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Investigation of Green Synthesis Conditions of Silver Nano-particles Using *A. eupatoria* extract

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pharmaceuticals MDPI

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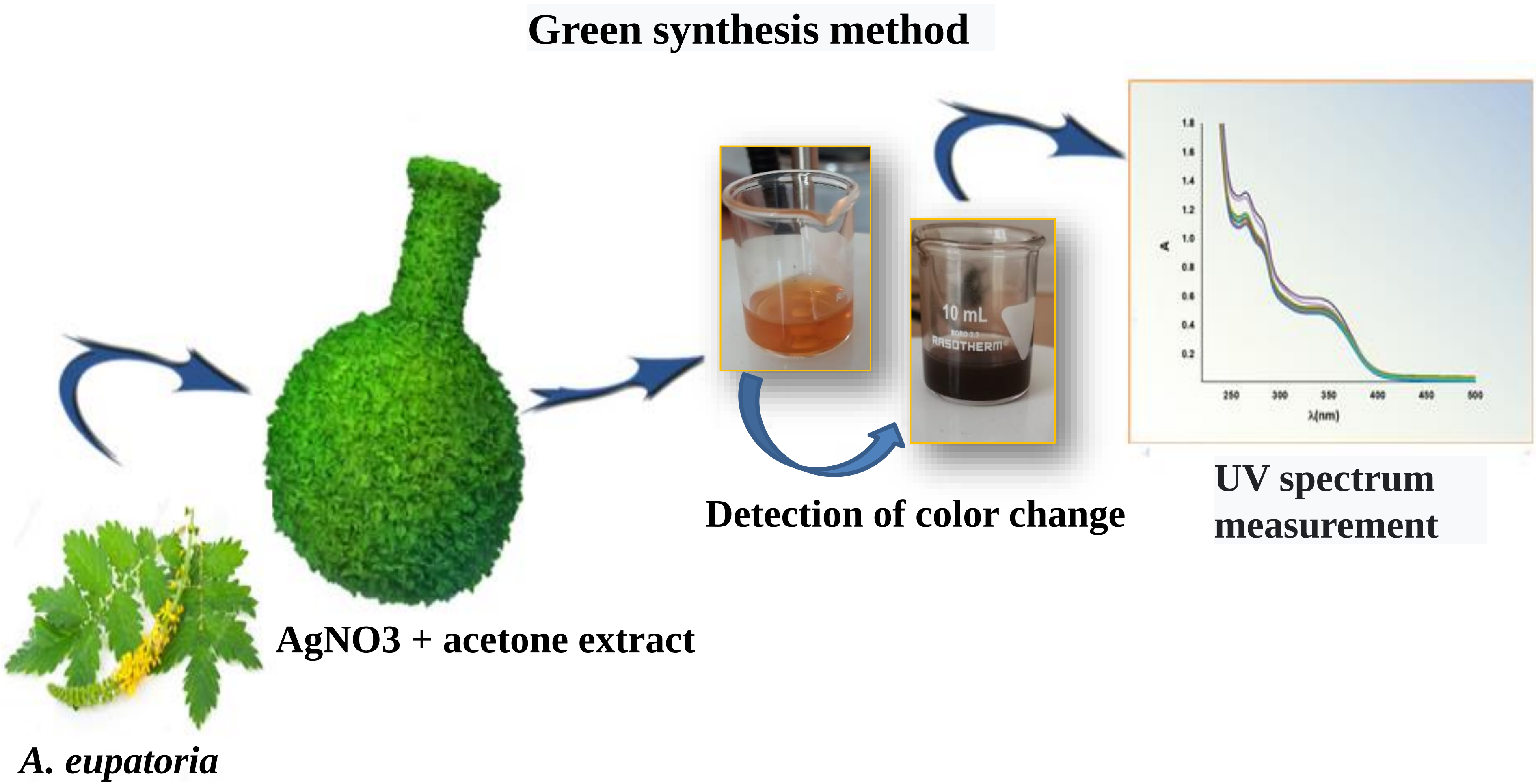
Grafical abstract:



Keywords: Silver, Nanoparticles, Green Synthesis, *A. eupatoria*

Abstract:

Introduction

Green synthesis of silver nanoparticles represents the great interest in pharmacology due to their wide range of applications. Silver nanoparticles have characteristic physical, chemical, and biological properties, which have potential applications in nanobiotechnological research. Green synthesis involves the synthesis of non-toxic nanoparticles, with cheap and ecological acceptable synthesis technology, and implies the use of plant extracts as a reducing agent of selected metal salts.

