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BIOSYNTHESIS OF SILVER NANOPARTICLES FROM *ARTOCARPUS HIRSUTUS* LEAF EXTRACTS ITS ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICAL ANALYSIS

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ABSTRACT

Nanotechnology is a fast emerging discipline not only in physics and chemistry but also in the field of biology. Nanoparticle synthesis and the study of their size and properties are of fundamental importance in the advancement of recent research in the field of medicinal search. The leaves of *Artocarpushirsutus* were used to synthesize silver nanoparticles. Silver nitrate is used as reducing agent as silver has distinctive properties such as good silver conductivity, catalytic and chemical stability. The aqueous silver ions when exposed to plant extracts were reduced in solution, there by leading to the formation of silver hydrosol. The time duration of change in color varies from plant to plant. The synthesis of SNPs had been confirmed by measuring the UV-Vis spectrum of the reaction media. The preliminary analysis of the extracts was performed to determine the presence or absence of the primary or secondary metabolites. The nanoparticles synthesis by green route was found toxic against bacterial species at a concentration of 100 micro liter Ag nanoparticles revealed higher antimicrobial activity against *Stephylococcusaureas and E.coli*.

KEYWORD

Nanotechnology, *Artocarpushirsutus*, UV-Visible spectrum, *Stephylococcusaureas* and *E.coli*.

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INTRODUCTION

Nanotechnology is a fast emerging discipline not only in physics and chemistry but also in the field of biology. In view of the tremendous applications of nanotechnology, there is a fillip among scientists to carry out research in this most vital discipline. Chemists are highly interested in synthesizing nanoparticles of different dimensions employing many of the precious metals. Already scientists have started exploiting the bio-based synthesis of

Nano-metalsusing leaf extracts and microorganisms (bacteria and fungi)[1].

Nano materials are, of course, abundant in nature as range. Numerous techniques are used to fabricate different nanomaterials. Nanoparticles can be produced from larger structures (top down) by use of ultrafine grinders, lasers, and vaporization followed by cooling. For complex particles, nanotechnologists generally prefer to synthesize nanostructures by a bottom-up approach by arranging molecules to form complex structures with new and useful properties. A variety of methods have been developed to achieve control over nanoparticles dimensions. Most of these methods are based on chemical reactions in solution ("wet chemistry"). The Brust-Schifrin method consists of two-phase synthesis and stabilization by thiols. Gold nanoparticles can be stabilized by other sulphur ligands, such as xantates and bisulfides, di- and trithiols and resorcinarenetetrathiols. In addition, other reagents can be used as ligands: phosphine, amine and carboxylate, isocyanides, acetone and iodine. Gold nanoparticles can be synthesized using micro emulsion, copolymermicelles, reversed micelles, surfactant, membranes and other amphiphiles. The seeding-growth method is very useful in Nano rods fabrication. Physical methods include photochemistry (UV, near-IR) sonochemistry[2].

Currently, biosynthesis of nanoparticles has attracted scientists' attention because of the necessity to develop new clean, cost-effective and efficient synthesis techniques. There are several organisms capable of synthesizing nanoparticles such as diatoms that produce siliceous materials or magneto static bacteria that synthesize magnetite nanoparticles. Biosynthesis of gold nanoparticles has been reported using bacteria, yeasts, actinomycetes, fungi and plants, such as the bacteria *Brevibacteriumcasei*, the fungus *Aspergillusoryzae* var. *viridis* or the plants tansy fruit and *Syzygium aromaticum*[2].

Silver nanoparticles play an important role in wide variety of applications. Silver-nano filters are used in air conditioners to make the outgoing air free from bacteria. Ag based Nano-compounds are used to purify water and to reduce the pollutants. Nano composites are temperature and corrosion resistant. Nano clothes, either Ag nanoparticles coated or mixed in the fibres, are under development. The wide applications have attracted the attention of scientists to produce them by different methods[3].

Various plant materials have the potential in synthesizing silver nanoparticles (AgNP), thus providing an alternative to the conventional chemical methods. Since no other chemicals are used in the process, this green synthesis method is safer and eco-friendlier.

As nowadays synthesis and characterization of nanoparticles is being an important area of research as selection of size and shape of nanoparticles provide an efficient control over many of the physical and chemical properties and their potential application in optoelectronics recording media sensing devices catalysis and medicine. Processes which are available presently for nanoparticles synthesis are chemical, physical and a recently developed Biomimetics. It provides an advancement over chemical and physical methods as it is a cost effective and environment friendly method for nanoparticles synthesis and in this method there is no need to use high pressure, energy, temperature and toxic chemicals[4].

