Plant based synthesis of silver nanoparticles and their effects on microorganisms

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***Abstract*-Silver nanoparticle synthesis has been gaining attention due to its availability. However, factors such as agglomeration and low yield have made it a difficult research area. The present study explores the use of four plants like *Hibiscus rosa-sinensis, Colocasia esculenta, Ocimum tenuiflorum* and *Epipremnum aureum* in synthesis of silver nanoparticles. The synthesized nanoparticles are characterized using UV-VIS Spectrophotometer and peaks are observed in the range of 400-500 nm. The characterized nanoparticles are explored for its anti-microbial activities against *Escherichia coli* and *Pseudomonas aeruginosa.* The synthesized nanoparticles from different plants sources have shown inhibition of growth in both the microbes.**

***Index terms*- Nanoparticles, Silver, microbe, plant, inhibition.**

I. INTRODUCTION

Nanobiotechnology has garnered a lot of attention as a division in nanotechnology. Nanotechnology deals with the process of synthesizing of nanomaterial with good monodisperity and chemical composition in a more convenient and consistent way.[1] Noble nanoparticles have made a significant mark in application in the field of material science, physics, chemistry and healthcare area.[2] Due to their physiochemical properties, nanoparticles have a high specific surface area and surface atoms. Metal nanoparticles have their applications in various fields such as electronics, medicine, energy, food etc.[3] Noble metal nanoparticles have been studied thoroughly as they have strong optical absorption in the visible range because of the group excitation of the free electron gas.[4] Silver nanoparticles have huge applications such as spectrally selective coating for solar energy absorption, bio labelling, intercalation materials for electrical batteries as optical receptors, catalyst in chemical reactions, antibacterial materials, chemically stable materials, nonlinear optics and good conductors.[5][6] They are the prime choice for researchers due to the novel method of synthesis of silver nanoparticles. Silver is renowned for showing inhibitory result towards bacterial strains and microorganisms commonly used in medical and industrial arenas.[7] Generally AgNPs are synthesized as colloidal dispersion in water or organic solvents.[6] Green synthesis method use renewable, non-toxic and eco-friendly solvent and resources.[8] Many experiments have been reported till date using plant leaf extracts from *Alternanthera sessilis,*[9] *Mukia scrabrella,*[10] *Iresine herbstii,*[11] *Morinda citrafolia,* [12] *Tribulus terrestris,* [13] *Azadirachta indica,*[14] *Cycas circinalis, Ficus amplissima, Commelina benghalensis, Lippia nodiflora*[15] and *Aloe vera.* [16] *Ocimum tenuiflorum,* an aromatic perennial plant, is a member of family Lamiaceae. It is native to the Indian subcontinent and widespread throughout the Southeast Asian Tropics. [17] It is commonly known as Holy Basil or Tulsi. Tulsi is widely used for traditional and medicine purposes. Essential oil extracted from Tulsi may have nematicidal properties against *Tylenchulus semipenetrans*, *Meloidogyne javanica*, *Anguina tritici* and *Heterodera cajani* .[18] *Epipremnum aureum* is a member of family Araceae, native to Mo'orea in the Society Islands of French Polynesia. It is a popular houseplant in temperate regions but also quite neutralized in tropical and sub-tropical forests including Southeast Asia, South Asia and West Indies where it has caused ecological damage severely. The plant is commonly known as money plant in many parts of India. It is mainly popular as a houseplant. The plant can remove indoor pollutants such as formaldehyde, trichloroethene, toluene, xylene and benzene in controlled environment. [19] It is sometimes used in aquarium allowed to grow roots in water. It absorbs many nitrates and use them for growth. *Colocasia esculenta* is a tropical plant commonly known as Taro. Colocasia esculenta, a member of the family Araceae, is mainly a native to Southern India and Southeast Asia but widely neutralized. It is primarily used for its edible corms throughout the nations. [20]The plant is toxic in its raw form due to the presence of calcium oxalate and needle-shaped raphides in the plant cells. *Hibiscus rosa-sinensis* is a species of tropical hibiscus in the Hibisceae tribe of the family Malvaceae. It is widely cultivated in the tropics and sub-tropics as an ornamental plant. The plant may have numerous medical uses in Chinese herbology. It has its uses in cosmetic skin care. An extract from Hibiscus rosa-sinensis has functioned as an anti-solar agent by absorbing ultraviolet radiation. [21] No experiment has been reported on the green synthesis of nanoparticles using *Ocimum tenuiflorum*, *Epipremnum aureum*, *Colocasia esculenta* and *Hibiscus rosa-sinensis* leaves extract. The presently study the synthesis of silver nanoparticles from the leaves extract from these four plant species and ascertain their properties.

