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Bio-fabrication of silver nanoparticles employing *Butea monosperma* (Lam.) Taub. var. *lutea* (Witt.) leaf extract and assessment of its antibacterial efficacy

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ABSTRACT

In the current investigation, the simplistic green bio-fabrication of silver nanoparticles (AgNPs) via aqueous leaf extract of Butea monosperma (Lam.) Taub. var. lutea (Witt.) has testified. This investigation exhibited a competent, viable and reproducible technique for the bio-fabrication of environmentally friendly, economically viable and long-time stable AgNPs and their potential claim as antibacterial agent against the Escherichia coli, Staphylococcus aureus, Pseudomonas sp., Bacillus subtilis. The development and steadiness of the reduced AgNPs in the colloidal solution were scrutinized by UV-Vi's spectrophotometer examination. Face-centred cubic structure with crystalline nature is confirmed by the peaks in the XRD pattern conforming to (257), (218), (141), and (207) reflections of the FCC structure of metallic silver planes. SEM analysis discloses individual spherical polydisperse AgNPs. Aggregations of particles having irregularity in shape was observed. The size of the AgNPs was found to be 5-50 nm, with an average size 15 nm. FTIR investigation also revealed the functional groups existing in the leaf extract such as OH, CH, C=C etc. induced the biosynthesis and also worked as a capping agent for the synthesized AgNPs.

Keywords: Eco-friendly; Green Synthesis; Nanotechnology; Plants

INTRODUCTION

Technology dealing with the particle 1-100nm in at least one dimension is known to lead in almost every utility sector of humankind. Due to unique proprieties like greater mass per volume, and optical and thermal properties now, we have quite a long list of nano-based consumer products, from cosmetics, food packaging, agrochemicals, and pharmaceuticals to wastewater treatment (Saxena, 2018). Among various nanoparticles, silver has attained more interest due to its antibacterial activity. AgNPs are widely used as catalysts, electrical conductors, optical receptors, and for antibacterial activity against several bacteria and microorganisms (Choudhary *et al.,* 2022). However, nanoparticles used in these sectors have been synthesized chemically or by physical methods, which involve using harsh and toxic chemicals, lots of energy expenses, and byproducts having unresolved toxic impact potency. these reasons not only restrict their use in agriculture and biomedical but also raise concerns about environmental health. Therefore, it's imperative to find alternatives for synthesising nano form of particles (Bao *et al.,* 2014; Choudhary *et al.,* 2022).

So, nowadays bioresource utilization for the synthesis of nanoparticle is emerging as sustainable solution for the technology development. As biogenic synthesis doesn't require the capping or stabilizing agent for nanoparticle synthesis as the biomolecules present within the bioresource have the potential to reduce and stabilize the synthesized nanoparticle. Energy consumption is low for the process, as no specific temperature and pressure is not required (Bandeira *et al.,* 2020; Kalia *et al.,* 2020). Various plant components such as leafs, leaves, stems, fruit, etc. have been reported to the successful synthesis of $AgNO_3$ (Sivakumar, 2021). The process is one step and doesn't require any aseptic or specific incubation conditions for the reduction of silver nitrate into nanoparticles (Ghaffari-Moghaddam *et al.,* 2014; Vanlalveni *et al.,* 2021). Moreover, phytochemicals present within the plant reduce and stabilize the nanoparticle. Having said that this study also investigated an ethnobotanical plant *Butea monosperma* (Lam.) Taub. var. *lutea* (Witt.) potency for developing silver nanoparticles organically and evaluation of them as antibacterial agents against a few bacterial strains. This study is the very first report of *Butea monosperma* (Lam.) Taub. var. *lutea* (Witt.) leaf extract to be used for AgNP synthesis.

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MATERIALS AND METHODS

Plant sample collection

Butea monosperma (Lam.) Taub. var. *lutea* (Witt.) was collected from a local area of Ambaji, Banas Kantha, Gujrat (Latitude: 24.3310079, Longitude: 72.8516471). The leaves were cleaned with water twice trailed by deionized water to get rid of the fine dust particles and shaded and desiccated for 1 week to eradicate the moisture completely.

