Biogenic Silver nanoparticles production and antimicrobial activity of *Rubus allegheniensis* leaf extracts

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Abstract

Biogenic syntheses of silver nanoparticles using plants and their pharmacological and other potential applications are gaining momentum owing to its assured rewards. Silver nanoparticles possess unique properties which find myriad applications such as antimicrobial, anticancer, larvicidal, catalytic, and wound healing activities. This work is aimed to unveil the phytomediated synthesis of silver nanoparticles from *Rubus allegheniensis* and its significant phytochemical and antimicrobial applications. The extract of *Rubus allegheniensis*, placed in a concentrated aqueous solution of AgNO3, resulted in the reduction of the silver ions and formation of silver nanoparticles. The UV-visible spectroscopy showed the synthesis of nanoparticles. Further the antimicrobial activity was determined.

Keywords: nanotechnology, *Rubus allegheniensis*, uv-visible spectrum, and antimicrobial activity

Introduction

Silver has long been recognized as having inhibitory effect on microbes present in medical and industrial process. The most important application of silver and silver nanoparticles is in medical industry such as topical ointments to prevent infection against burn and open wounds. It is well known that silver nanoparticles exhibit yellowish brown color in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticle [Jain *et al*., 2010] [1].

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). [Leela and Vivekanandan 2008] [6].

Novel approaches for synthesis of gold nanoparticles (AuNPs) are of utmost importance owing to its immense applications in diverse fields including catalysis, optics; medical diagnostics and therapeutics. Most of the available chemical processes for synthesis of gold nanoparticles (AuNPs) involve toxic chemicals that get adsorbed on the surface, leading to adverse effects in medical applications. Presently there is a growing need to develop environmentally benign process for rapid synthesis of nanoparticles (Ghosh, *et al*., 2014) [10].

Many techniques of synthesizing silver nanoparticles, such as chemical reduction of silver ions in aqueous solutions with or without stabilizing agents (Liz-Marzan and Lado-Tourino, 1996] [8].

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 [Zhang, Yongfang *et al*. 2007] [19].

In this study, synthesis of gold and silver nano- particles using *Rubus allegheniensis* has been investigated. The biosynthesis of pure metallic nanoparticles by the reduction of Au+ and Ag+ ions with the aqueous, dried and boiled extracts of *Rubus allegheniensis* leaves was studied. A single-step environmental friendly approach is employed to synthesize the nanoparticles. The biomolecules found in plants induce the reduction of Ag+ ions. UV-visible spectrum of the aqueous medium containing metal ions demonstrated a peak at 560nm and 425nm corresponding to the Plasmon absorbance of silver nanoparticles. Various parameters like optimum reaction temperature, pH, and time required for the synthesis of metal nanoparticles, concentration of gold salts as well as plant extracts were taken into consideration.

Further these biologically synthesized nanoparticles were found toxic against human pathogens like *E. coli & Staphylococcus aures.*

Materials & Methods

Chemical used

Silver nitrate, Methanol, Formaldehyde, Hexane etc.

Instrument Used

UV-VIS Spectrophotometer, Water bath, Autoclave, Hot Air oven, Soxhlet apparatus, weighing machine, conical flasks, Beakers and test tubes, No. 1 Whatmann filter paper, pestle mortar, Scissors, blade.

Sample collection

The selection of plant materials and sampling area were crucial, the plant *Rubus allegheniensis*is traditionally well known for its medicinal property. This plant was reported to be found in the Madikeri (India) during the month of January 2012. The plant leaves were 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

*Rubus allegheniensis*is a perennial shrub growing to 3 m (9ft 10in) at a medium rate. It is hardy to zone 3 and is not frost tender. It is in flower from May to July, and the seeds ripen from Aug to September. The plant prefers light (sandy), medium (loamy) and heavy (clay) soils and requires well-drained soil. The plant prefers acid, neutral and basic (alkaline) soils. It can grow in semi-shade (light woodland) or no shade. It requires moist soil.

Fig 1

Common name	**:**	**Blackberry**
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Subclass	:	Rosidae
Order	:	Rosales
FamilyRosaceae	:	Rose family
Genus	:	Rubus L
Species	:	*Rubusallegheniensis*

Herbal Use and Medicinal Properties of *Rubus allegheniensis*

Blackberry is edible and medicinal. The leaf is more commonly used as a medicinal herb, but the root also has medicinal value. The root-bark and the leaves are astringent, depurative, diuretic, tonic and vulnerary. They make an excellent alternative medicine for dysentery, diarrhea, hemorrhoids, and cystitis, Antirheumatic, Astringent, Diuretic, Ophthalmic, Stimulant, TB, Tonic. The most astringent part is the root. Orally, they are used the presence of large amounts of tannins that give blackberry roots and leaves an astringent effect useful for treating diarrhea are also helpful for soothing sore throats. Medicinal syrup is also made from Blackberry, using the fruit and root bark in honey for a cough remedy.