Many techniques of synthesizing silver nanoparticles, such as chemical reduction of silver ions in aqueous solutions with or without stabilizing agents.

Biological route of synthesis nanoparticles is mainly used because of its extensive advantages over other traditional methods. The advantages such as defined and mild reaction conditions suited to the environment, adequate range of material sources present and good nature of reduction takes place to form nanoparticles. The time for the completion of the reaction, which is an obvious advantage of the biosynthetic procedures compared to the chemical methods. While the chemical and physical methods continue to be investigated in nanoparticle synthesis, the use of microorganisms and plant materials in similar nanoparticle synthesis methodologies is an exciting possibility that is relatively unexplored and under exploited.

MATERIALS AND METHODS

Silver nitrate, Methanol, Formaldehyde, Distilled water.

Sample collection

The selection of plant materials and sampling area were crucial, the plant *Artocarpushirsutus* is traditionally well known for its medicinal property. This plant was reported to be found in the Madikeri (India) and collected during the month of February 2012. The aril and tender leaves of the plant was collected and washed thoroughly in the tap water removing the dirt on it. It was washed again with the distilled water for plant extract.

Plant Description

It is an evergreen, tall tree grows up to 75 meters in height. *Artocarpushirsutus* (Figure No.1), known by a variety of names, such as Aini, Aini-maram, Aani, Anhili, Anjili is a tropical evergreen tree species found in Karnataka, Kerala and Tamil Nadu.

This Plant pacifies vitiated vata and pitta, anorexia, burning sensation and sexual weakness. Unripe fruit cause vitiation of tridosha and rakta, and cause indigestion and impotency. Ripe fruits are used for cooling, and aphrodisiac. Bark cures diarrhea, pimples and ulcers. *Artocarpushirsutus* were used to make the different types of extracts like aqueous, methanolic, dried and boiled extracts.

Preparation of aqueous extract

Artocarpushirsutus leaves weighing 20g were thoroughly washed in distilled water, dried, cut into fine pieces and were crushed into 100ml sterile distilled water and centrifuged at 5000rpm for 10 mins. Filtered. The supernatant was collected. The extract was stored at 4^0C experiment (Figure No.2).

Preparation of boiled extract

Artocarpushirsutus weighing 20g were used to prepare boiled extracts was thoroughly washed in distilled water, cut into fine pieces and 100ml distilled water is added, boiled for 20 minutes, and filtered through Whatman No.1 filter paper, extract was collected. The extract was stored at 4^0C for further experiment (Figure No.3).

Preparation of dried extract

Leaves weighing 500g were thoroughly washed in distilled water, dried for 1month under shade. Than it was crushed using pestle motar and from that 20g were weighed and crushed into 100ml sterile distilled water, boiled for 20 minutes and filtered through Whatman No.1 filter paper 2-3 times. The extract was stored at 4^0C (Figure No.4).

Preparation of methanolic extract

Crude plant extract was prepared by Soxhlet extraction method (Okeke, *et.al*, 2001) about 50g of powder material was uniformly packed into a thimble and run in Soxhlet extractor. It was exhaustible extracted with Methanol (70%) for the period of about 48hrs or 22 cycles or till the solvent in the siphon tube of an extractor become colourless. After that, extracts were filtered with the help of filter paper and solvent was evaporated from extract in rotary evaporator to get the syrupy consistency. The extract was stored at 4^0C for further experiment (Figure No.5).

Biosynthesis of silver nanoparticles

Silver Nitrate ($AgNo_3$) was purchased from Merck India Pvt. limited and was used.1mM aqueous solution of silver nitrate was prepared and used for the synthesis of silver nanoparticles. To the 1ml of aqueous, boiled, methanol and dried *Artocarpushirsutus* extracts 9ml of aqueous solution of 1mM silver nitrate was added for reduction into silver ions and kept at room temperature and absorbed for colour change.

UV-Visible absorption spectroscopy analysis

UV-visible spectroscopy analysis was carried out on a JASCO UV-visible absorption spectrophotometer with a resolution of 5 nm between 300 and 900 nm. Equivalent amounts of the suspension (0.5 ml) were diluted in a constant volume of water (2.5 ml) and subsequently analyzed at room temperature. The progress of the reaction between metal ions and the leaf extracts were monitored by UV–visible spectra of Ag nanoparticles in aqueous, boiled, methanolic solution with different reaction times (Figure No.6). The reduction of silver ions and the formation of stable nanoparticles occurred rapidly within an hour of reaction, making it one of the fastest bio-reducing methods to produce Ag nanostructures reported till date[5].