Methodology

In our research, we used the cosmopolitan plant species *A. eupatoria*, which is known to be used for medicinal purposes, as well as silver nitrate with known antimicrobial properties. Different reaction conditions were applied for the synthesis of nanoparticles using the extracts of *A. eupatoria*. Silver nitrate was dissolved in different concentrations (5, 10, and 20mM). The reaction mixtures were stirred on a magnetic stirrer and heated at different temperatures (25 ^{0}C and 50 ^{0}C) until metal nanoparticles were formed. To modify the pH of the reaction mixture (pH 2, 4, and 6) 0.1M HNO_3 or 0.1M NaOH were used. Visual color change (from light yellow to dark brown) and UV-Vis spectrophotometry were used to observe the production of AgNPs throughout the synthesis.

Results and Discussion

The UV-Vis absorption spectra of formed nanoparticles were recorded (300–800 nm) and the highest peaks were positioned within 300–375 nm (characteristic peak for AgNPs), suggesting the formation of AgNPs. The best conditions for the highest AgNPs yield production were a 5 mM concentration of $AgNO_3$, a reaction temperature of 25 ^{0}C, pH=4, and a reaction time of 3h for synthesis.

Introduction

- ***Agrimonia eupatoria L.*** belongs to the family Rosaceae. The species is widespread throughout Europe, Asia, Africa, and North America.
- *A. eupatoria* is a well-known medicinal plant, which is traditionally used in folk medicine to treat various inflammatory diseases.
- The plant possesses anti-inflammatory, neuroprotective, antidiabetic, antiobesity, hepatoprotective, and anticancer properties.
- It is known that the plant synthesizes secondary metabolites that exhibit antioxidative and antimicrobial activity. Besides, some studies have demonstrated the antioxidant activity of water, ethanol, acetone, and diethyl ether extracts of this plant as well as the antibiofilm activity of these extracts.
- Besade antioxidative activity plant has reduced power[1].

[1] Muruzovic M et al. Journal of Food and Drug Analysis, 2016, 24: 539–547

Green synthesis of silver nanoparticles

- Extracts of plant can be used as reducing agents and as stabilizers in the synthesis of nanoparticles. Silver ion reducers such as amino acids, polysaccharides, proteins and vitamins found in the extract are environmentally acceptable.
- The appearance of a brown/dark color to black-purple in the previously yellow liquid (depends on the color of the plant extract) is an indicator of nanoparticle synthesis.
- Nanoparticles are primarily detected using a UV spectrophotometer that reads the characteristic spectrum of nanoparticles. $AgNO_3$ is the most frequently used salt, which served as the starting compound for mixing with the plant extract. Reduction of Ag^+ ions is performed by a combination of biomolecules from the extract such as enzymes/proteins, amino acids, polysaccharides, vitamins, alkaloids, flavonoids, etc., capable of reducing Ag^+ to Ag^0 [2,3].