1. Preparation of boiled extract

Rubus allegheniensis were used to make boiled extracts weighing 20g were thoroughly washed in distilled water, cut into fine pieces & 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.

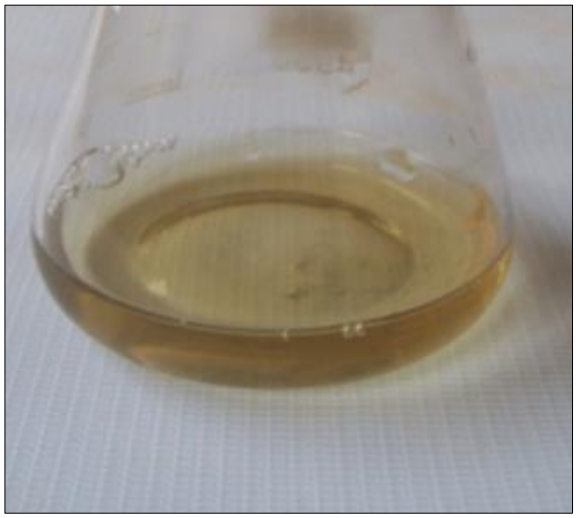


Fig 2

2. 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 & filtered through Whatman No.1 filter paper 2-3 times. The extract was stored at 4^0C.

Fig 3

3. 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 theiphon tube of an extractor become colourless. After that, extracts were filtered with the help of filter paper.

Fig 4

Biosynthesis of silver nanoparticles

Silver Nitrate ($AgNO_3$) by Merck India Pvt. limited was used 0.1mm aqueous solution of silver nitrate was prepared and used for the synthesis of silver nanoparticles. To the 1ml of aqueous, boiled, methanol and dried *Rubus allegheniensis* extracts, 9ml of aqueous solution of 1mM silver nitrate was added for reduction into silver ions and kept at room temperature and observed 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 silver nanoparticles in aqueous, boiled and methanol solutions. in different reaction times. 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 [Shankar, *et al*., 2003; Begum, *et al*., 2009; Philip, 2009].

Stability Studies

Temperature

Aqueous, boiled, dried and methanolic extracts were studied under 40c, 50c 70c and,100c temperature.

pH

Aqueous, boiled, dried and methanol extracts were studied under different pH range, 5, 5, 6, 7 and 8.

Phytochemical Analysis

All the extracts of *Rubus allegheniensis* 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.

Results and Discussions

The Aqueous, boiled, methanolic and dried extract from the leaves of the plant *Rubus allegheniensis*was successfully extracted. To 1ml of extract & 9 mlof silver nitrate. 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.