Leaf extract Preparation

The desiccated sample leaves were blended to prepare a fine powder. 5 g of powder was mixed into 100 ml of deionized water. Later, the blend was boiled at 60° C for 30 min using a hot magnetic stirrer. Afterwards cooling the leaf extract was filtered via Whatman No. 1 and filtrate kept at 4 C for further usage (Ghaffari-Moghaddam *et al.,* 2014).

Synthesis of nanoparticles

For synthesizing AgNPs, 5 ml of extract was added into Erlenmeyer flask comprising 50 ml of 1 mM silver nitrate solution and held in reserve for incubation for 2 h at 50 C. complete reaction was performed in a dark room to minimize the photoactivation activity of silver nitrate (Ranoszek-Soliwoda *et al.,* 2019).

Characterization of synthesized nanoparticles

The bio-reduction of silver nitrate to AgNPs was measured intermittently by UV-Vi's spectroscopy (Department of Botany, Gokul Global University, Sidhpur, Gujrat). Reading was constantly monitored as the colour of the solution changed by using UV-Vis Spectrophotometer in between 420-450 nm. XRD have been performed for validating the crystalline structure. Synthesized AgNPs were studied using FTIR for the detection of function groups involved in reducing $AgNO_3$. Size and morphology as well as aggregation pattern was observed using scanning electron microscope (SEM) (Vaibhav Analytics Services, Ahmedabad) (Ghaffari-Moghaddam *et al.,* 2014).

Antibacterial efficacy of synthesized silver nanoparticles

The antibacterial potential of the selected extract concentration was evaluated according to their zone of inhibition against the*, Pseudomonas sp., Bacillus subtilis, Escherichia coli Staphylococcus aureus*. and the result were compared with the activity of the control water and the standard antibiotic streptomycin (Sivakumar, 2021).

RESULTS AND DISCUSSION

Synthesis of nanoparticles

The biogenic synthesis of AgNPs using *Butea monosperma (Lam.) Taub. var. lutea (Witt.)* leaf extract was primarily observed by the change in colour. The aqueous mixture turns from light yellow to dark brown within the 24h period at room temperature (Fig. 1&2).

Leaf extracts were added in the aqueous solution of the 1mM silver nitrate. The study reported a gradual variation in the color from light yellow to brown due to the reduction of Ag^+ to Ag^0(Ghaffari-Moghaddam *et al.,* 2014). The colour intensity was found directly in proportional with reaction time and results observed that with the increasing time span, intensity of the colour also found increased (Ranoszek-Soliwoda *et al.,* 2019).

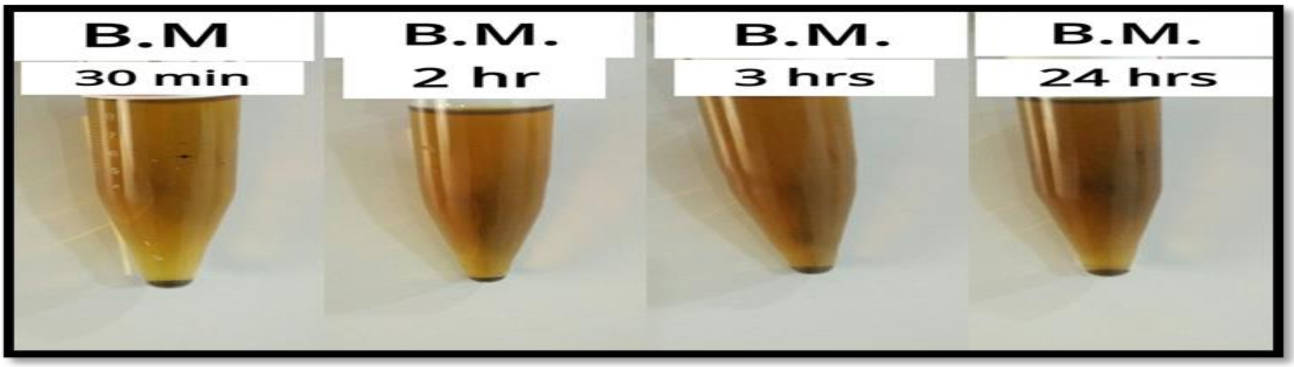

Fig.1: *Butea monosperma (Lam.) Taub. var. lutea (Witt.)* change in colour monitored after (1) 30mins, (2) 2h, (3) 3h, (4) 24h.

