is potent for the green and eco-friendly synthesis of Silver (Ag) nanoparticles which has various applications. The syntheses of synthesized (Ag) silver nanoparticles were analyzed through the techniques like UV, SEM, EDX, TEM, XRD, and FTIR. It showed that the silver nanoparticles (AgNPs) were spherical in shape with an average size of 50 nm.

The present study revealed that silver nanoparticle synthesized using aqueous leaf extract of *Aristalochia bracteata* showed augmented antibacterial activities. The antibacterial affect of the silver nanoparticle synthesized is directly proportional to the aqueous leaf extract of *Aristalochia bracteata* concentration for the highest zone of inhibition. The results obtained in the present study where in increased leaf concentration in the reduced smaller size silver nanoparticles.

In conclusion, the green synthesized silver nanoparticles using the leaf extract of *Aristalochia bracteata* exhibited antibacterial and MIC. Green synthesis of silver nanoparticles (AgNPs) revealed the most significant bacterial effect and thus it could be used as a (potential) bacterial pathogen for various medical applications.

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