II. MATERIALS AND METHODS

Preparation of Leaf extract

Among different plants available in our campus, we have selected four plants - *Ocimum tenuiflorum* (Tulsi), *Hibiscus rosa-sinensis* (China Rose), *Colocasia esculenta* (Taro) & *Epipremnum aureum* (Money Plant). Leaves were washed with distilled water, weighed and crushed in mortar pestle. Different concentrations of leaf extract (15, 20 and 30 % w/v) were prepared in double distilled water and used for biosynthesis of AgNP. The pH of the freshly prepared leaf extract was found to be in the range of 4.7–4.9.

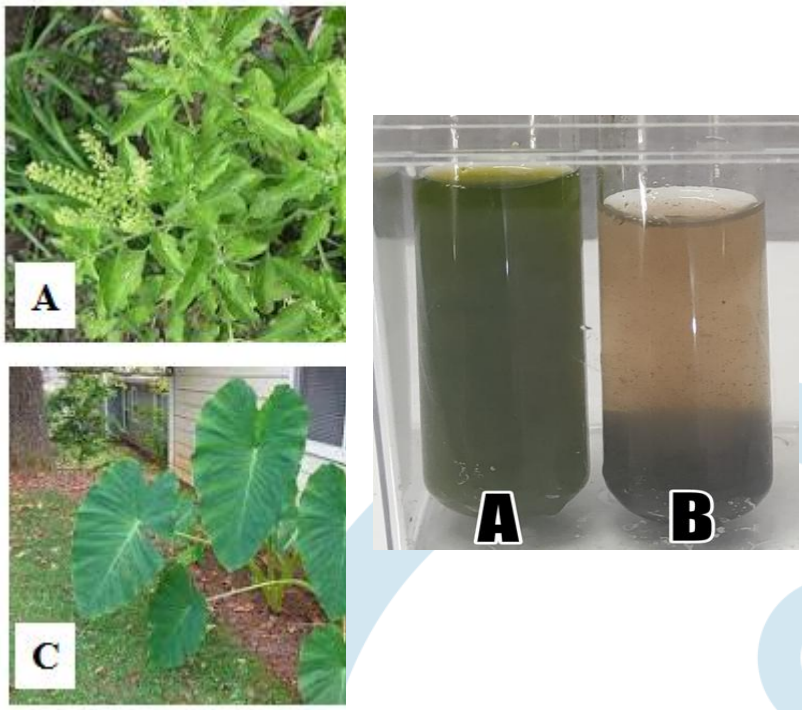

Figure 1: Representative image showing A: *Ocimum tenuiflorum*, B: *Hibiscus rosa-sinensis*, C:*Colocasia esculenta* & D: *Epipremnum aureum* plants

Synthesis of AgNP

The prepared leaf extracts was filtered using Whatman No.1 filter paper to make different concentrations of aqueous solution. The reaction mixture consisted of aqueous solution of leaf extract and silver nitrate solution in the ratio of 1:9 and the pH was adjusted by adding 3-4 drops of sodium hydroxide (NaOH).[22] The formation of silver nanoparticles is then indicated by the appearance of yellowish brown color within 5 min. The reaction was performed at room temperature. The powdered form of the nanoparticles was prepared by drying in freeze dryer (Instrumentation India) for 12 h.[23]

