E-ISSN: 2706-8927
P-ISSN: 2706-8919
www.allstudyjournal.com
IJAAS 2023; 5(8): 16-20
Received: 18-05-2023
Accepted: 22-06-2023

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Qualitative evaluation of inhibition of β-hematin formation antimalarial and radical scavenging activity of synthesized *Butea monosperma* silver nanoparticles

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DOI: https://doi.org/10.33545/27068919.2023.v5.i8a.1023

Abstract
In this research, silver nanoparticles were synthesized from *Butea monosperma* for *in vitro* Qualitative evaluation of inhibition of β-hematin formation and Radical scavenging activity. Silver nanoparticles are deemed the most positive, considering their strong volume surface region, and are of concern for study because of the improved microbial tolerance to antibiotics and medicines. Therefore, green synthesis of nanoparticles of silver using biomolecules derived from various plant sources in the form of extracts can be applied for the screening of different diseases which trigger microorganisms and for the physical and biological characterization of plant-derived silver nanoparticles. The experiment involved the green synthesis of silver nanoparticles (AgNPs) from *Butea monosperma* leaf extract. Biosynthesized *Butea monosperma*-AgNPs were characterized by UV-visible spectroscopy, Fourier-transform infrared (FTIR) spectroscopy and scanning electron microscopy (SEM). The intensity of peak broad range 200-800nm in UV-vis spectra, EDS test. The SEM shows the actual size of the nanoparticles Sample Code A, B, C, D samples showed good percent inhibition *in vitro* Qualitative evaluation of inhibition of β-hematin formation and Radical scavenging activity1mg/ml, compound showed good antioxidant property.

Keywords: *Butea monosperma*, UV-visible spectroscopy, fourier-transform infrared (FTIR) spectroscopy, scanning electron microscopy (SEM), silver nanoparticles, radical scavenging activity antioxidant, β-hematin inhibition antimalarial activity

1. Introductions
Metals such as metals and metal oxides, silicates, non-oxide ceramics, polymers, organic materials, biomass and biomolecules may be used for producing nanoparticles. In various morphologies, nanoparticles occur, including balls, cylinders, platelets, tubes etc. Inorganic nanoparticles such as golden and silver metal nanoparticles have superior material properties with mechanical flexibility, with broad availability, comprehensive mobility, strong compatibility, selective therapeutic products and regulated drug release capabilities. For the synthesizing and stabilisation of silver nanoparticles, many physical, chemical and biological methods were used. The word biofilm has been used to refer to the thin coated condensations of microbes (for example bacteria, fungi, protozoa, etc.) which can appear in different types of surface structures. Antifungal performance may be calculated by means of well diffused methods on various fungal strains. Free floating bacteria, classified as planktonic microorganisms in an aqueous climate, are a requirement for the development of biofilms. Thus, such films may be formed on every organic or inorganic substratum where planktonic microorganisms prevail in a water solution (. Because of its unusual physical and chemical properties, silver nanoparticles (AgNPs) are progressively being used in numerous fields, including medical, fruit, patient treatment, consumption and industrial uses. This involves visual, electronic, thermal, heavy electrical and biological characteristics. Because of its unusual properties, it has been used for many applications in the medicinal, food processing, surgical, orthopedic, medication distribution, anticancer industries, as well as for numerous applications such as non-bacterial agents, automotive, domestic and health goods, electronic products, medical equipment jackets, optical sensors and cosmetics AgNPs have been widely used lately in numerous textiles, keyboards, wound dressings and biomedical instruments. The nanosized metallic particles are peculiar and, because of their surface to volume ratio, can greatly alter physical, chemical and biological properties; thus, nanoparticles have been

