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GREEN NANOPARTICLES FROM *BUTEA MONOSPERMA* AND EVALUATION OF THEIR ANTIMICROBIAL ACTIVITY

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ABSTRACT

Biosynthesis (Green synthesis) of nanoparticles by plant extract is currently under exploitation. The development of such biological experimental processes for the preparation of AGNPs is evolved into an important branch of Nanotechnology. The present study reveals preparation of silver nanoparticles (AgNPs) utilizing bark extract of *Butea monosperma* and evaluation of its antimicrobial activities against various microorganisms. *Butea monosperma* showed strong potential for synthesis of silver nanoparticles, by rapid reduction of silver ions (Ag+ to Ag0) and the formed AgNPs demonstrated a strong potential against *Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa and Streptococcus pneumonea*. The results were compared with the effects of antibiotics, and were found to be more potent than antibiotics. It was also recorded that small concentration of nanoparticles strongly inhibited the microbial growth rate.

Keywords: Silver nanoparticles, *Butea monosperma*, UV-VIS spectroscopy, Antimicrobial activity.

1. INTRODUCTION

In 21st century, nanotechnology is expected to be the basis of many technological innovations. The studies reveals that noble metal nanoparticles have been currently under exploitation due to their unique optical, electronic, mechanical, magnetic and chemical properties that are significantly different from those of bulk materials [1].These special and unique properties could be attributed due to nanoparticles small sizes and large surface areas. Due to these reasons, metallic nanoparticles exploited for many applications in different fields such as catalysis, photonics, and electronics. Formation of silver nanoparticles has considerable attracted attention due to their diverse properties and uses like electrical conductivity [2] antimicrobial and antibacterial activities [3, 4] DNA sequencing [5] and surface-enhanced Raman scattering (SERS) [6].

Different procedures of preparing silver nanoparticles, like chemical reduction of silver ions in aqueous solutions with or without stabilizing agents [7], thermal decomposition in organic solvents [8] chemical reduction and photoreduction in reverse micelles [9,10] and radiation chemical reduction [11,12] have been reported in the literature. Almost all these methods are quite expensive and also utilises toxic, hazardous chemicals, which impose heavy environmental and biological risks. Since noble metal nanoparticles are widely used in the areas with human contact [13] there is an urge to develop eco-friendly

processes to synthesis nanoparticle without use of any toxic chemicals. Biological methods of preparing Nanoparticle using microorganisms [14-16] enzymes [17] fungus [18] and plants or plant extracts [13] have been regarded as possible eco-friendly alternatives to chemical and physical methods. Literature reveals that silver Nanoparticles have been prepared by using various natural products like green tea *Camellia sinensis* [19], *Azadiracta indica* leaf broth [20] natural rubber [21], Aloeavera plant extract [22] Latex of Jatropacureas [23] etc.

Butea monosperma (Lam) or Taub (*Butea frondosa*) commonly known as Palas in Sanskrit language, belongs to Family Fabeceae. In South Asia it is a traditionally used medicinal plant for treatment of diseases. Seeds, leaves bark, flowers all have medicinal properties. The Seeds are purgative, ophthalmic, anthelmintic, depurative and tonic. They are useful in curing herpes, skin disease, ringworm, arthritis and diabetes [24]. Leaves are believed to have astringent, diuretic and aphrodisiac properties. Gum is used in external astringent. Besides all this, it has also been reported to have antibacterial, antifungal properties [25]. Presence of flavinoids and steroids in Bark extract is reported to have anti-diabetic properties [24, 25].

In the present study, synthesis of silver nanoparticles was done by biological method, by utilising aqueous bark extract of *B. monosperma* is being reported, and the prepared Green silver particles were explored for their potential against different pathogenic bacteria.

2. MATERIALS AND METHODS

2.1. Sample and strain collection:

The bark of *B. monosperma* was collected from local market of Gondia, India, and aseptically taken to laboratory. The experimental bacterial strains of Escherichia coli (NCIM 2831), *Bacillus subtilis* (NCIM 3466), *Pseudomonas aeruginosa* (NCIM 2035), and *Streptococcus pneumonea* (NCIM 2127) were obtained from National Centre for cell science (NCCS), Pune Antibiotics (Vancomycin and Erythromycin) were purchased from Hi-media Mumbai, India.

2.2. Extract Preparation

The bark was sun dried for a week and then grinded into fine powder. 10 gm. of dried powder was mixed with 100ml of double distilled water and filtered through Whatman filter paper (0.22µm). Then the extract was boiled for10 min and then stored at 4ºC for further experiment.

2.3. Synthesis of Silver Nanoparticles

For the synthesis of silver Nanoparticles, 10ml of Bark Extract (*B. monosperma*) was mixed with 90ml of 0.1 mM of AgNO3 and then heated at 80ºC for 15min for reduction of Ag ions. Colour change was observed, and its stability in aqueous colloidal solution was observed using UV-VIS spectrophotometer (Shimadzu UV- 2450) at room temperature. The reduction of silver ions was confirmed by qualitative testing of supernatant obtained after centrifugation with a pinch of NaCl.