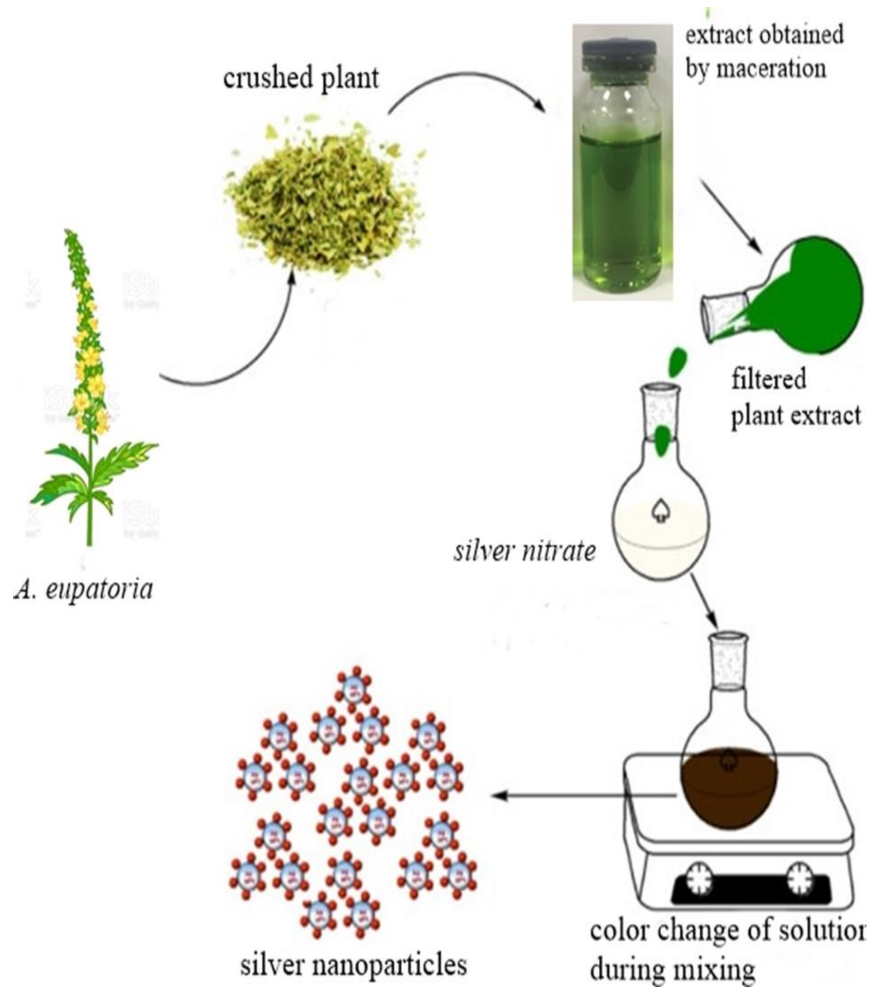


Figure 1*. Graphic representation of the green synthesis of silver nanoparticles*

[2] Sharma KV et al. Advances in Colloid and Interface Science 2009, 145: 83-96

[3] Srikar SK et al. Green and Sustainable Chemistry, 2016, 6:34-56

Methodology

Biosynthesis of AgNPs

- ✓ Different reaction conditions were applied for the synthesis of nanoparticles using the extracts of *A. eupatoria.*
- ✓ Silver nitrate was dissolved in different concentrations (5, 10, and 20mM).
- ✓ The reaction mixtures were stirred on a magnetic stirrer and heated at different temperatures (25 ^{0}C and 50 ^{0}C) until metal nanoparticles were formed.
- ✓ To modifies the pH of the reaction mixture (pH 2, 4, and 6) 0.1M HNO_3 or 0.1M NaOH were used.
- ✓ Visual color change (from light yellow to dark brown) and UV-Vis spectrophotometry were used to observe the production of AgNPs throughout the synthesis.
- ✓ After the synthesis of AgNPs, the suspensions were centrifuged at 4 500 rpm for 20 min.
- ✓ Obtained residue after centrifugation resuspended in demineralized water, centrifugated again, and the precipitated nanoparticles were then dried in a hot air oven (40 ^{0}C) and stored at 4 ^{0}C. [4]

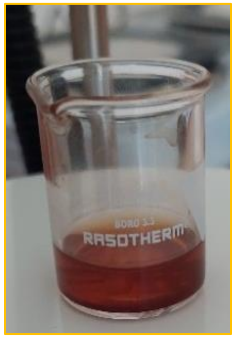
[4]Srećkivić NZ et al. RSC Advances, 2021, 11: 35585

- ✓ Perkin Elmer Lambda365 spectrophotometer was used to monitor the synthesis of AgNPs in the wavelength range 200–800 nm.
- ✓ DM0412 MicroCentrifuge from Scilogex | Lab. Equipment is used for centrifugation of suspensions at 4 500 rpm for 20 min.
- ✓ Obtained residue after centrifugation resuspended in demineralized water and centrifugated again at 4 500 rpm for 20 min.
- ✓ The precipitated nanoparticles were then dried in a hot air oven (40 ^{0}C) and stored at 4 ^{0}C.

