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Biosynthesis of Gold and Silver Nanoparticles and Antimicrobial Activity of Physalis Minima Leaf Extracts

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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 whole Physalis minima plan was 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 extract of Physalis minima, placed in a concentrated aqueous solution of AgNO3, resulted in the reduction of the silver ions and formation of silver nanoparticles, and that an extract in aqueous solution of AuCl4 resulted in the reduction of the gold ions and formation of gold nanoparticles. The UV-visible spectroscopy showed the synthesis of nanoparticles. Further the antimicrobial activity was determined, along with the nitrate reductase activity and the total protein estimation of the samples.***

Keyword: Nanotechnology, Physalis minima, UV-Visible spectrum, and antimicrobial activity

I. 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-metals using leaf extracts and microorganisms (bacteria and fungi). [Leela and Vivekanandan, 2008].

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.,2011)

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)

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. [Shen et.al. 2007]

In this study, synthesis of gold and silver nanoparticles using Physalis minima 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 Physalis minima leaves was studied. A single-step environmental friendly approach is employed to synthesize the nanoparticles. The biomolecules found in plants induce the reduction ofAu+ and 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 Staphylococcus aureus.

II. MATERIALS & METHODS

A. Chemical used

Silver nitrate, Methanol, Formaldehyde, Chloroauric acid

B. 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,

C. Sample Collection

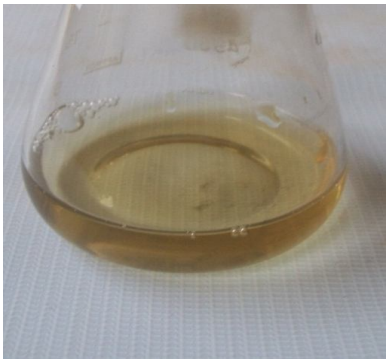
The selection of plant materials and sampling area were crucial, the plant *Physalis minima* is traditionally well known for its medicinal property. This plant was reported to be found in the Madikeri (India) during the month of January 2013. The entire plant was collected and washed thoroughly in the tap water removing the dirt on it. It was washed again with the distilled water for plant extract.

D. Plant Description

Physalis minima are herbaceous annual pant of about 30cm hight. With striate stems, often pubescent. Leaves alternate, ovate-soinute, more or less pubescent, apex acute, base Circinate, margins shallowly toothed or lobed; petiole 1.3 to 3.2 cm long. Flowers solitary, pedicels very slender, noding, 3 to 8 mm long. Fruit (berry) round, 8 to 12 mm in diameter, green, many-seeded.

Plant pacifies vitiated pitta, burning sensation, hepatitis, splenomegaly, ascites, ulcer, sexual weakness and cough. The fruit is appetizer, bitter, diuretic, laxative and tonic. Extracts from the plant have anticancer activity. The juice of the leaves, mixed with mustard oil and water, has been used as a remedy for ear ache.

1) *Preparation Of Boiled Extract:* Physalis minima 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 40C for further experiment.



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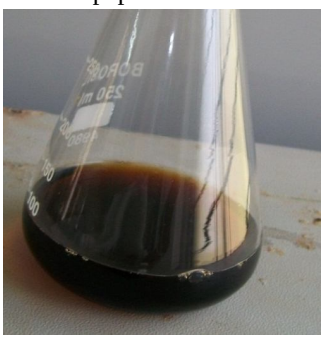
2) *Preparation of Dried Extract:* Leaves weighing 500g were thoroughly washed in distilled water, dried for 1month under shade. Than it was crushed using pestle motar and from that 20g were weighed and crushed into 100ml sterile distilled water, boiled for 20 minutes & filtered through Whatman No.1 filter paper 2-3 times. The extract was stored at 40C.



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 the siphon tube of an extractor become colourless. After that, extracts were filtered with the help of filter paper & solvent was evaporated from extract in rotary evaporator to get the syrupy consistency. The extract was stored at 40C for further experiment.

E. 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 ***Physalis minima***. 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.

F. Biosynthesis of Gold Nanoparticles

Chloroauric acid (HAuCl4) purchased from Merck India Pvt. limited was used. 1 mM aqueous solution of Chloroauric acid was prepared and used for the synthesis of gold nanoparticles. To the 260µ of Physalis minima leaf extract 10ml of aqueous solution of 1mMChloroauric acid was added for reduction into gold ions and kept at room temperature and observed for color change.

