**In vitro α- amylase inhibitory and anti-inflammatory activity of Butea monosperma silver nanoparticles**

Akshay Patil, Ganesh Janvale, Shrutkirti Shinde, Dhanvarsha Bhusari, and Sanghmithra Kadam

**Abstract**

In this research, silver nanoparticles were synthesized from Butea monosperma for in-vitro α-amylase inhibitory and anti-inflammatory activity. Because of the improved microbiological resistance to antibiotics and medications, silver nanoparticles are rated the most positive due to their large volume surface region. As a result, green synthesis of silver nanoparticles using biomolecules derived from various plant sources in the form of extracts can be used for disease screening and physical and biological characterization of plant-derived silver nanoparticles. The green synthesis of silver nanoparticles (AgNPs) from Butea monosperma leaf extract was the subject of the experiment. UV-visible spectroscopy, used to analyse biosynthesized Butea monosperma-AgNPs. The intensity of the peak broad range 200-800 nm in UV-Visible spectra, an in vitro anti-diabetic activity was evaluated by α-amylase inhibition method while anti-inflammatory by albumin denaturation assay. At the concentration of 1000 µl of compound, the sample A (water) and D (acetone) showed good α-Amylase inhibitory activity and showed more percent inhibition of protein denaturation as compared with standard.

**Keywords:** Monosperma, Silver nanoparticles, α-Amylase Inhibition activity, Anti-inflammatory activity

1. Introduction

condensations of microbes such as bacteria (Shinde S. S. et al. 2023) [41-42], fungus, protozoa, etc. that can develop on various kinds of surface structures are referred to as biofilms. On a variety of fungal strains, antifungal performance can be calculated using well-established techniques (Yazdanian, Mohnsen, et al. 2022; Karthikeyan, G., et al. 2020; Polash, Shahil Ahmed, et al. 2021) [54, 15, 53]. For biofilms to form, free-floating bacteria, also known as planktonic microorganisms in an aquatic environment, are necessary. This means that where planktonic microbes predominate in a water solution, such films may form on any organic or inorganic substrate (Subramanayan, S. B., 2021; Al-Momani, H., et al. 2023) [45-52]. Silver nanoparticles (AgNPs) are increasingly being used in a variety of disciplines, including medicine, food, patient treatment, consumption, and industrial usage due to their distinctive physical and chemical characteristics. This involves biological, heavy electrical, thermal, electronic, and optical aspects (Widatalla, H. A., et al. 2022; Habeeb Rahuman, H. B., et al. 2022; Zhang, X. F., et al. 2016; You, C., et al. 2012) [51, 11, 58, 56]. Due to its unique characteristics, AgNPs are widely used in the pharmaceutical, food manufacturing, surgery, orthopedics, medication distribution and anticancer industries (Shariatinia, Z. 2018; Panja, A., et al. 2021; Zhang, X. F., et al. 2016) [39, 31, 58]. AgNPs are also used in a variety of other applications such as Non-Bacterial Agents, Automotive (Sacharczuk, J. 2021) [38]. In recent years, AgNPs can be found in many textiles and keyboards as well as wound dressings as well as biomedical instruments (Li, W. R., et al. 2010; Li, C., et al. 2014; Sondi, I., & Salopek-Sondi, B. 2004) [43, 20, 44]. These nano-sized metallic particles are unique and, due to their surface-to-volume ratio, have the potential to significantly change physical, chemical because nanoparticles have biological qualities, they are used in a wide range of applications. Different synthesis routes have been devised to meet the AgNPs requirement. In general, existing physics and chemistry procedures appear to be highly costly and dangerous. It is crucial to highlight, however, that bio-processed AgNPs have a high yield, solubility, and stability (Navya, P. N., & Daima, H. K. 2016; Shokrlu, Y. H., & Badabaghi, T. 2014; Shokrlu, Y. H., & Babadagli, T. 2014) [28, 40]. Biological approaches for AgNPs appear to be simple, fast, nontoxic, dependable, and ecologically sound. UV spectroscopy (Huang, X., et al. 2007; Leung, A. B., et al. 2006; Tomaszewska, E., et al. 2013) [12, 19, 47]. X ray diffractometry (XRD) (Das, R., et al. 2009; Zawrah, M. F., et al. 2013 Yazdian, N., et al. 2013; Vaia, R. A., & Liu, W. 2002) [6, 57, 55, 48]. Fourier infrared transform spectroscopy (FTIR) (Jung, C. 2000; Kim, S., & Barry, B. A. 2001; Mantele, W. G., et al. 1988) [14, 17, 25]. X-ray photoelectron spectroscopy (XPS) (Joshi, M., et al. 2008; Demathieu, C., et al. 1999) [13, 8]. DLS scanning, SEM, transmission electron microscope (TEM), and atomic force microscopy (AFM) are among the analytical procedures employed (Wang, Z. L. 2000; Yao, H., & Kimura, K. 2007; Lin, P. C., et al. 2014) [50, 53, 22]. Several relevant books and studies have identified various approaches for characterizing AgNPs. UV-Visible Spectroscopy is a highly effective and accurate technique for the main characterization of synthesized nanoparticles used for tracking the creation and stabilisation of AgNPs (Rajeshkumar, S., & Bharath, L. V. 2017) [56].