Stability Studies

Artocarpus hirsutus were used to make the different kinds of extracts like boiled, aqueous methanol and dried.

Phytochemical Analysis

All the extracts were subjected to preliminary phytochemical qualitative screening for the presence or absence of various primary or secondary metabolites such as Sterols, Triterpinoids, Carbohydrates, Flavanoids Alkaloids, Tannins, Proteins and Saponins.

Antimicrobial activity

Antimicrobial activity of the synthesized Ag nanoparticle was studied by agar well diffusion method. 20ml of molten agar was poured in petriplates. After solidification, 100µl of bacterial suspension was spread over the media. 4 wells were made and labeled A, B, C, D. for A- Add sample containing AgNO, B-raw-sample, C-$AgNO_3$, D-H_2O. Then petriplates were kept for incubation.

RESULTS AND DISCUSSIONS

The Aqueous, boiled, methanolic and dried extract from the leaves of the plant *Artocarpus hirsutus* was successfully extracted. To 1 ml of extract 9 ml of silver nitrate solution was added. A colour change was observed after the 30 mins of incubation at room temperature. The synthesis of nanoparticles was analyzed by the UV-Visible Spectrophotometry by adding 0.5 ml of suspension (containing $AgNO_3$) diluted by 2.5 ml of distilled water. The highest activity of silver nanoparticles was observed in boiled extract. The lowest activity of silver nanoparticles was observed in methanol extract.

Stability Studies

A Stability studies was carried out for Aqueous, Boiled, methanol and dried extract at different temperature and pH.

Aqueous Extract

The highest and lowest activity of the aqueous extract was observed at 70^0 and 100^0 (Figure No.7). It was determined by the colour changes from yellow to dark yellow after the addition of 1 Mm silver nitrate to the extract at different temperatures. The mixture was observed by UV-Visible spectroscopy.

The highest and lowest activity was observed at pH 5.5 and 6 due to the reduction of silver to silver ions (Figure No.8).

The highest and lowest activity was observed at 70^o and 100^o due to the reduction of silver to silver ions (Figure No.9).

Boiled Extract

The highest and lowest activity of the boiled extract was observed at 50^0 and 100^0. It was determined by the color changes from colorless to dark brown after the addition of

1 Mm silver nitrate to the extract at different temperatures.

The mixture was observed by UV-Visible spectroscopy. The highest and lowest activity was observed at pH 8 and 6 due to the reduction of silver to silver ions (Figure No.10). The highest and lowest activity was observed at 50^o and 100^o due to the reduction of silver to silver ions (Figure No.11).

Methanol Extract

The highest and lowest activity of the methanol extract was observed at 100^0 and 4^0. It was determined by the color changes from colorless to brown after the addition of 1 Mm silver nitrate to the extract at different temperatures. The mixture was observed by UV-Visible spectroscopy. The highest and lowest activity was observed at pH 6 and 8 due to the reduction of silver to silver ions (Figure No.12). The highest and lowest activity was observed at 100^o and 4^o due to the reduction of silver to silver ions (Figure No.13).

Dried Extract

The highest and lowest activity of the dried extract was observed at 50^0 and 4^0. It was determined by the colour changes from colourless to dark brown after the addition of 1 Mm silver nitrate to the extract at different temperatures. The mixture was observed by UV-Visible spectroscopy.

The highest and lowest activity was observed at pH 8 and 5.5 due to the reduction of silver to silver ion (Figure No.14).

The highest and lowest activity was observed at 50^o and 4^o due to the reduction of silver to silver ions (Figure No.15).