G. 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 & Au nanoparticles in aqueous, boiled and methanol solutions. With 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)

H. Stability Studies

The stability of Physalis minima extract was studied at different temperature and PH.

pH	Temperature			Color change		
	Direct	30	100	Direct	30	100
4	0.056	0.104	0.285	24hrs	24hrs	1hrs
6	0.445	0.501	0.292	24hrs	24hrs	1hrs
8	0.474	0.472	0.197	24hrs	24hrs	15min
10	0.329	0.041	0.114	24hrs	24hrs	1 hrs

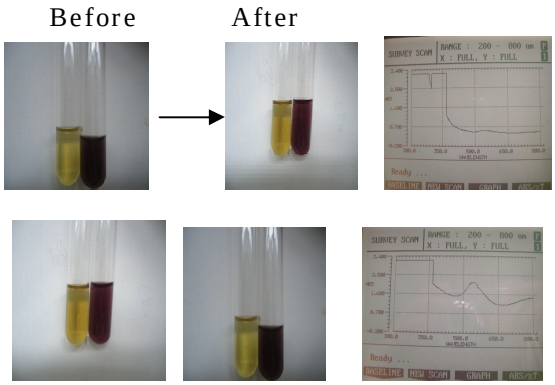
Impact of pH on Biosynthesis of GNPs on Physalis minima

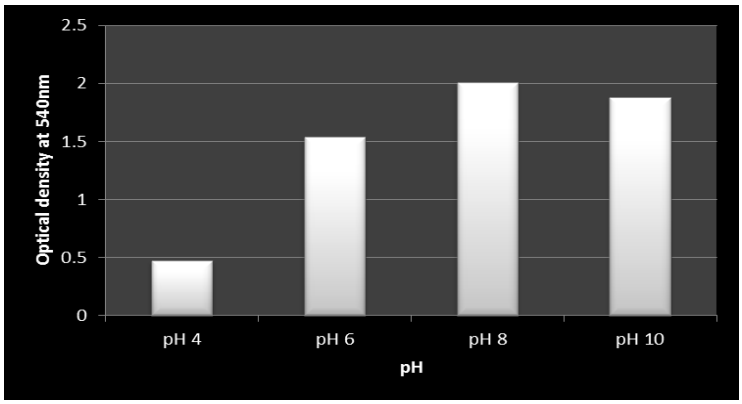
I. Phytochemical Analysis

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.

III. RESULTS AND DISCUSSIONS

The Aqueous, boiled, methanolic and dried extract from the leaves of the plant Physalis minima was successfully extracted. To 1 ml of extract 9 ml of silver nitrate / Chloroauric acid (HAuCl4) 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 AgNO3 / Chloroauric acid HAuCl4) diluted by 2.5 ml of distilled water.





Graph shows the absorbance peak at 540nm of leaf extract at different ph.

The highest activity of silver nanoparticles was observed in boiled extract.
The lowest activity of silver nanoparticles was observed in methanol extract.
The highest and lowest activity was observed at ph 6 and 8 due to the reduction of silver to silver ion.

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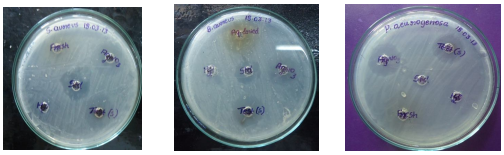
IV. ANTIMICROBIAL ACTIVITY

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 Pseudomonas aeurogenosa 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 Pseudomonas aeurogenosa 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 Pseudomonas aeurogenosa The inhibition zone was observed

A. Antimicrobial Activity

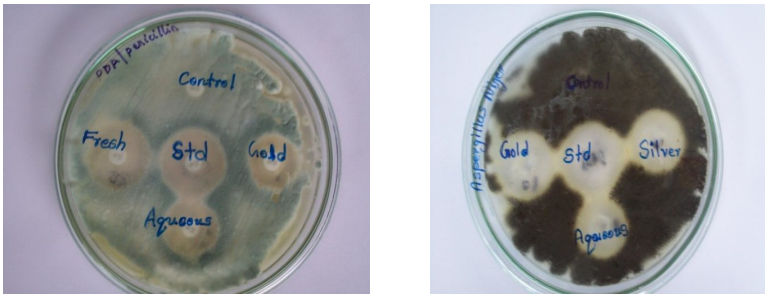
1) *Antibacterial Activity:* The different leaf extracts of *Physalis minima* turned out to be toxic against the *S.aureus* and non toxic against *P.aero genosa* at a concentration of 20µl on each sample extract disc



Name of the bacterial strain	Zone of Inhibibition in Millimeter (test samples)				
	Standard Tetracycline	Fresh	Aqueous Dried	Silver nitrate	SILVER SYNTHESIZED ((SNP)
Staphylococcus aureus	8	-	-	6	7
Pseudomonas aeurogenosa	-	-	-	8	5

Showing The Zone Of Inhibition

2) *Antifungal Activity:* The different leaf extracts of *Physalis minima* turned out to be toxic against the *Penicillium sp* and *Aspergillus niger* at a concentration of 20µl on each sample extract disc. The gold synthesized sample extract showed the highest zone of inhibition against the fungi. The other extracts showed inhibition zone to a lesser extent.