Characterization of AgNP

Synthesis of AgNP by bio reduction of silver nitrate solution was monitored with UV–Vis spectroscopy. The absorption spectra of the synthesized AgNP were measured using Systronics Double Beam UV–Vis Spectrophotometer in 300–700 nm range of electromagnetic spectrum.[24]

Antimicrobial susceptibility test

The antibacterial properties of the biosynthesized AgNPs were evaluated by a qualitative method against the aforementioned microorganisms using the agar disk diffusion method. For this experiment *P. aeuriginosa* & *E. coli* are used to examine the antimicrobial activities of AgNPs synthesized from different leaf extracts. Both the strains are grown on LB agar medium. The surfaces of the solidified agar plates were allowed to dry in the incubator prior to streaking of microorganisms onto the surface of the agar plates. Next, 100 µL of the microbial culture suspension in broth was streaked over the dried surface of the agar plate and spread uniformly using a sterilized glass rod and allowed to dry before applying the loaded disks. As the yield from *Epipremnum aureum* plant was less so not considered for further experiments. Different concentrations (5, 10 and 20 ppm) of AgNPs samples are prepared using sonicator in distilled water. Discs were prepared from Whatman filter paper and dipped in the different concentrations of AgNP. The loaded disks were applied carefully to the surface of the seeded agar plates using sterile forceps. The experiment was carried out in triplicate and the diameters of the zones of inhibition were measured after 24 hours of incubation at 37°C. [25][26]

III. RESULTS & DISCUSSION

Biosynthesis of silver nanoparticles

In this study four types of plant species are used for the production of AgNPs. The plant species considered are *Ocimum tenuiflorum* (Tulsi), *Hibiscus rosa-sinensis*, *Epipremnum aureum* (Money plant) and *Colocasia esculenta* (Taro). The reaction mixture containing the respective leaf extracts and silver nitrate solution are allowed to react in the ratio of 1:9. The green colored leaf extract changes to brown color indicating the formation of AgNPs in the reaction mixture within a few minutes of incubation at room temperature. The production of AgNPs was further analyzed using UV-VIS spectroscopy. The amount of AgNPs synthesized is measured as yield per unit volume of leaf extract and are shown in Tabl 1. Among all the sources of AgNPs used it has been observed that the yield obtained from *Ocimum tenuiflorum* was highest in comparison to other three samples used.

Figure 2: Formation of silver nanoparticles and identification by color change. A. Aqueous leaf extract without silver nitrate, B. Synthesis of AgNP by bioreduction of silver nitrate

Table 1. Summary of yield of AgNPs from different plant sources

Sl No.	Plant Name	Concentration of Leaf Extract (%)	Concentration of Silver Nitrate (mM)	Yield of AgNPs (mg/ml of leaf extract)
1	*Ocimum tenuiflorum*	20%	2mM	0.31475
2	*Hibiscus rosa-sinensis*	20%	2mM	0.05625
3	*Epipremnum aureum*	15%	2mM	0.01675
4	*Colocasia esculenta*	30%	2mM	0.06025

Figure 3 Yield obtained from different nanoparticles synthesized from A: *Ocimum tenuiflorum*, B: *Hibiscus rosa-sinensis*, C: *Epipremnum aureum* & D: *Colocasia esculenta* leaf extract

Characterization of silver nanoparticles

Confirmation of biosynthesis of AgNPs was mainly done by the excitation at electromagnetic field of SPR using UV-VIS spectroscopy. AgNPs in colloidal form yields absorption peak at around 430 nm.[27] The broad SPR of AgNPs was mainly due to the formation of anisotropic particles. The absorption spectra of the synthesized nanoparticles have been shown in Fig. 4.