Phytochemical Analysis

Preliminary Phytochemical Screening: All the extracts were subjected to preliminary phytochemical qualitative screening for the presence or absence of various primary or secondary metabolites. The phytochemical analysis showed the presence of Carbohydrate, Flavonoids, Alkaloids and Proteins in aqueous, boiled, methanolic and dried extract of *Artocarpus hirsutus.*

Antimicrobial Activity

The nanoparticles synthesis by green route was tested by disc diffusion method and found toxic against bacterial species at a concentration of 100 micro litre Ag nanoparticles revealed higher antimicrobial activity against *Stephylococcus* aureas and *E.coli.* The extracts were examined for evidence of zones of inhibition, which appears as a clear area around the wells. The bactericidal effect of silver nanoparticles was compared based on diameter of inhibition zone in disk diffusion tests. Bacterial sensitivity to nanoparticles was found to vary depending on the microbial species. Disk diffusion studies with *E.coli* and *S.aureas revealed* greater effectiveness of its silver nanoparticles than other microorganisms. The nanoparticles synthesis by green route was found toxic against bacterial species revealed higher attributed activity against *Stephylococcus aureus and E.coli*. The inhibition zone was observed (Figure No.16 and (Table No.1).

SUMMARY

Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size distribution and morphology. Typically, the methods employed for their synthesis of nanoparticle include physical mechanical and chemical methods. However, these methods are very expensive and some of them which involve hazardous chemicals. Therefore ,there is emergent need to develop environmentally benign and sustainable methods for nanoparticle synthesis .Green chemistry processes led to environmental friendly method of synthesis and safe process as compared to other methods .On challenging leaf broth of *Artocarpus hirsutus* and aqueous $AgNO_3$ (1mM) solution changed from yellowish to light brown, the final color appeared grad usually with time. Formation of silver nanoparticles were confined by UV - visible spectroscopy, exposure to varying temperature, pH and substrate concentration influences, directly or indirectly, the rate of intracellular NPs synthesis. The rate of reduction of metals ions using plants has been found to be much faster as compared to microorganism and stable formation of metal nanoparticles has been y reported. Different extracts were taken for the synthesis of silver nanoparticles which was confirmed by UV-Visible Spectroscopy. All Extract was studied under different temperature (4^0C, 50^0C, 70^0C, 100^0C) and pH (5.5, 6, 7 and 8). Different extracts were taken for the phytochemical analysis and its antibacterial activity by well diffusion method.

Table No.1: Zone of inhibition

S.No	Organism	Concentration	Zone of inhibition in mm	
			STD	Extract
1	*E.coli*	25µl	20	12
2	*S. aureas*	25µl	20	16