Results and discussion

UV-Vis spectral analysis

- ✓ The generation of AgNPs in solution during their synthesis using extracts was monitored spectrophotometrically.
- ✓ The color change of solutions from light yellow to dark brown characteristic for AgNPs as a result of the surface plasmon resonance (SPR) effect was observed.
- ✓ The UV-Vis absorption spectra of formed nanoparticles were recorded (300–800 nm) and the highest peaks were positioned within 300–375 nm (characteristic peak for AgNPs), suggesting the formation of AgNPs.
- ✓ The maximum absorption values during biosynthesis were obtained at 3h, and thereafter the absorption peaks did not increase, thus indicating the end of the synthesis process.
- ✓ Then, the influence of concentration of $AgNO_3$, temperature, and pH on the biosynthesis of nanoparticles using *A. eupatoria* acetone extracts were evaluated.



Time-dependent UV-Vis absorption spectra and color change

- ✓ The initial conditions for the synthesis of both types of nanoparticles were 5 mM $AgNO_3$, 25 ^{0}C, and 10% extract concentration without adjustment of pH values.

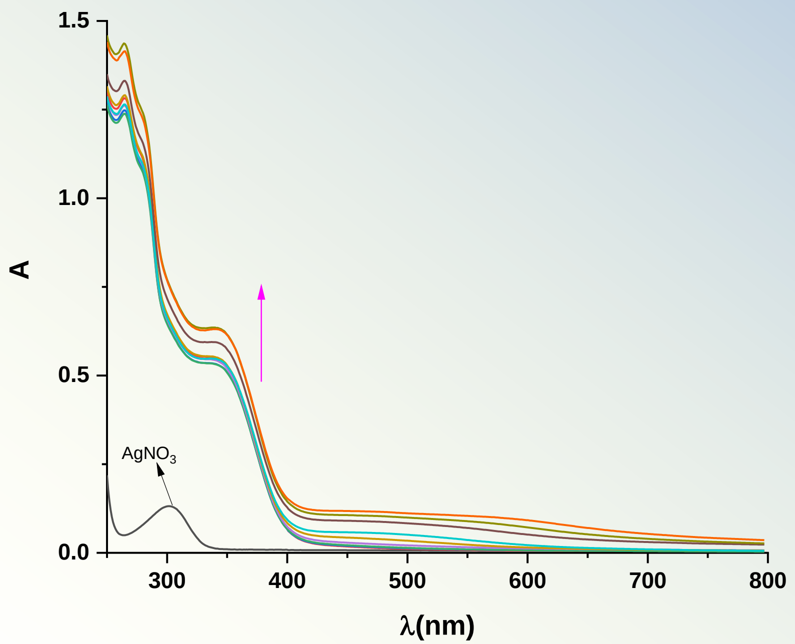
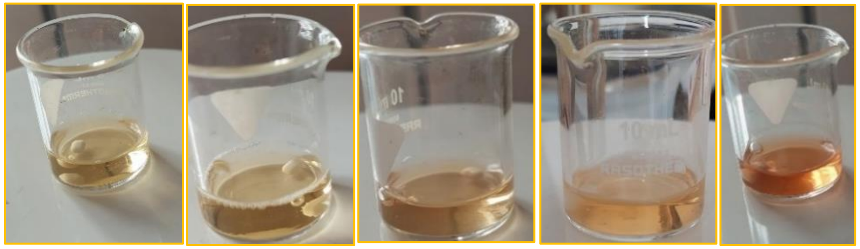