Absorption of different extracts of *Rubus allegnensis*

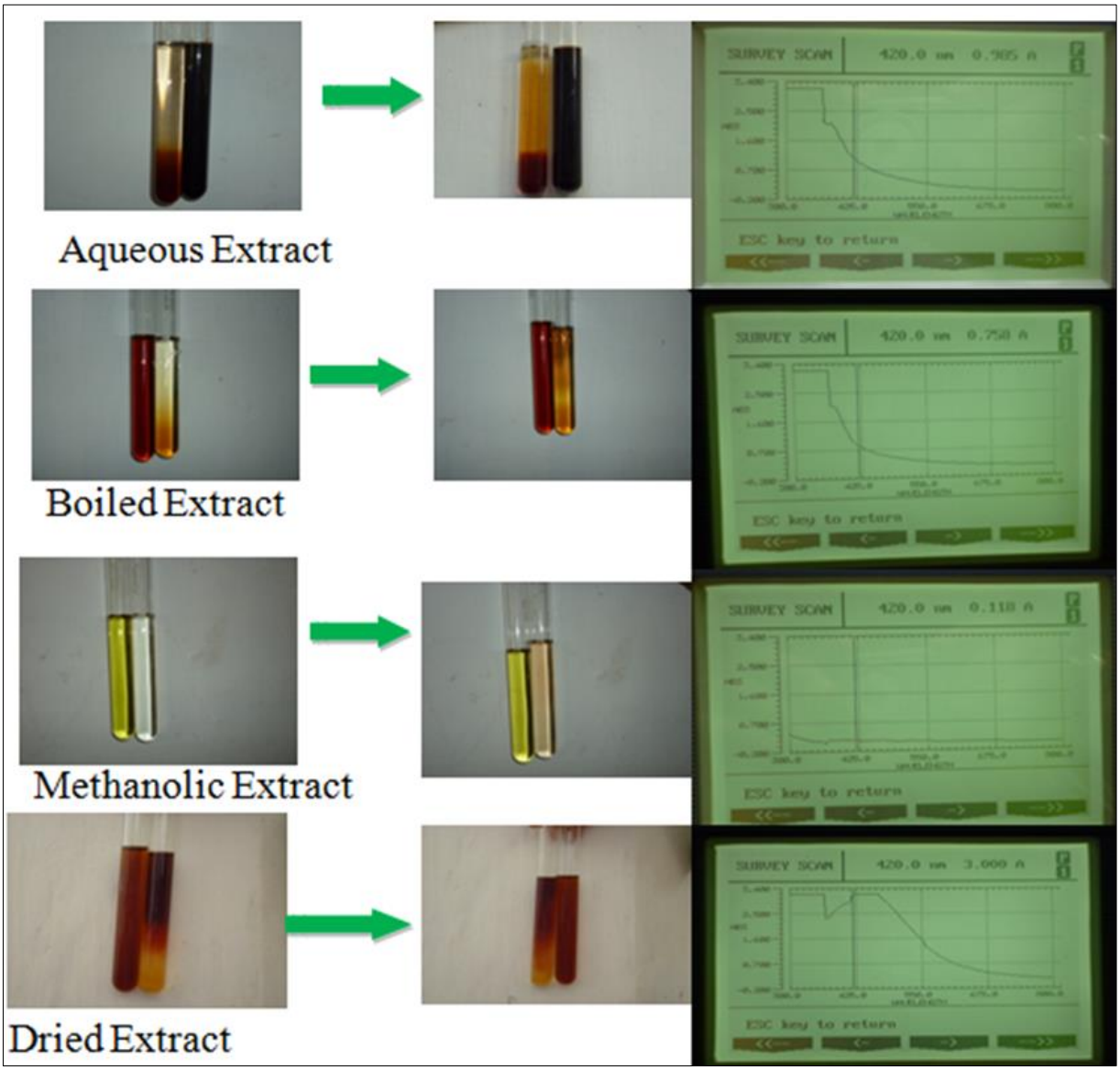


Fig 5: shows the change in colour of Rubus allegheniensis extracts like, aqueous, boiled, methanolic and dried from colourless to brown and methanolic showing colourless to light brown colour this is due the reduction of Ag+ to Ag nanoparticles

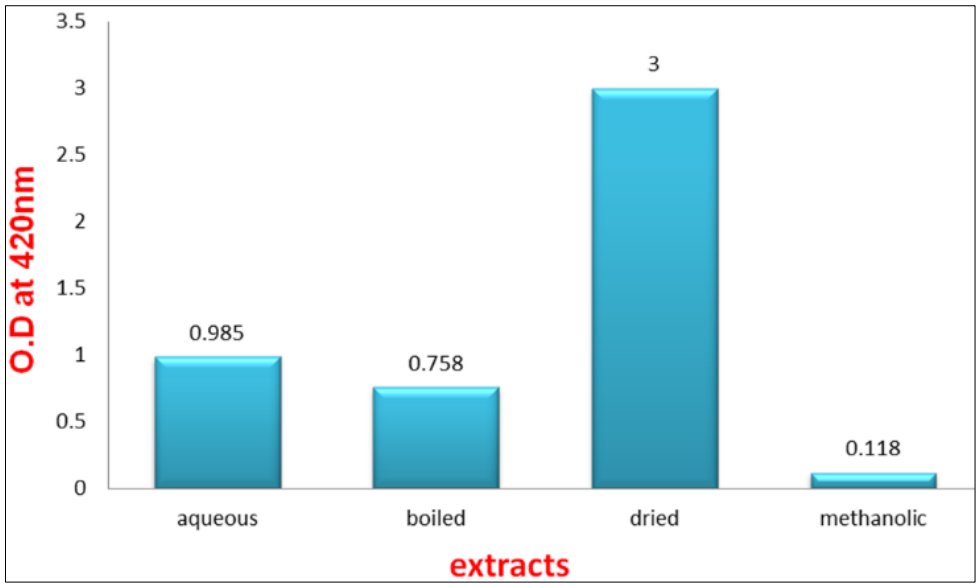


Fig 6: showing activity of silver nanoparticles in different extracts. The highest activity of silver nanoparticles was observed in dried extract. The lowest activity of silver nanoparticles was observed in methanolic extract

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 *Stephylococcusaureas* 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.