Characterization of synthesized nanoparticles

UV Visible spectra

The UV-visible spectra of the synthesized AgNPs via *Butea monosperma (Lam.) Taub. var. lutea (Witt.)* The UV-Vis examination was recorded after time interludes of 30 min, 2 hrs, 4 hrs and 24 hrs (Fig.3.) The sharp peak of AgNPs was observed around 424 nm, after 30 min. That peak was owing to surface plasmon resonance (SPR) which indicated the free electrons presence generated during reduction. SPR provides an absorption band because of the collective vibration of electrons of metal nanoparticles in resonance through a light wave, during the study at 419nm same peak was found significant confirming the reduction and formation of silver nanoparticles. The sharp bands of silver colloids for the Plasmon resonance appeared in the visible range at 417nm which is cumulative with time(Ghaffari-Moghaddam *et al.,* 2014; Sivakumar, 2021).

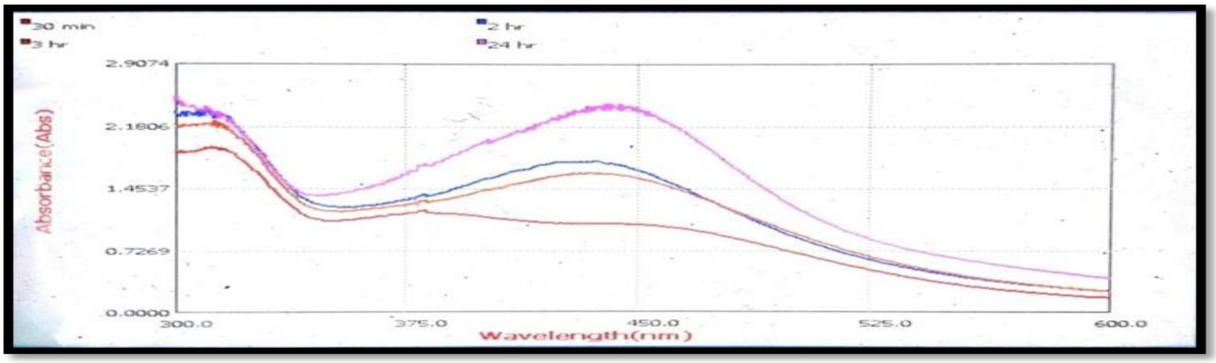

Fig.2: UV-Vis Spectra with respect to the time interval

XRD

The bio-synthesized AgNPs are extremely crystalline in nature with diffraction peaks could be noticeably assigned to the face-centered cubic (FCC) phase of metallic silver. Figure 4 demonstrations five main distinctive diffraction peaks for Ag were detected at 2θ values of 32.06°, 37.99°, 44.28°, and 77.29° are indexed to the (257), (218), (141), and (207) reflections of the FCC structure of metallic silver. Though, the diffraction peaks are wide-ranging which specifies that small crystalline size is attained was matched with a database. (Joint Committee on Powder Diffraction Standards; file No. 04e0783(Ranoszek-Soliwoda *et al.,* 2019).