used for different purposes. In order to satisfy the AgNPs criterion, different methods for synthesis have been introduced. In general, current approaches of physics and chemistry appear rather costly and risky. It is important to notice the high yield, solubility and high stability of biologically prepared AgNPs. Biological methods for AgNPs seem simplistic, quick, nontoxic, reliable and green among. A range of analytical methods are used, including UV spectroscopy, X Ray Diffractometer (XRD), Fourier infrasound transform spectroscopy (FTIR), X-ray photoelectron spectroscopy (XPS), DLS scanning, SEM, transmission electron microscope (TEM), atomic force microscopy (AFM) (K Venugopal *et al*., 2017) [8] (Raju Vivek, *et al*., 2012) [17], (V. Kathiravan *et al*., 2014) [24], (Shahnaz Majeed, *et al*., 2019) [20], (Sabina Yeasmin *et al*., 2017) [19], (R.M. Gengan *et al*., 2013) [14], (R.R. Remya *et al*., 2015) [16]. Several competent books and studies have identified different styles of methodological methods for characterizing AgNPs. A highly effective and accurate technique for the primary characterization of synthesised nanoparticles used for tracking the production and stabilisation of AgNPs is UV-Visible Spectroscopy. Malaria is one of the major threats concerning world public health. There are around 250 million clinical cases every year and almost one million deaths, mostly children, which are attributable to this disease The main reasons that explain this worsening situation are therapy problems), (R.M. Gengan *et al*., 2013) [14], One possible source for such affordable treatments lies in the use of traditional herbal remedies. The use of plants for the treatment of malaria extends over at least three continents, including several countries in Africa, in the Americas and in Asia.

2. Materials and Methods

2.1 Sample preparation: The young and disease-free leaves of *Butea monosperma* were selected.

2.1.1 Drying of leaves: Leaf samples were dried in room temperature for more than two weeks, so that they may be converted into fine powder.

2.1.2 Preparation of fine powder: After proper drying of leaves, thick mid ribs of the leaves was removed, dried leaves were grinded into fine powder using a grinder.

2.1.3 Preparation of extracts: Aqueous extracts using distilled water, 50% ethanol, 50% methanol & 50% acetone were prepared.

2.2 Synthesis of Silver Nanoparticles from *Butea monosperma* extracts

AgNPs were synthesized by the following method. 10mM $AgNO_3$: plant extracts in different solvent in 9:1 ratio in a reagent bottle mixed thoroughly, forming a uniform mixture. The mixture was then rested at room temperature for 24 hours at 37 °C, with continuous monitoring. After about few minutes, the mixture was observed to start changing from pale green to yellowish brown. After about 24 hours, the mixture had completely changed colour to brown in all solvents. This color change is visual evidence of formation of AgNPs. (Kasthuri *et al*., 2009) [10].

2.3 Characterization of silver nanoparticles

For determination of the time point of maximum production of silver nanoparticles, the absorption spectra of the samples were taken 200–8000 nm using a UV–vis spectrophotometer. The silver nanoparticles were synthesized by novel green chemical route. The nanoparticles were characterized by UV- spectral analysis, SEM -EDAX analysis (Scanning Electron Microscopy) was performed for studying the surface morphology & to predict the size of the nanoparticle. Also, FTIR analysis was conducted for identifying the presence of functional groups. (Anuja *et al*. 2020) [6]

2.4 Antioxidant activity

2.4.1 Sample Description: **Four Samples (A, B, C, D.) Activity**: Antioxidant activity by DPPH (96 well method) Procedure: Determination of Anti-oxidant activity Antioxidant activity in the sample A, B, C, D compounds were estimated for their free radical scavenging activity by using DPPH (1, 1-Diphenyl-2, Picryl-Hydrazyl) free radicals. 100 µL of A, B, C, D (1mg/ml) compounds were taken in the microtiter plate. 100 µL of 0.1% methanolic DPPH was added over the samples and incubated for 30 minutes in dark condition. The samples were then observed for discoloration; from purple to yellow and pale pink were considered as strong and weak positive respectively and read the plate on Elisa plate reader at 490nm Radical scavenging activity was calculated by the following equation:

DPPH radical scavenging activity (%) = [(Absorbance of control - Absorbance of test sample) / (Absorbance of control)] x 100