Figure 2*. Time-dependent UV-Vis absorption spectra of AgNPs biosynthesized A. eupatoria acetone extracts*

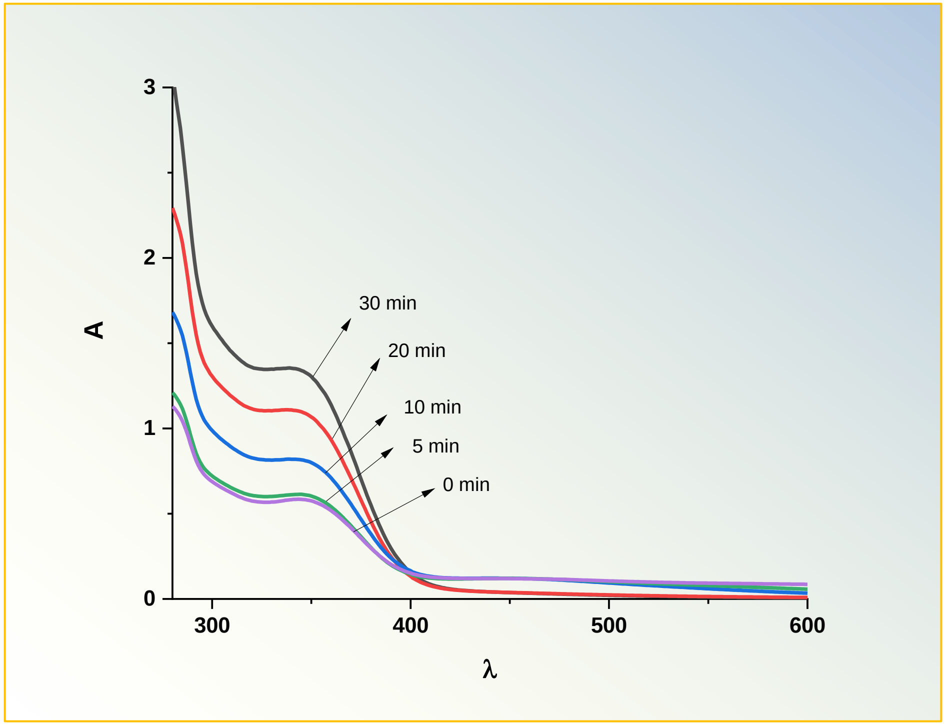
light yellow



dark brown

Temperature dependence

- the conditions for testing the temperature sensitivity were 5 mM $AgNO_3$, 50 ^{0}C, 10% extract concentration without adjusting the pH value.
- When the temperature was increased to 50 ^{0}C, the reaction accelerates significantly.



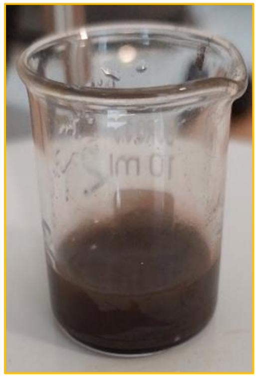


***Figure 3**. Time-dependent UV-Vis absorption spectra of AgNPs biosynthesized A. eupatoria acetone extracts at 5 0^{0}C.*

pH dependence

✓ the conditions for testing the temperature sensitivity were 5 mM $AgNO_3$, 25 ^0C, 10% extract concentration and

1) **OH =2** - a few drops of the 0.1M HNO_3 solution are added
2) **pH=4** - without adjusting the oH value
3) **pH=6** - a few drops of the 0.1MNaOH solution are added