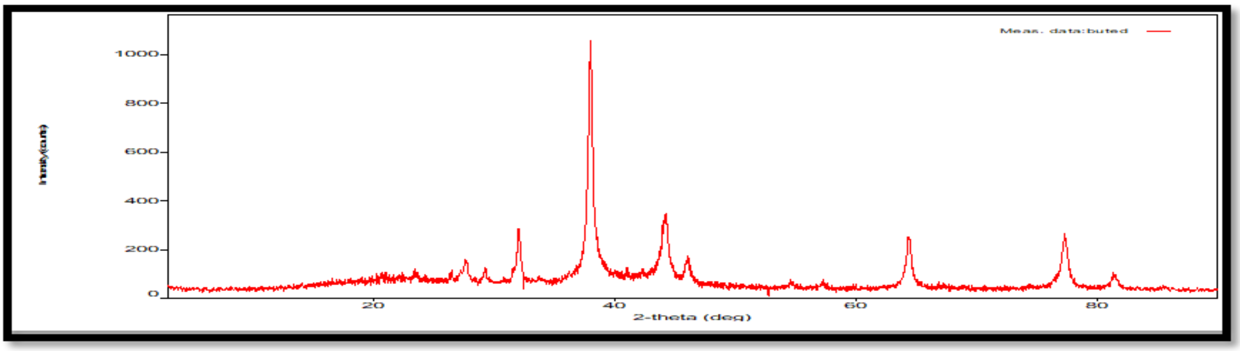

Fig.3: XRD Spectra confirming the formation of silver nanoparticles

FTIR

The FTIR investigation was done for both plant extract and AgNPs. Aqueous leaf extract revealed the peaks at 3317 cm^{-1}, 2121 cm^{-1}, 1636 cm^{-1}, 571 cm^{-1}, the peak at 3317 cm^{-1}shows (Fig.5). The peaks were signifying the participation of multiple functional groups in the reduction and synthesis of AgNPs. Peak identify O-H stretching for Hydroxyl group, C-H Stretching for Alkane, C=C Stretching for Alkanes, NO_2 for Alkanes, C-H Stretching for alkyl, and C-N stretching for Amine groups respectively (Ranoszek-Soliwoda *et al.,* 2019).

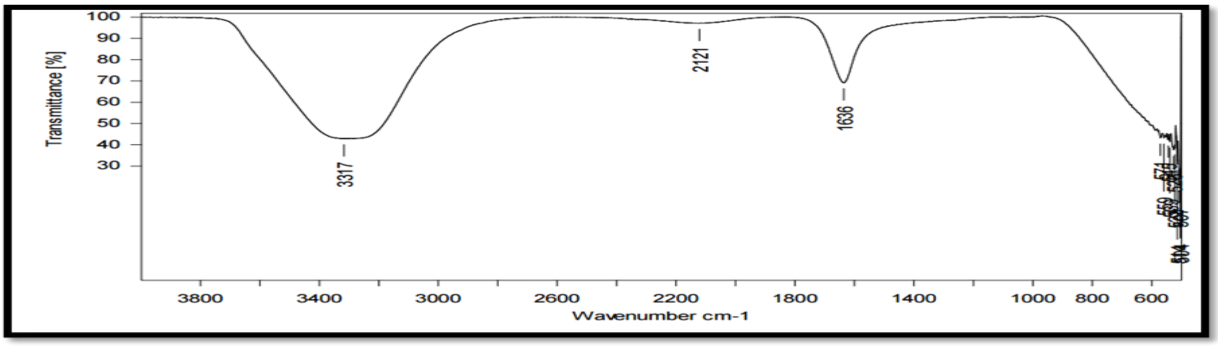

Fig.4: FTIR spectra showing the various functional groups involved in the reduction of $AgNO_3$

Table 1: FTIR peaks and corresponding functional group in AgNPs synthesized from the *B. monosperma* leaf extract

FTIR observed peaks (Cm^{-1})	Functional group
3307	O-H: Hydroxyl group
2920	C-H: Alkane
1609	C=C: Alkanes
1514	No_2
1384	Alkanes
1253	C-H: alkyl
1068	C-N: Amine group

SEM Analysis

SEM examination displays uniformly distributed AgNPs on the surfaces of the cells. SEM study reported spherical polydisperse shape of synthesized AgNPs. Mostly nanoparticles were aggregate and displayed irregular shape. Synthesized nanoparticle size was in between 5-50nm. And average size is 15 nm. Aggregation resulted into the larger sizes (Fig-6)(Sivakumar, 2021).