2.5 Anti malarial Qualitative evaluation of inhibition of β-hematin formation

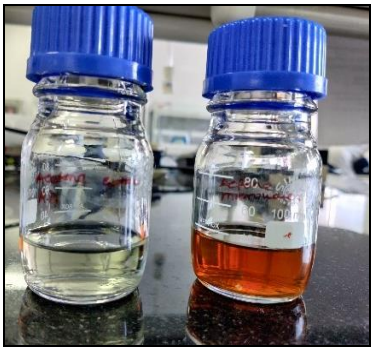
2.5.1 Stock solution preparation: A solution of hematin was prepared by dissolving 5 mg hemin (bovine, ≥ 90.0 %, obtained from Hi Media) in 1.0 mL of 0.1 M NaOH. This solution was incubated at 60 °C, followed by the addition of previously incubated 0.1 mL 1.0 M HCl and 0.58 mL of 12.9 M acetate (pH 5) at 60 °C. The mixture was incubated at 60 °C for 1 h. After cooling in an ice bath for 5 min, the solid obtained was filtered using a 8 µm cellulose acetate/nitrate filter disk Millipore filter and washed thoroughly with water. The solid collected was dried under vacuum for several hours before analysis by infrared spectroscopy in a Bruker FT-IR spectrometer.

3. Results

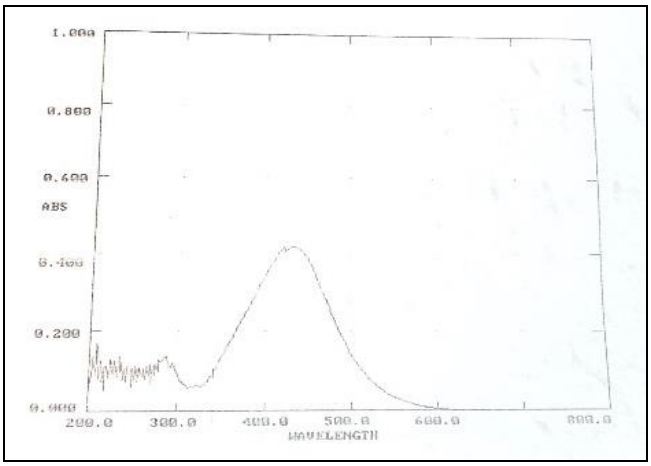
The detailed study on biosynthesis of silver nanoparticles by natural *Butea monosperma* extracts such It was observed that the color of the solution turned from yellow to bright yellow and then to dark brown after 1, 24 and 48 h of the reaction, which indicated the formation of silver nanoparticles (fig 1).

The formation and stability of the reduced silver nanoparticles in the colloidal solution was monitored by UV–vis spectrophotometer analysis. The UV–vis spectra show maximum absorbance at 420 nm, which increased with time of incubation of silver nitrate with the plants extract. The curve shows increased absorbance in various time intervals (1 h, 24 h and 48 h) and the peaks were noticed at420 nm corresponding to the surface plasmon resonance of silver nanoparticles. The observation indicated that the reduction of the Ag+ ions took place extracellularly. It is reported earlier that absorbance at around 430 nm for

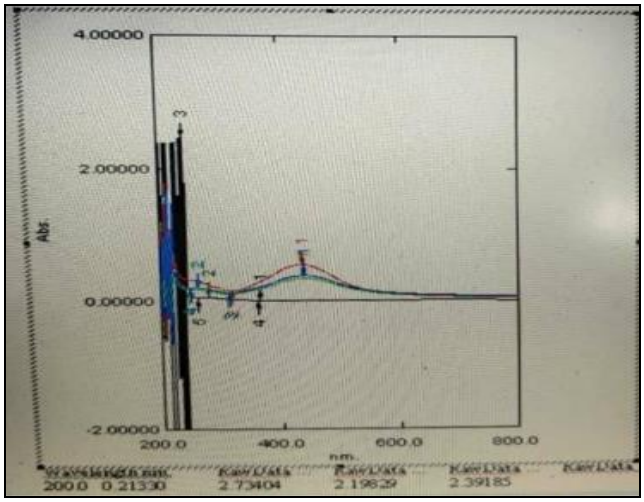
silver is a characteristic of these novel metal particles (Nestor *et al*., 2008). The synthesis of AgNPs from the ethanolic, aqueous, methanol & acetone extract of leaves of *Butea monosperma* was further confirmed by ultraviolet - visible spectroscopy (UV/VIS) in the range of between 200 nm to 800 nm and solvents were used as a blank. The spectrum has a maximum absorption peak at a which is reported to have an absorption maximum of between about 400nm to about 450nn. The presence of the maximum peak absorption peak at 400nm to about 450nn is therefore an indication and confirmation that the AgNPs were present. (Fig 1)



a)



b)



c)