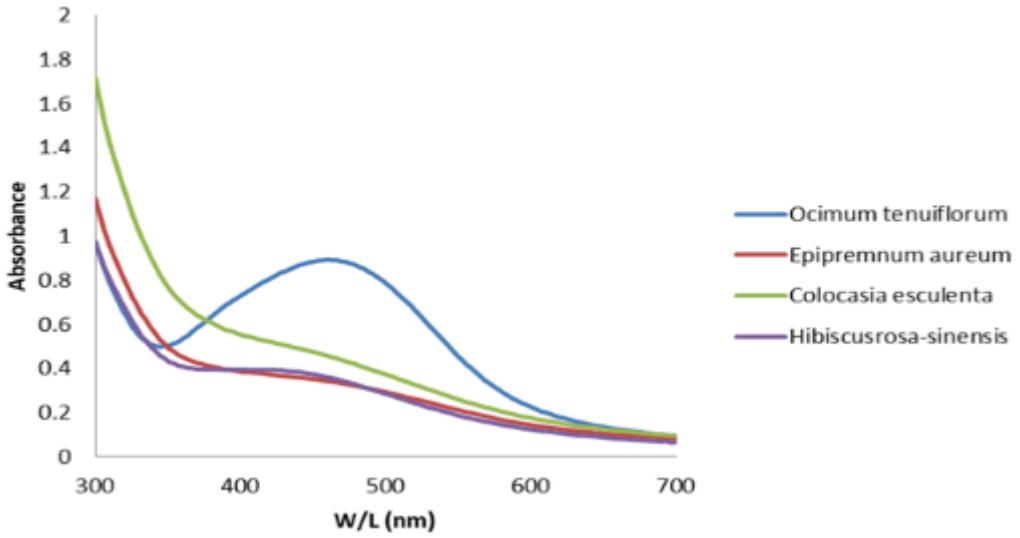

Figure 4: UV-VIS spectra of synthesized AgNP from different leaf extracts

Antimicrobial susceptibility test

For antimicrobial susceptibility test, ccommon pathogenic bacterial strains like *Escherichia coli* and *Pseudomonas aeruginosa* cultures are grown in LB media. Different concentrations of biosynthesized AgNPs were prepared and their antimicrobial activities are tested against these microbes. Yield of AgNPs obtained from *Epipremnum aureum* was significantly low so further experiments are not conducted with these AgNPs. Concentrations of AgNPs (5, 10 and 20 ppm) produced from other three leaf extracts are sonicated for further experiments on disc diffusion assay. Discs are prepared using punching machine from Whatman filter paper. They are dipped into different concentration of nanoparticles taken (5, 10 and 20 ppm) and they are placed into the petriplates using forceps in four quadrants. Discs dipped in double distilled water are considered as control.

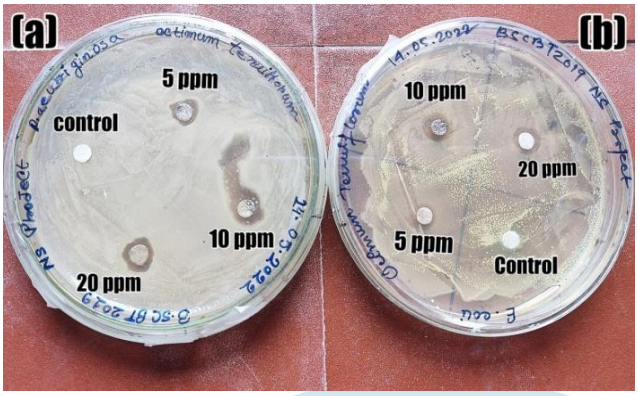

Figure 5: LB Agar plates showing antimicrobial activity of AgNP synthesized from *Ocimum tenuiflorum*

The biosynthesized nanoparticles showed aantimicrobial effect against the gram negative bacteria, *E.coli* and *P. aeruginosa*. The antibacterial activity of AgNPs indicated by the zone of inhibition was clearly observed in every sample. The concentrations taken for the disc diffusion test were respective 5, 10 and 20 ppm. In Table 2, the diameters of the zone of inhibition measured are recorded. The AgNPs obtained from *Hibiscus rosa-sinensis* leaf extract showed the highest zone of inhibiton in *E.coli* bacteria. That indicated the highest antibacterial activity of AgNPs in *E. coli* bacteria. for the nanoparticles produced from the leaf extract of *Colocasia esculenta* also showed the highest antibacterial activity in *E.coli*. But the AgNPs obtained from the plant extract of *Ocimum tenuiflorum* showed the highest zone of inhibition in *P.aeruginosa* which indicated highest antibacterial activity. All the nanoparticles showing high inhibition rates are proved to have effective antibacterial activity.