2.4. Anti-bacterial activity

The antibacterial assay was performed by standard disc diffusion method. The fresh bacterial culture was prepared in nutrient broth. The Nutrient Agar media was autoclaved to sterility, then the media was poured in sterile petri discs and was kept for 30 minutes for solidification. After 30 minutes, the fresh overnight cultures of inoculums (100µl) of four different organisms (*Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, and Streptococcus pneumonea*) were spread on nutrient agar plates evenly on respective plates. Sterile paper discs made of Whatman filter paper (5mm) dipped in different concentration of

aqueous solution of silver nanoparticle such as 0.2mM, 0.4mM, and 0.8mM along with two standard antibiotics Vancomycin and Erythromycine (Hi-media Mumbai, India) disc were placed in each plates as control. The media petri plates were incubated at 37oC for 24 hr. After 24 hr. of incubation the zone of inhibition was observed.

3. RESULTS AND DISCUSSION

3.1. Synthesised Silver nanoparticle:

The reduction of pure Ag+ ions was monitored as the colour changed from yellow to dark brown. It is well known that silver nanoparticles exhibit yellowish brown colour in aqueous solution due to excitation of surface plasmon vibration in silver nanoparticles [26, 27]. As the *B. monosperma* bark extract was mixed with aqueous solution of the silver nitrate, it started to change the colour from yellowish brown to dark reddish brown due to reduction of silver ions, which

(A) **(B)**

Fig 1. Colour changes before (A) and after (B) the process of reduction of Ag+ to Ag nanoparticles

indicated the formation of silver nanoparticles. The synthesised nanoparticle was stable and the UV-VIS spectrum showed the characteristic surface plasmon absorption bands at 478nm with resolution of 1nm. Excitation spectra of silver synthesis from AgNo3 are shown in (Figure 2). It is generally known that UV-VIS spectroscopy could be used to examine size and shape controlled nanoparticles in aqueous

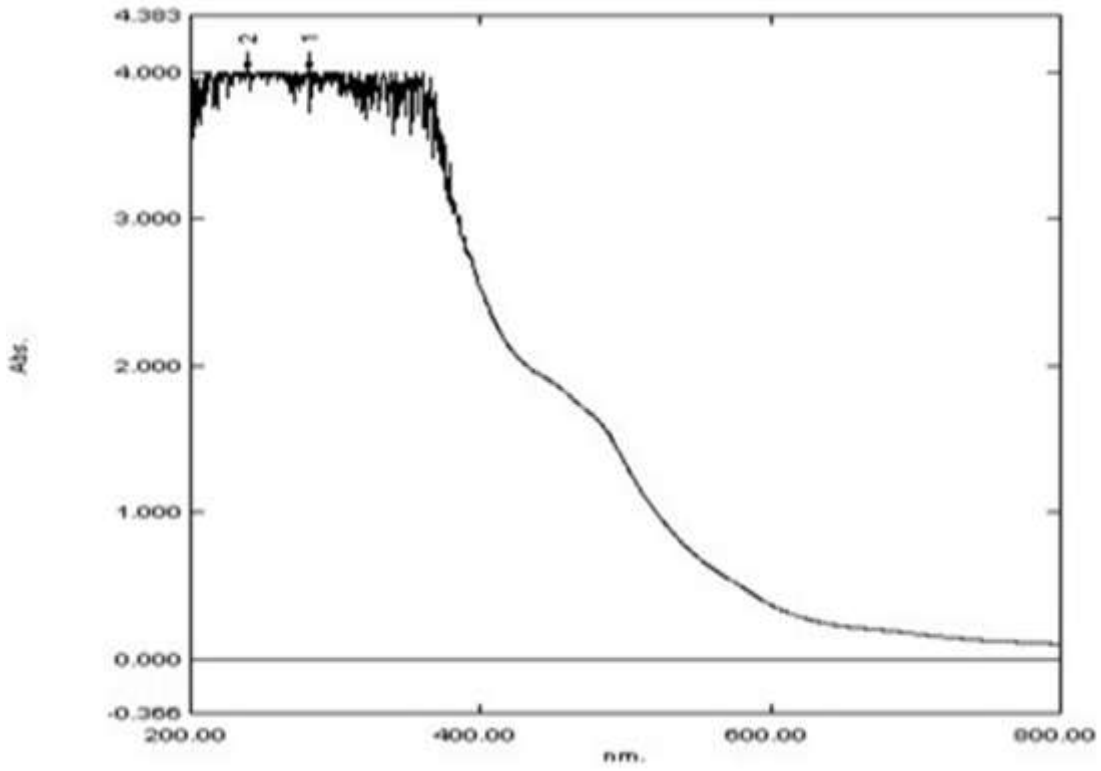
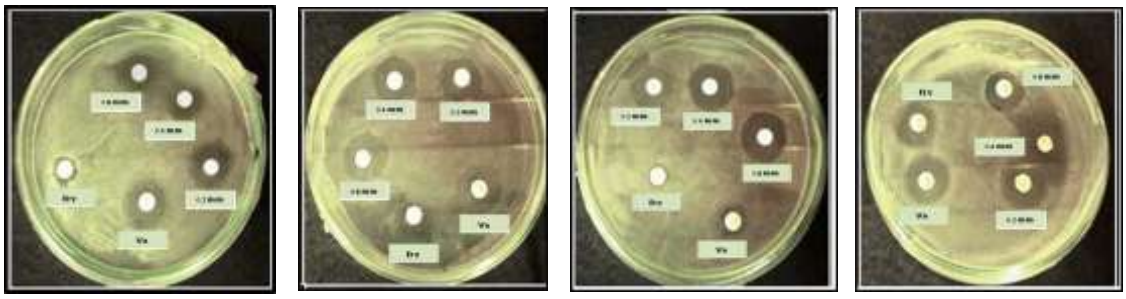