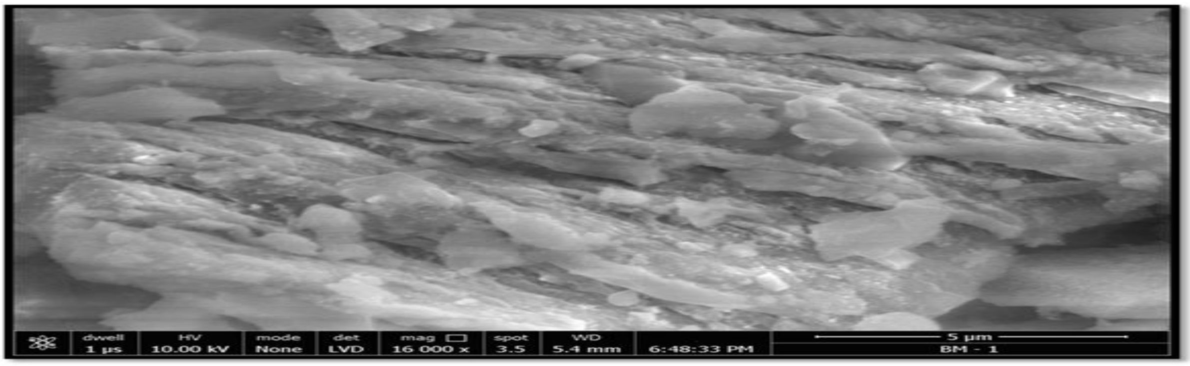

Fig. 5: SEM Micrograph

Antibacterial potential of synthesized AgNPs

It was observed by means of the Agar well diffusion method by measuring the diameter of the developed inhibition zone. They exhibited antibacterial activity against *Bacillus subtitls*, Staphylococcus and *Pseudomonas sp., Escherichia coli.* The antibacterial capability of the selected extracts was assessed according to their zone of inhibition against various bacteria and the result were compared with the activity of the control water and the standard antibiotic streptomycin (Vanlalveni *et al.,* 2021). Among all the types of concentration, a minimum inhibitory zone was obtained in 50 µl of the leaf extracts and synthesized AgNPs, whereas the maximum inhibitory zone was found in 70 µl of both the plant leaf extracts and synthesized AgNPs (Ghaffari-Moghaddam *et al.,* 2014). After development for 24 hrs, a growth inhibition zone was detected around discs saturated with AgNPs and streptomycin as positive control, but the negative control discs could not produce the same zone (table 1) (Sivakumar, 2021). AgNPs unveiled robust antibacterial activity against both Gram-positive and gram-negative bacteria such as, *S. aureus, B. subtilis*, *E. coli, P. aeruginosa.* The Maximum zone of inhibition (18.90±0.45mm) by AgNPs prepared from *B. monosperma* leaf extracts show inhibition for Gram-negative *P. aeruginosa* followed *S. aureus* (18.26±0.17mm) and *B. subtilis* (18.33±0.28mm), *E. coli* (17.93±0.15mm) as shown in Table.

Table 2: Antibacterial efficacy of synthesized AgNPs using *Butea monosperma (Lam.) Taub. var. lutea (Witt.)* leaf extract against various microorganisms

Sr.No.	Name of the organism	Concentrations (µl)	Zone of Inhibition(mm)	
			Leaf extract	Silver nanoparticles
1.	*Escherichia coli*	50 µl	11.50±0.40	16.53±0.29
		100 µl	17.40±0.23	17.53±0.27
		Positive Control	25.33±0.33	
		Negative control	0	
2.	*Pseudomonas sp.*	50 µl	10.76±0.39	17.26±0.31
		100 µl	18.46±0.29	18.30±0.35
		Positive Control	24.33±0.33	
		Negative control	0	
3.	*Bacillus subtilis*	50 µl	11.60±0.15	15.60±0.30
		100 µl	14.83±0.12	16.96±0.52
		Positive Control	28.33±0.33	
		Negative control	0	
4.	*Staphylococcus aureus*	50 µl	14.17±0.59	16.43±0.23
		100 µl	16.40±0.26	17.83±0.42
		Positive Control	28.33±0.33	
		Negative control	0	

NOTE: Values are the mean of three replicates± standard error

CONCLUSION

The AgNPs green synthesis process via *Butea monosperma (Lam.) Taub. var. lutea (Witt.)* is eco-friendly and cost-effective. Phytochemicals found in leaf extract are believed to reduce silver ions in metallic nanoparticles. Synthesized AgNPs are found to have significant antibacterial efficacy.

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