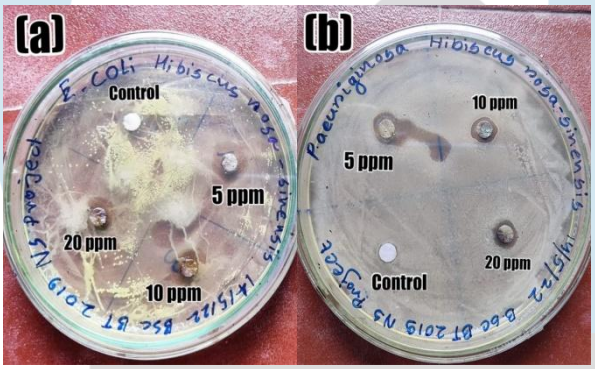

Figure 6 LB Agar plates showing antimicrobial activity of AgNP synthesized from *Hibiscus rosa-sinensis*

Slower diffusion of the AgNPs released from the LB medium in the sample is responsible for antibacterial activity. AgNPs of 20 ppm concentration have better smaller-sized nanoparticle distribution than that of nanoparticles in the 5 ppm concentration, which contributes to the higher antibacterial activity. Optimum concentration of stabilizer for the antimicrobial analysis can also be suggested by this observation.[28] It is generally assumed that the nanoparticles stabilized by biopolymers have the prolonged release-time which improves their antibacterial activity. The result of the present study is in agreement with other reports that indicate the antibacterial activity of silver nanoparticles synthesized from plant extracts.

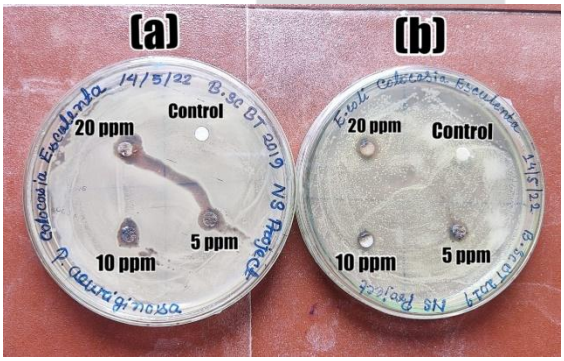

Figure 7 LB Agar plates showing antimicrobial activity of AgNP synthesized from *Colocasia esculenta*

Table 2 Summary of mean diameters of LB Agar plates showing antimicrobial activity of AgNP

Plant used for AgNP	Microbes used	Diameter of Inhibition(mm)	Mean diameter

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		5 ppm	10ppm	20ppm	
Ocimum tenuiflorum	***E. coli***	3	4	2	3.00
	P. aeruginosa	7	5	3	5.00
Hibiscus rosa-sinensis	***E. coli***	2	3	5	3.33
	P. aeruginosa	2	4	3	3.00
Colocasia esculenta	***E. coli***	4	6	7	5.66
	P. aeruginosa	5	3	2	3.33