Fig 1: a) Synthesis of nanoparticles using *Butea monosperma* b) and c) presence of the maximum peak absorption peak at 400 nm to about 450nn

Fourier Transform Infra-Red Spectrometer (Equipped With ATR) Model Tensor 600 Brucker. As seen in figure given below, FTIR spectra of all samples shows similar pattern.

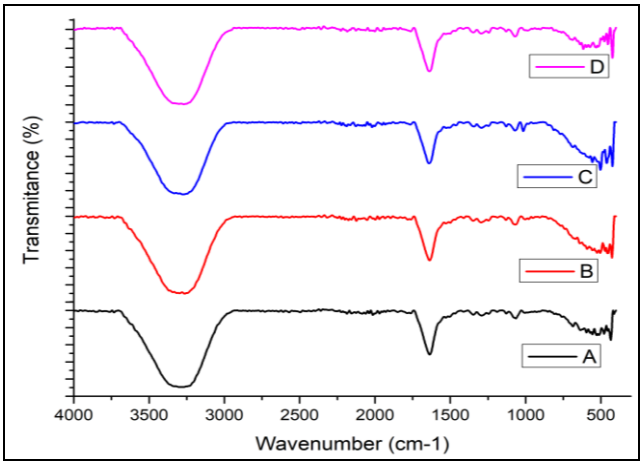


Fig 2: FTIR spectra of all four solvents of *Butea monosperma* synthesized Sliver AgNPs

FTIR spectra depicts bands at ~3200-3300 corresponding to alcoholic O-H stretching, bands at ~ 1620 corresponds to; where as small band at ~1100 is corresponds to alcoholic C-O bond, metallic silver bond is seen at ~450 cm-1. Fig 2 where A-water, B Ethanol, C methanol & D acetone solvents respectively.

3.1 antioxidant activity

Antioxidant activity in the sample A, B, C, D compounds were estimated for their free radical scavenging activity by using DPPH (1, 1-Diphenyl-2, Picryl-Hydrazyl) free radicals. 100 µL of A, B, C, D (1mg/ml)