Figure No.1: ***Artocarpus hirsutus***

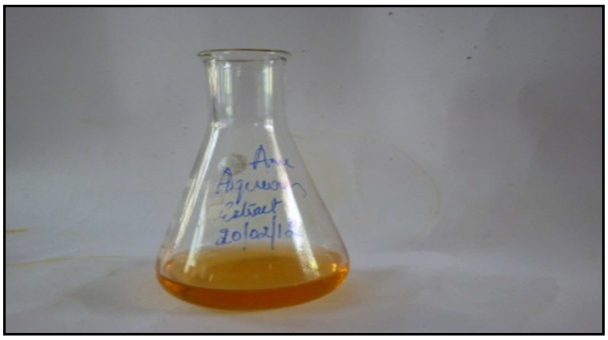

Figure No.2: Preparation of aqueous extract

Figure No.3: Preparation of boiled extract

Figure No.4: Preparation of dried extract

Figure No.5: Preparation of methanolic extract

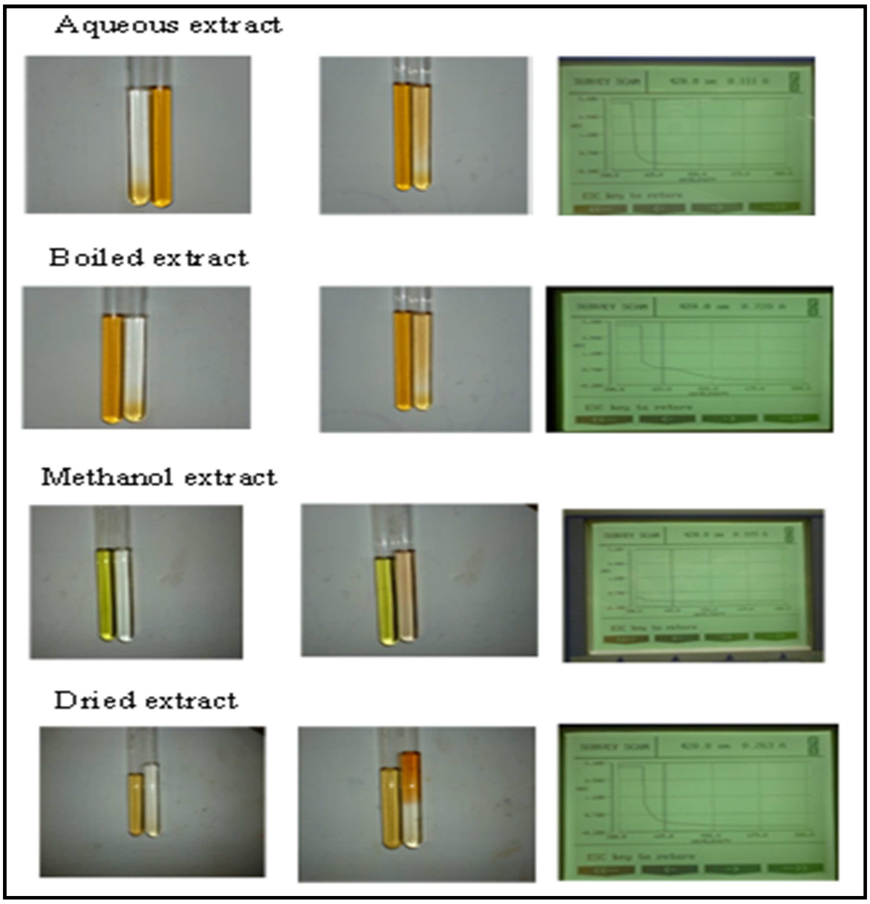

Figure No.6: Shows the change in colour of *Artocarpus hirsutus* extracts and Spectral analysis of different extracts containing $AgNo_3$ by UV-Visible spectroscopy. This Shows the absorbance at 420nm revealing the formation of nanoparticles

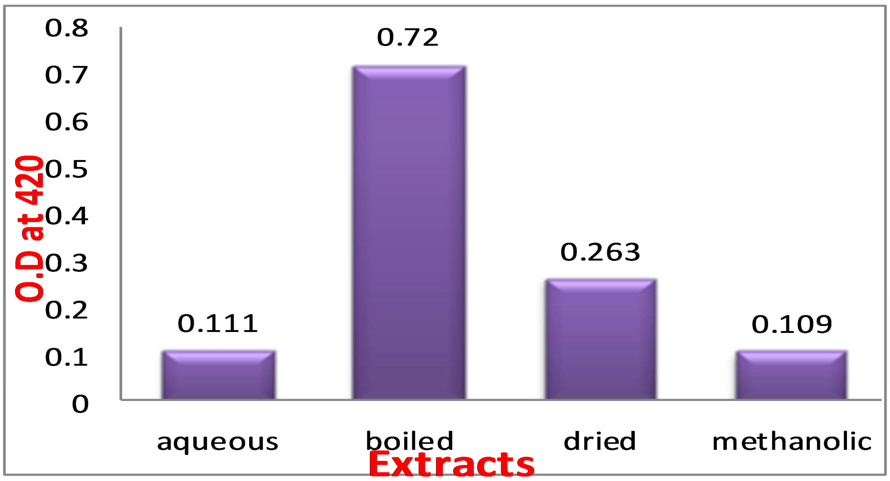

Figure No.7: Graph showing activities of silver nanoparticles in different extracts

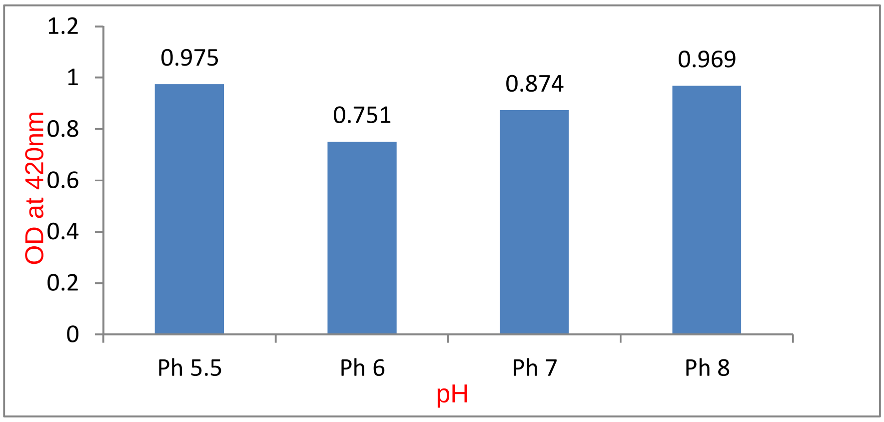

Figure No.8: Graph showing activities of silver nanoparticles in aqueous extract at different pH