2. Materials and Methods
2.1 Sample preparation: Young and actively growing leaves of Butea monosperma were collected from different regions of Maharashtra. After thoroughly drying the leaves, the thick midribs were removed and the dried leaves were ground into a fine powder with a grinder. Aqueous extracts were made with distilled water, 50% ethanol, 50% methanol, and 50% acetone.

2.2 Synthesis of Silver Nanoparticles from Butea monosperma extracts
AgNPs were produced from the plant extract of Plant extracts in various solvents in a 9:1 ratio were thoroughly mixed to create a homogeneous mixture containing 10 mM AgNO3 in a reagent bottle. The combination was then allowed to settle for 24 hours at 37°C under constant observation. The combination began to turn from pale green to yellowish brown after a short while. After around 24 hours, the mixture had completely turned brown in all solvents. The change in colour is tangible evidence that AgNP was produced. (Mohammad Ali, et al., 2016) [24].

2.3 Characterization of silver Nanoparticles
Characterization of silver nanoparticles is done by UV-Visual spectrophotometer, SEM-EDAX analysis and FTIR analysis.

Sample Description: Sample A (Water), B (Ethanol), C (Methanol), D (Acetone).

Assay of Amylase Inhibition
The Bernfeld technique was used to study in vitro amylase inhibition. In a nutshell, 500 µl of the 0.1 M phosphate buffer pH 6.9 containing -amylase enzyme and 1000 µl of the test substance were allowed to react. 500 µl of 1% starch solution in 0.1M phosphate buffer pH6.8 was added after a 10-minute incubation period at 25 °C. The same procedure was used for the controls, where 500 µl of the enzyme was swapped out for buffer and incubated once more for 10 minutes at 25°C. 1000 µl of the dinitrosalicylic acid reagent were added to both the control and the test after incubation. They spent 10 minutes in a bath of boiling water before being cooled. Utilising a spectrophotometer to measure absorbance at 540 nm, the percentage of the -amylase enzyme that was inhibited was estimated using the formula.

\[
\text{Inhibition} (\%) = \frac{\text{Abs 540(control)} - \text{Abs 540(extract)}}{\text{540 (Control)}} \times 100
\]

Suitable reagent blank and inhibitor controls were simultaneously carried out.

Anti-inflammatory activity
In-vitro anti-inflammatory activity was performed by Protein denaturation method. The reaction mixture (10 ml) consisted of 0.4 ml of egg albumin (from fresh hen’s egg), 5.6 ml of phosphate buffered saline (PBS, pH 6.4) and 1000µl Sample A, B, C, D. A comparable amount of double-distilled water was used as the control. The mixtures were then heated for 5 minutes at 70°C after 15 minutes of incubation at (370 c ±2) in the incubator. Their absorbance at 660 nm was measured after cooling, using a vehicle as a reference. For the purpose of determining absorbance, diclofenac sodium at a concentration of 1000 g/ml was employed as a reference medication and handled identically. Using the following formula, the % inhibition of protein denaturation was determined.

\[
\%\text{inhibition} = \frac{\text{absorbance of control} - \text{absorbance of test}}{\text{absorbance of control}} \times 100
\]
3. Results and Discussion