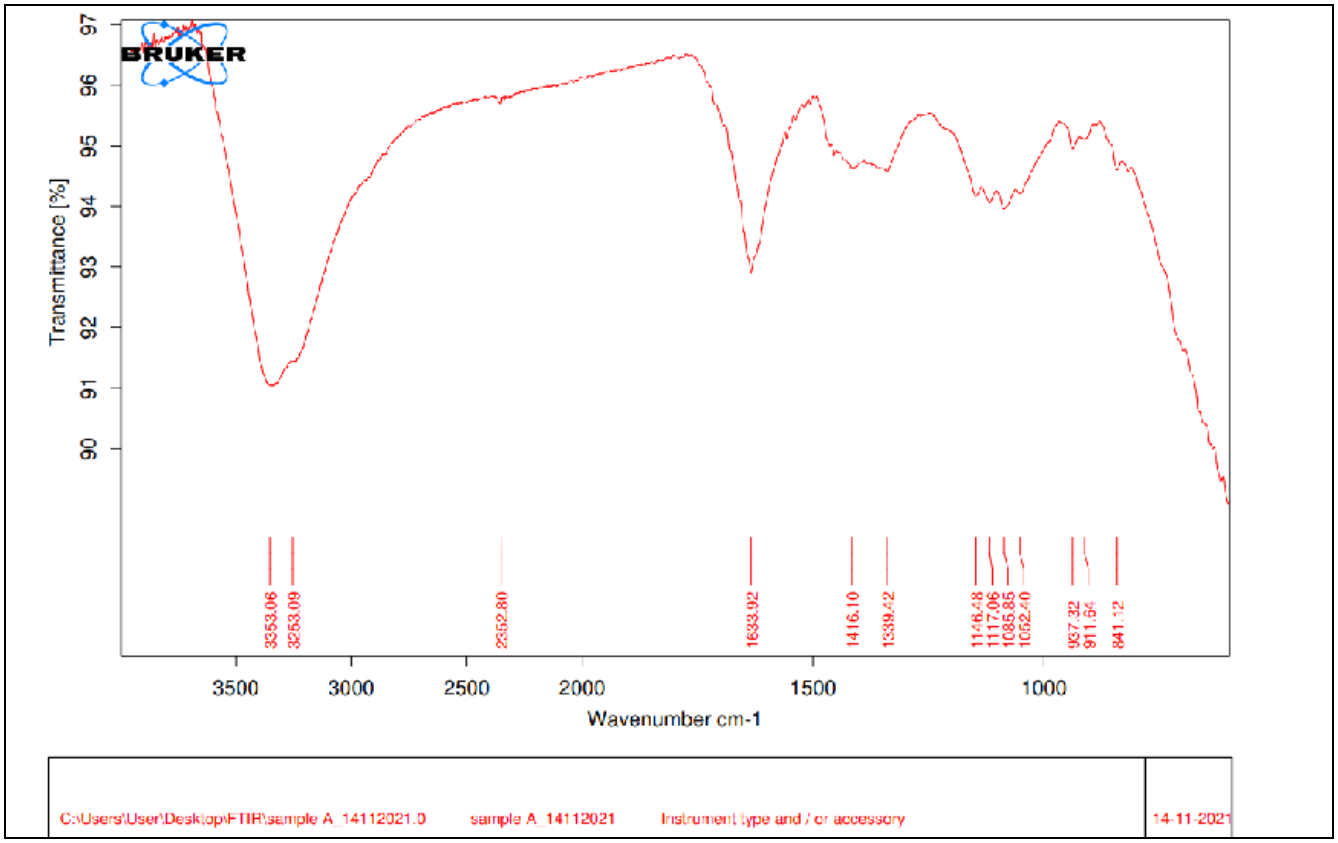
Table 1: Effects of different compounds against DPPH

Sr. no.	compounds (1 mg/ml)	ABS (OD)	Mean	Percentage of DPPH radical scavenging
	Control	0.815		
		1.087	1.014	
		1.140		
	Std Ascorbic acid	0.288		
		0.269	0.28	72.38
		0.283		
	Sample A	0.283		
		0.584	0.493	43.05
		0.613		
	Sample B	0.496		
		0.565	0.547	46.05
		0.580		
	Sample C	0.469		
		0.546	0.529	47.83
		0.574		
	Sample D	0.443		
		0.506	0.494	51.28
		0.533		

Conclusion: At the concentration of 1 mg/ml, compound showed good antioxidant property

3.2 Antimalaria

As per the IR graph value on the control value 1635, and 3300 indicates formation of β-hematin formation. Test compound A to D sample showed similar peak as compared to control. so it indicates that the said Qualitative compound inhibit the β-hematin formation.



4. Conclusion

In this study, different temperatures were used for silver nanoparticle biosynthesized by using *Butea monosperma* extract. The greatest Plasmon resonance was acquired at 60 °C previously, improved the procedure parameters for incorporating AgNPs by utilizing aqueous extracts of *Butea monosperma*. UV–Vis spectroscopy and FT-IRanalysis confirmed the formation of the silver nanoparticles. The size of AgNPs was confirmed by TEM analysis, which illustrates that AgNPs were spherical in shape with size ranging from 5 to 40 nm. Synthesized AgNPs appeared polydispersed and well scattered. The synthesized silver nanoparticles and *Butea monosperma* extracts were compared and showed promising which suggests the potential therapeutic use of these nanoparticles. At the Concentration of 1mg/ml, compound showed good antioxidant a Antimalarial property. As compared to standard drug.

5. Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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