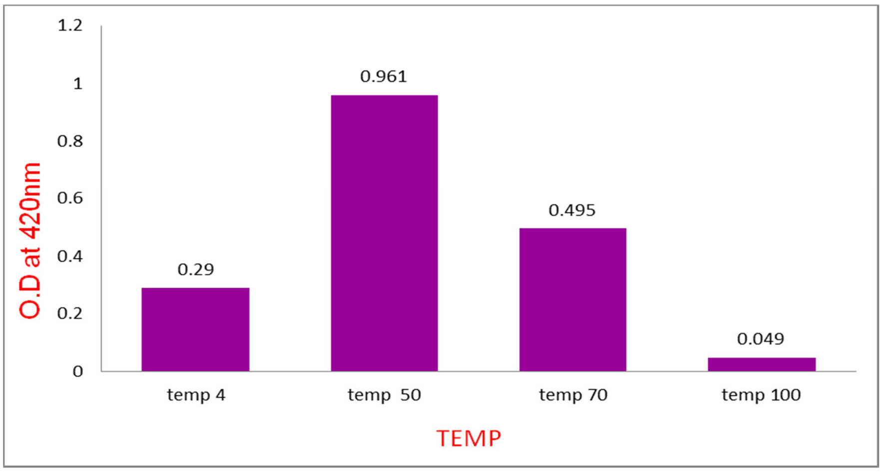

Figure No.9: Graph showing activities of silver nanoparticles in aqueous extract at different temperatures

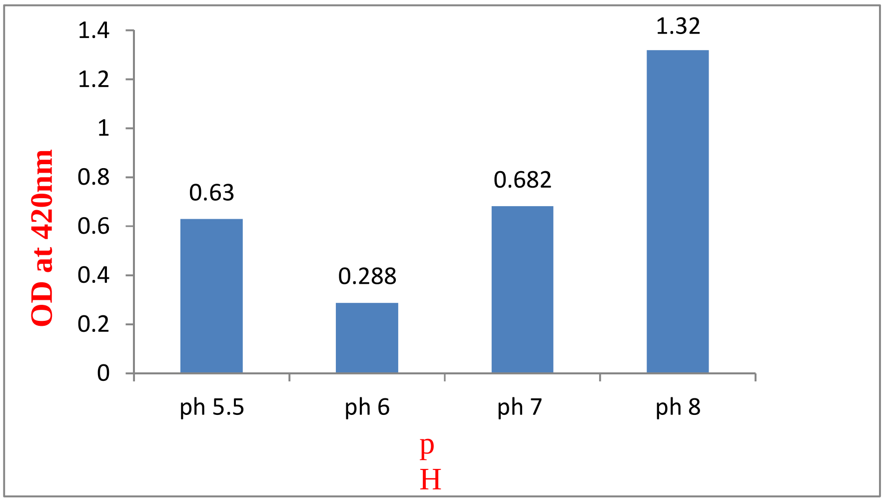

Figure No.10: Graph showing activities of silver nanoparticles in boiled extract at different pH

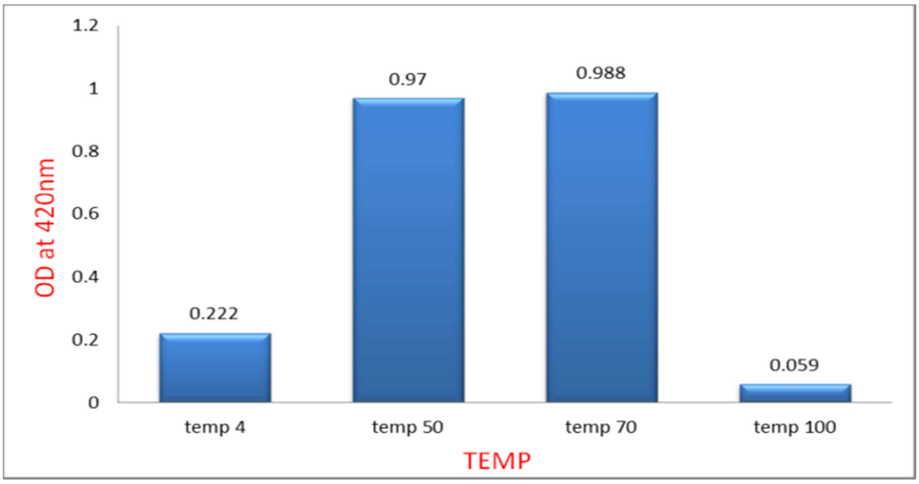

Figure No.11: Graph showing activities of silver nanoparticles in boiled extract at different temperatures