B. 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 Physalis minima and aqueous $AgNO_3$ (1mM) / Chloroauric acid HAuCl4 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

V. CONCLUSION

Physalis minima 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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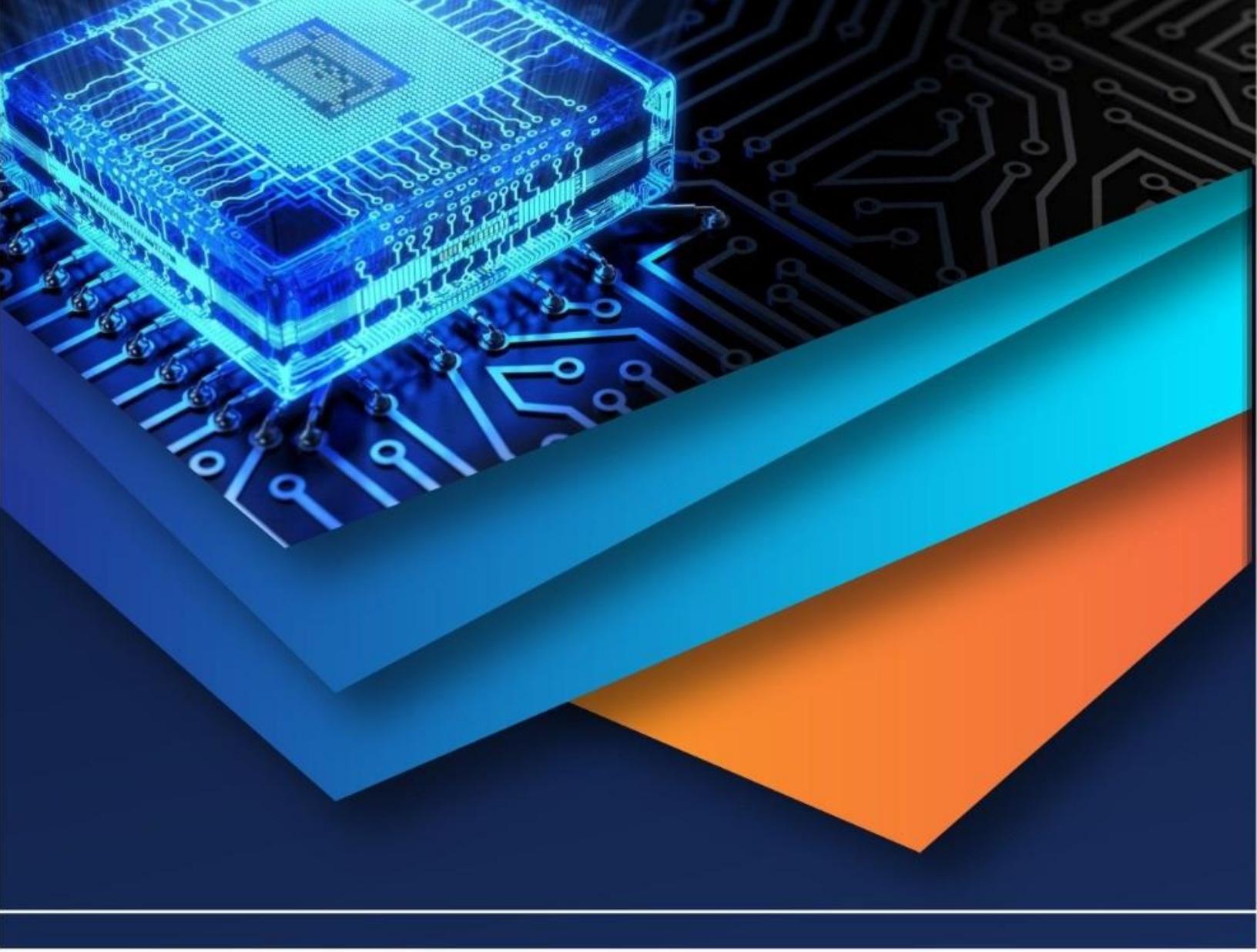

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