The thorough investigation of the production of silver nanoparticles using natural *Butea monosperma* extracts, including after 1, 24, and 48 hours of the reaction, it was noticed that the colour of the solution changed from yellow to brilliant yellow and subsequently to dark brown, indicating the creation of silver nanoparticles (Figure 1). Through UV-vis spectrophotometer examination, the production and stability of the reduced silver nanoparticles in the colloidal solution were tracked. Maximum absorbance at 420 nm was seen in the UV-Visual spectra, and it increased over time as silver nitrate was incubated with plant extract. The curve indicates an increase in absorbance with time (1 hour, 24 hours, and 48 hours), and the peaks were found at 420 nm, which corresponds to the silver nanoparticles' surface plasmon resonance. The observation suggested that the Ag⁺ ions were reduced extracellularly. It has previously been stated that one hallmark of these new metal particles is their absorbance at about 430 nm for silver. By using solvents as a control, ultraviolet-visible spectroscopy (UV/VIS) was performed to further confirm the production of AgNPs from the ethanolic, aqueous, methanol, and acetone extract of *Butea monosperma* leaves. The spectrum has a maximum absorption peak at which the highest absorption is reportedly between 400 and 450 nm. Therefore, the occurrence of the greatest peak absorption peak from 400 nm to around 450 nm provides proof that AgNPs were present. In Figure 1. Table 1 and figure 2 display the results of the inhibitory activity of the -amylase enzyme.

![Fig 1: AgNPs from Leaves extracts of Butea monosperma shown in brown colour and UV–Vis absorption spectrum of silver nanoparticles (AgNPs) with λ = 300–600 nm](image)

**Table 1: Inhibition α-amylase assay of standard and Butea monosperma leaves extract**

<table>
<thead>
<tr>
<th>Sample code and Concentration</th>
<th>Absorbance at 540 nm</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.98</td>
<td></td>
</tr>
<tr>
<td>Standard Acarbose (1000 µg/ml)</td>
<td>0.38</td>
<td>80.80</td>
</tr>
<tr>
<td>Sample A (1000 µl)</td>
<td>0.61</td>
<td>69.19</td>
</tr>
<tr>
<td>Sample B (1000 µl)</td>
<td>1.06</td>
<td>46.46</td>
</tr>
<tr>
<td>Sample C (1000 µl)</td>
<td>0.97</td>
<td>51.01</td>
</tr>
<tr>
<td>Sample D (1000 µl)</td>
<td>0.65</td>
<td>67.17</td>
</tr>
</tbody>
</table>

![Fig 2: In-vitro α-amylase inhibitory potential of B. monosperma extracts for standard Acarbose, Sample A-Water, and Sample B-Ethanol. Sample C-Methanol and Sample D-Acetone](image)
### Table 2: The anti-inflammatory activity/ Percent Inhibition (%) at different concentrations

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Concentration</th>
<th>Anti-inflammatory activity</th>
<th>Absorbance at 660 nm</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.89</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard Diclofenac sodium</td>
<td>1 mg/ml</td>
<td>0.28</td>
<td>68.53</td>
<td></td>
</tr>
<tr>
<td>Sample A</td>
<td>1000µl</td>
<td>0.33</td>
<td>62.92</td>
<td></td>
</tr>
<tr>
<td>Sample B</td>
<td>1000µl</td>
<td>0.49</td>
<td>44.94</td>
<td></td>
</tr>
<tr>
<td>Sample C</td>
<td>1000µl</td>
<td>0.44</td>
<td>50.56</td>
<td></td>
</tr>
<tr>
<td>Sample D</td>
<td>1000µl</td>
<td>0.38</td>
<td>57.30</td>
<td></td>
</tr>
</tbody>
</table>

**Fig 3:** The anti-inflammatory activity percent inhibition (%) at different concentrations of B. monosperma extracts for standard Acarbose, Sample A-Water, Sample B-Ethanol, Sample C-Methanol, and Sample D-Acetone

### 3. Conclusion

In this study, different temperatures were used to biosynthesize silver nanoparticles using *Butea monosperma* extract. The highest Plasmon resonance was obtained before at 60 °C, which increased the technique parameters for integrating AgNPs by utilizing *Butea monosperma* aqueous extracts. The production of the silver nanoparticles was confirmed by UV-Vis spectroscopy and FT-IR measurement. TEM investigation confirmed the size of the AgNPs, which were spherical in shape and ranged in size from 5nm to 40 nm. Synthesized AgNPs appeared polydispersed and evenly distributed. Anti-inflammatory activity of AgNPs showing at sample A at a concentration 1000 µl where the percentage of inhibition is high i.e. 68.53 as shown in table 2 and figure no. 3. The synthesized silver nanoparticles and *Butea monosperma* extracts were compared and showed good anti-diabetic and anti-inflammatory activity.

### References


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