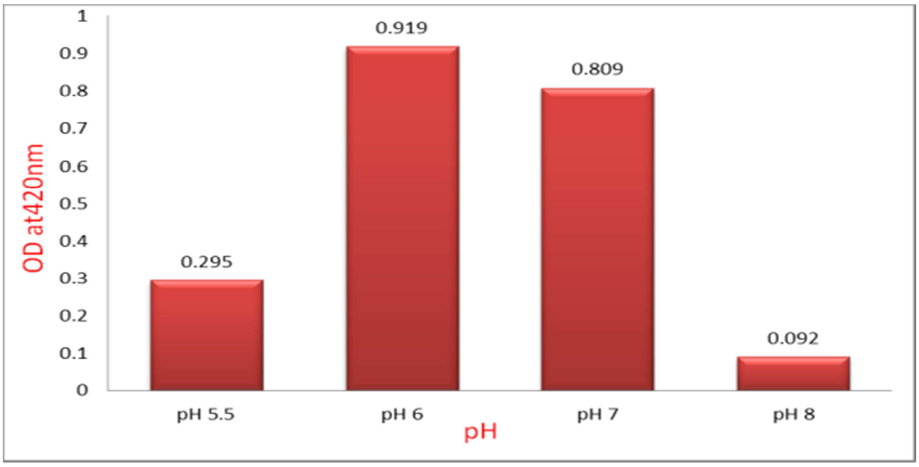

Figure No.12: Graph showing activities of silver nanoparticles in methanol extract at different pH

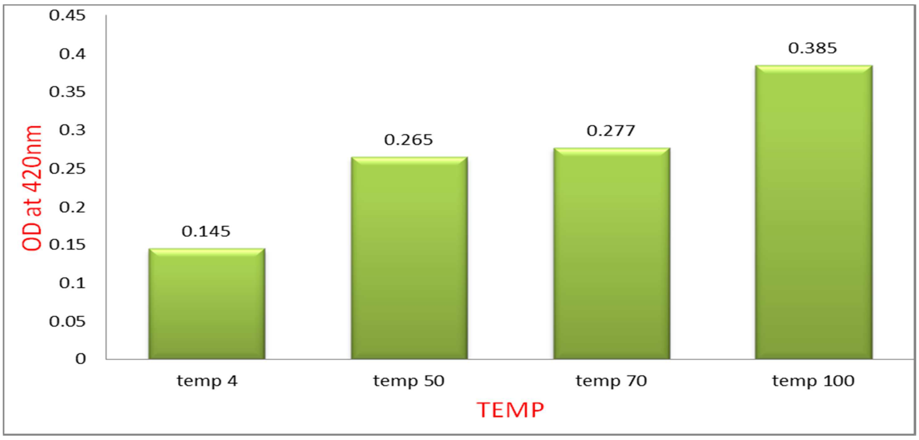

Figure No.13: Graph showing activities of silver nanoparticles in Methanol extract at different temperatures

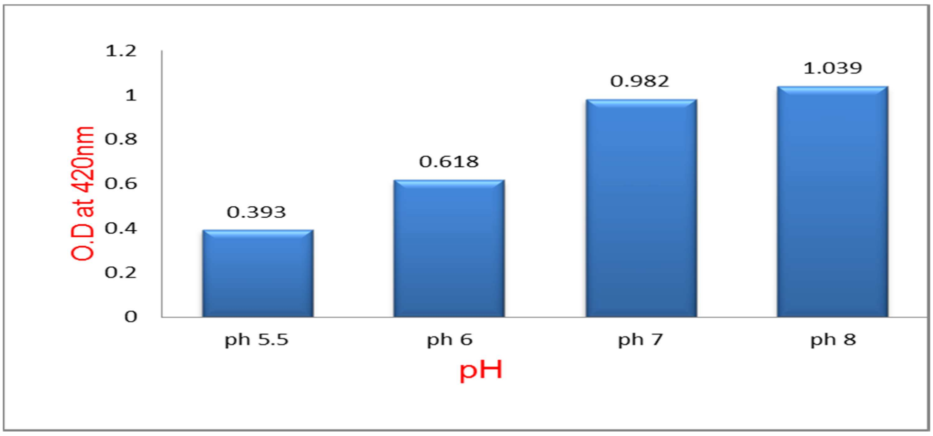

Figure No.14: Graph showing activities of silver nanoparticles in Dried extract at different pH