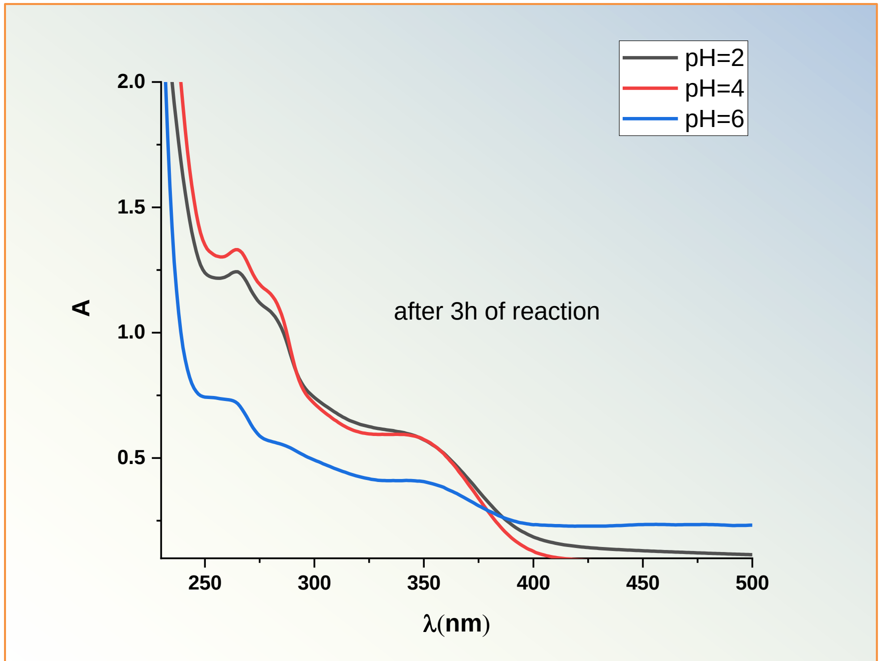
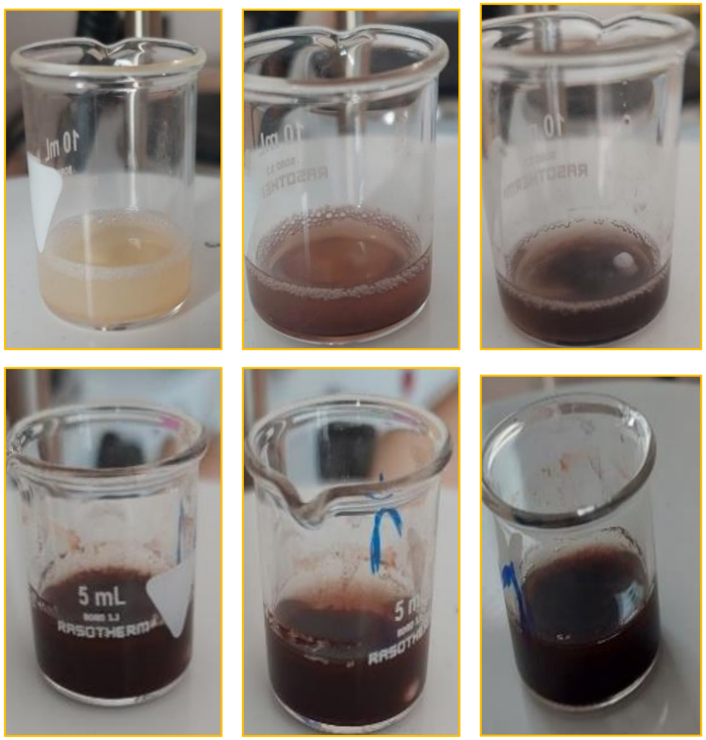


Figure 4 *.UV-Vis absorption spectra of LSA-AgNPs as a function of npH value*

pH =2

pH =6



Concentration dependent

✓ the conditions for testing the temperature sensitivity were, 25 ^{0}C, 10% extract concentration, pH=4 and