Our results also indicate that the nanoparticles synthesized from *Hibiscus rosa-sinensis* and *Colocasia esculenta* have higher antibacterial activity against the gram negative bacteria, *E.coli.* and biosynthesized nanoparticles from *Ocimum tenuiflorum* shows higher antibacterial activity against the gram negative bacteria, *P. aeruginosa*. This can be explained by the influx of smaller sized nanoparticles into the cell wall of gram negative bacteria which has an unique outer membrane layer and a single peptidoglycan layer.[29][30] Thus, it is more exposed to the outer bacterial membrane. This leads to the death of the bacterium due to the interaction with the bacterial cell membrane through its surface.[31] Therefore, the size of the nanoparticles has its importance for their antibacterial activity. AgNPs synthesized from *Pedalium murex* leaf extract of three different concentrations also have shown antibacterial effect. The antibacterial activities have been studied on *E.coli, K.pneumoniae, P.aeruginosa, M.flavus, B.subtilis, B.pumilus* and *S.aureus*. AgNPs showed higher antibacterial activity against E.coli and B.subtilis respectively. Lesser antibacterial activity has been reported against others. [27

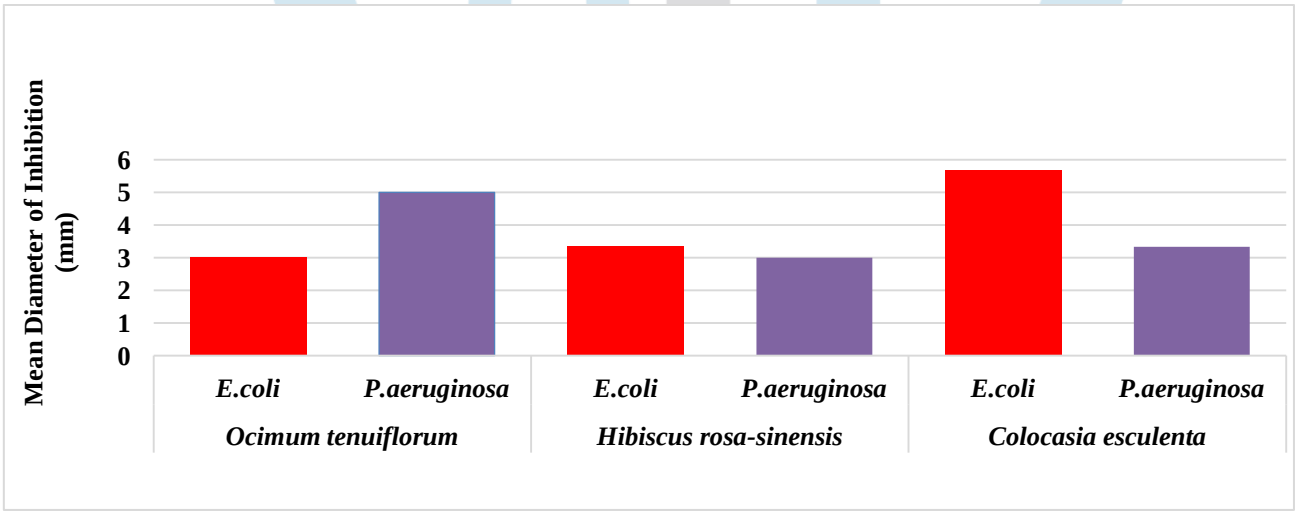

Figure 8 Comparative analysis of antimicrobial activity of different AgNP

CONCLUSION:

The study of the production of AgNPs from *Ocimum tenuiflorum, Hibiscus rosa sinensis, Epipremnum aureum and Colcasia esculenta* plant species have been used for the experiment. Nanoparticles have been synthesized from these plant extracts using silver nitrate at room temperature. The maximum yield has been extracted from the leaves of *Ocimum tenuiflorum* and the minimum from *Epipremnum aureum*. Further we have observed the characteristics of AgNPs and performed the Antimicrobial Susceptibility Test. Disc Diffusion Test was then performed to analyse the antibacterial activity of the biosynthesized AgNPs and it has showed positive results. The use of nanoparticles in various fields such as pharmaceutical, food, healthcare, agriculture etc. have garnered a lot of attention, mostly focusing on the development of more convenient ways of producing nanoparticles using green technology. The results have showed antibacterial activity of biosynthesized nanoparticles. So we conclude that the nanoparticles synthesized from plant extract can work as antibacterial agent and can be used in medicinal field as well as in other aspects.

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