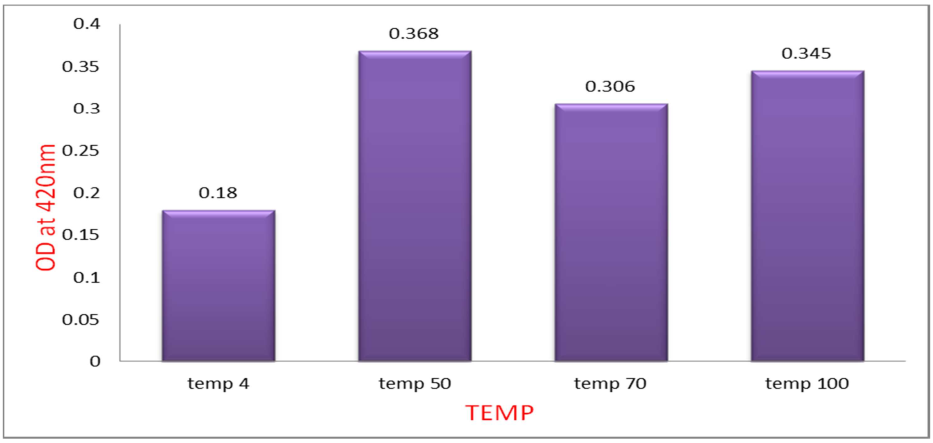

Figure No.15: Graph showing activities of silver nanoparticles in Dried extract at different temperatures

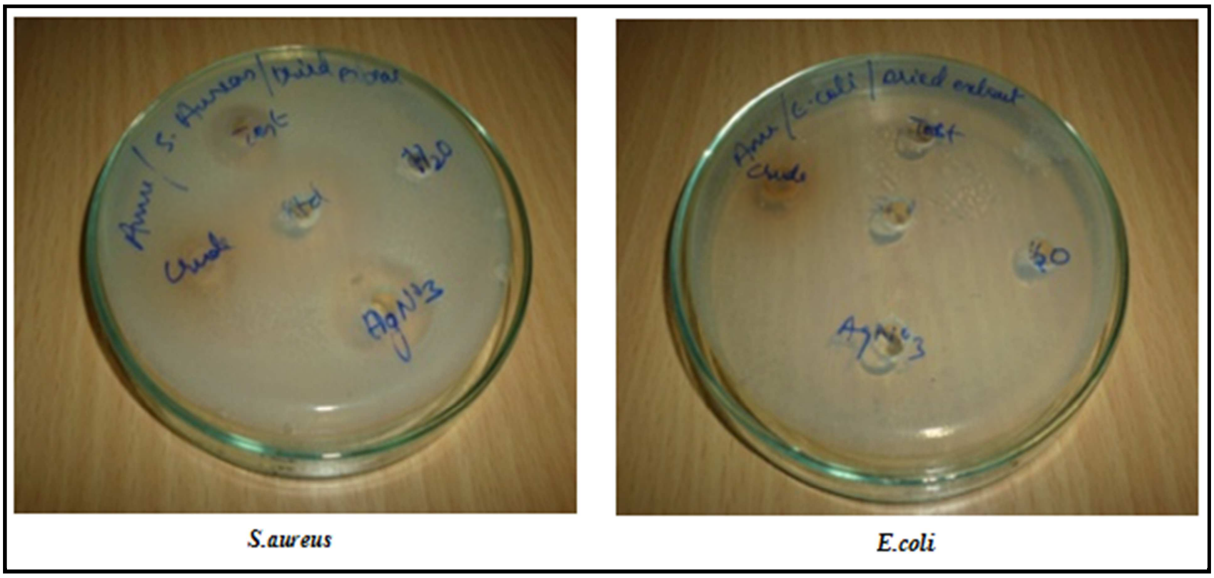

Figure No.16: Appearance of clear zone in leaf extracts

CONCLUSION

Artocarpushirsutus demonstrates strong potential for synthesis of silver nanoparticles by rapid reduction of silver ions. This study provides evidence for developing large scale commercial production of value added products for biomedical or nanotechnology industry. The temperature and pH was also found to have drastic effect on the production of nanoparticles. Further characterization of nanoparticles through TEM, XRD is necessary for determination their exact size and shape.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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