Fig 2. UV-visible spectroscopic analysis for formation of Silver nanoparticles.



(A) (B) (C) (D)

Fig 3. Images of antibacterial activities of discs of different concentration of Ag nanoparticles (0.2mM,0.4mM and 0.8mM) and other antibiotics on (A) *E. coli* (B) *P. aeruginosa* (C) *B. subtilis* (D) *S. pneumonia,* N= nanoparticles, VA= Vancomycin, E= Erythromycin

suspension [28]. Absorption spectra of silver nanoparticles formed in the reaction media has a strong absorbance peak at 478 nm and broadening of peak indicated that the particles are polydispersed.

3.2. Anti-microbial activity

Biosynthesized silver nanoparticles were evaluated for antimicrobial activity against pathogenic microorganisms by using standard zone of inhibition. A clear inhibition zone treated with silver nanoparticles was recorded. (Table 1). Highest zone of inhibition was recorded for E. coli (4.5mm) at 8mM concentration. The standard antibiotics like Vancomycin, Erythromycin shows smaller zone of inhibition as compared to the Nanoparticles treated discs for all the organisms (Table 1). It is observed that there is increase in zone of inhibition with respect to increase in concentration. Silver nitrate which is readily soluble in water has been exploited as an antiseptic agent for many decades. It is being used as a safe inorganic antibacterial agent since centuries and is capable of killing about 650 microorganisms that causes diseases. Silver has been described as being 'oligodynamic' that is, its ions are capable of causing a bacteriostatic (growth inhibition) or even a bactericidal (antibacterial) impact. It has also having ability to exert a bactericidal effect at minute concentration [29]. The exact mechanism of the anti-bacterial effect of silver ions was partially understood. Literature survey reveals that the bactericidal behaviours of nanoparticles is attributed to the presence of electronic effect that are brought about as a result of change in local electronic structure of the surface due to smaller sizes. These effects are considered to be contributing towards

Table 1. Zone of inhibition of Silver Nanoparticles, Erythromycin and Vancomycin against various organisms

Bioactive agent	Concentration	Zone of inhibition (Diameter, mm)			
		E.coli	*B. subtilis*	*P. aeruginosa*	*S. pneumonia*
Ag nanoparticle	0.2mM	2.5	3.2	3.1	3.2
	0.4mM	3.4	4.2	3.3	3.4
	0.8mM	4.5	4.4	4.3	3.8
Erythromycin	(10mcg/disc)	nil	nil	0.6	4.1
Vancomycin	(10mcg/disc)	0.8	nil	0.8	3.8

enhancement of reactivity of silver nanoparticles surface. Silver in ionic form strongly interact with thiol group of vital enzyme and inactivates them [30] studied antibacterial activity against E. coli, S. aureus and S. typhi. They have reported that the effect was dose dependant and was more pronounced against gram negative organisms than gram positives ones. They reported that the major mechanisms through which silver nanoparticles manifest antibacterial property was either by anchoring or penetrating the bacterial cell wall or modulating cellular signalling by dephosphorylating putative key peptide substrates on tyrosine residues. The antibacterial efficacy of the biogenic silver nanoparticles reported in the present investigation may be ascribed to the mechanism described above but it still remains to clarify the exact effect of the nanoparticles on important cellular metabolism like DNA, RNA and protein synthesis.

4. CONCLUSION

An urgent need in the field of nanotechnology is to develop a reliable and eco-friendly process for the synthesis of silver nanoparticles. We have demonstrated for the first time formation of silver nanoparticles utilizing bark extract of *B. monosperma* through efficient green technique, without using hazardous and toxic solvents. The green synthesized silver nanoparticles from bark extract of *B. monosperma* attributed potential antimicrobial activity. Hence forth demonstrating a rapid, economical, simple, rapid, economical Green route for synthesizing noble metal Nanoparticles.

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6. CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

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