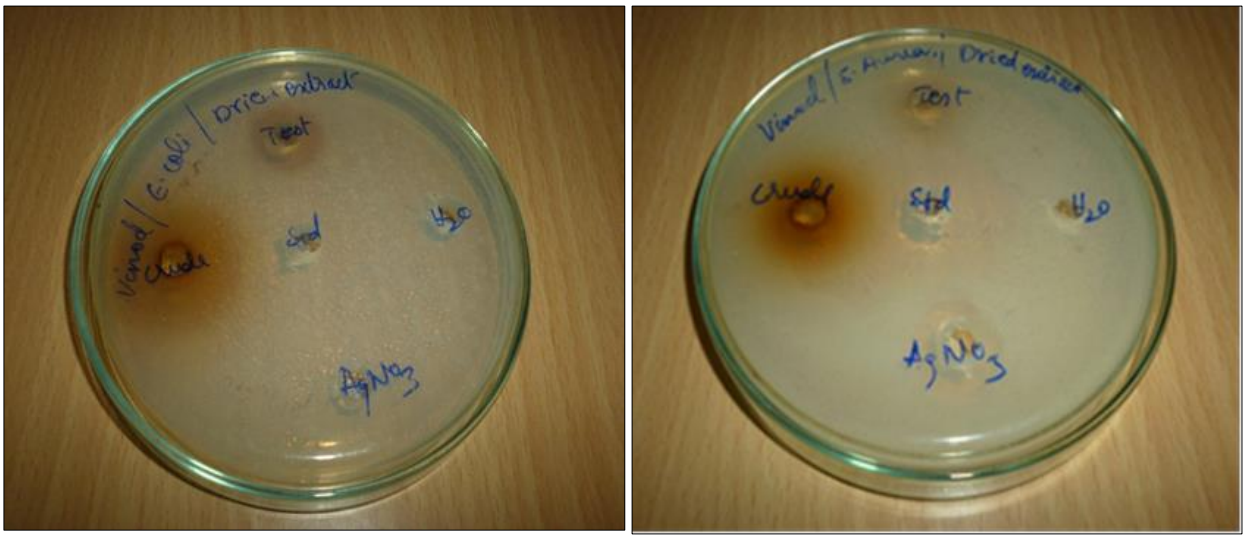
1. Antibacterial activity

The different leaf extracts of *Rubus allegheniensis* turned out to be toxic against the *S. aureus* and *E. coli* at a concentration of 25µl on each sample extract disc.

Table 1

Organism	Concentration	Zone of inhibition in mm	
		STD	Extract
E. coli	25µl	20	10
S. aureus	25µl	20	16

Showing the zone of inhibition



E. coli *S. aureus*

Fig 7

2. Antifungal activity

The different leaf extracts of *Rubus allegheniensis* turned out to be toxic against the *Penicillium sp* and *Aspergillus niger* at a concentration of 25µl on each sample extract disc. The silver synthesized sample extract showed the highest zone of inhibition against the fungi. The other extracts showed inhibition zone to a lesser extent.

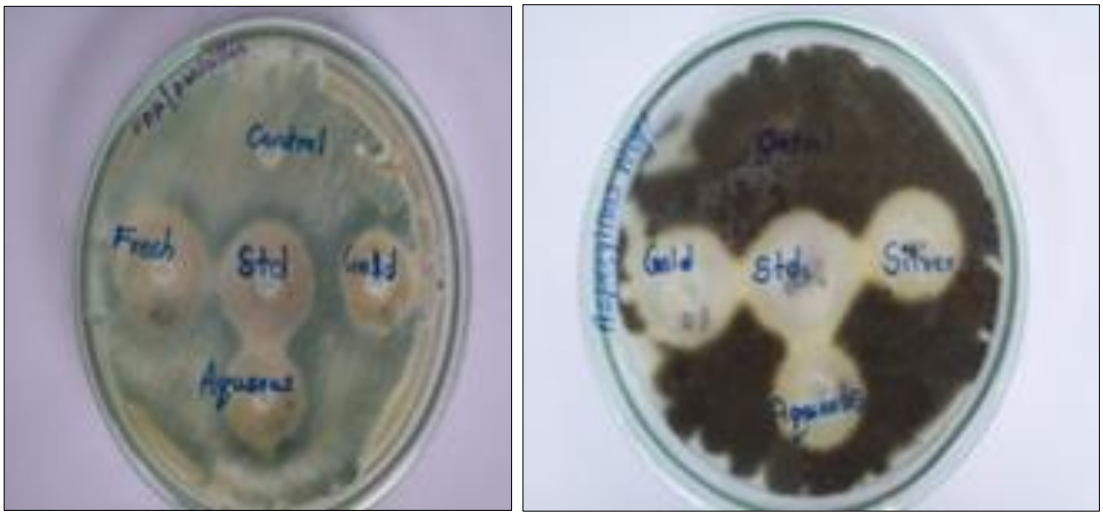


Fig 8: Showing the zone of inhibition in fungi

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 *Rubus allegheniensis* 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.

Conclusion

*Rubus allegheniensis*demonstrate strong potential for synthesis of silver and gold 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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