1) 5 mM $AgNO_3$ - initial conditions
2) 10 mM $AgNO_3$
3) 20 mM $AgNO_3$

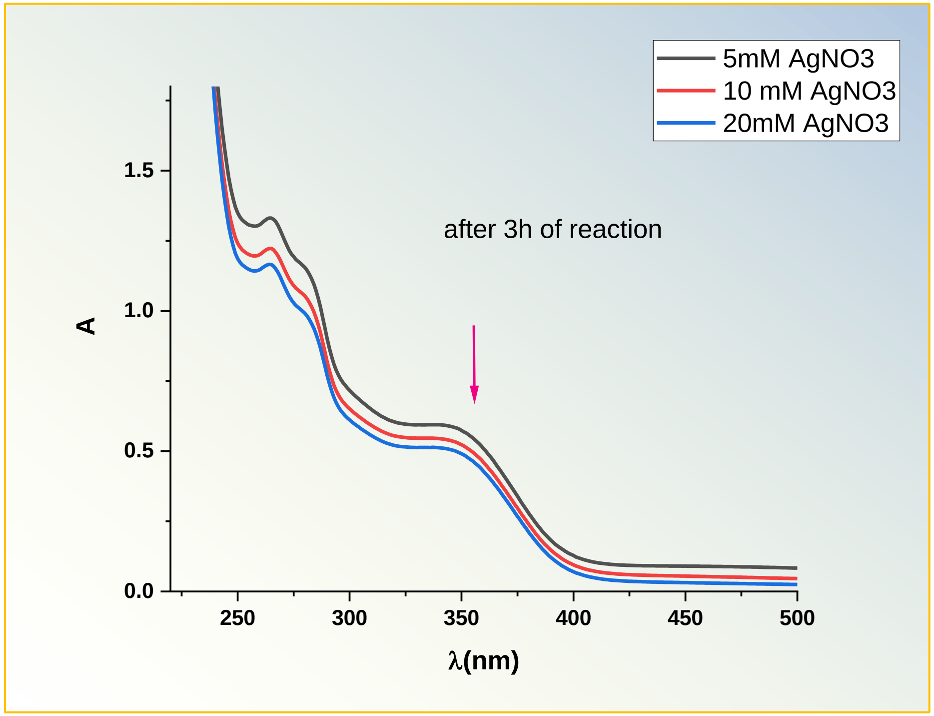


***Figure 5** UV-Vis absorption spectra of biosynthesis AgNPs as a function of concentration $AgNO_3$*

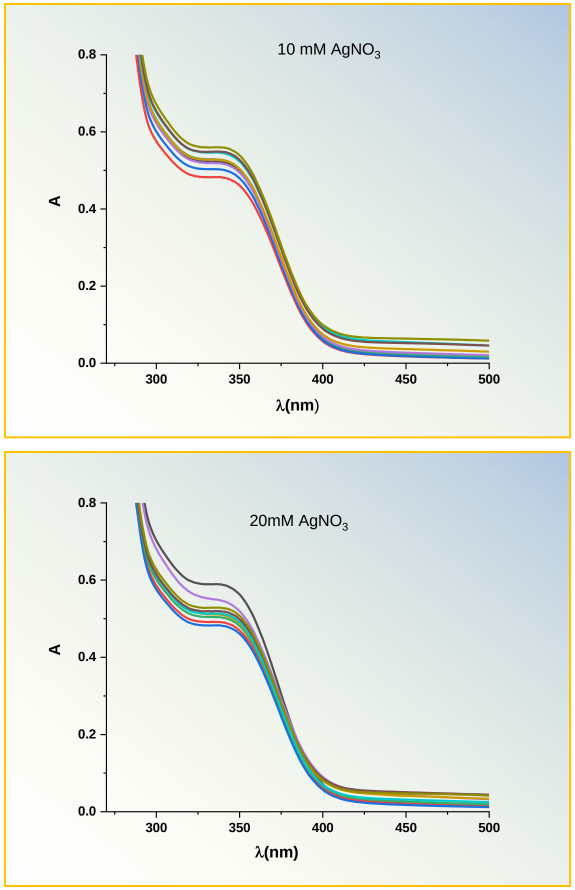


***Figure 6**. Time-dependent UV-Vis absorption spectra of AgNPs at different concentrations of $AgNO_3$*

Conclusions

- ✓ The plant ***A. eupatoria*** proved to be a good choice for the green synthesis of silver nanoparticles.
- ✓ *Time-dependent UV-Vis absorption spectra and* ***color*** **change** reaction solution are indications of gradual synthesis of silver nanoparticles.
- ✓ By increasing the **temperature**, the rate of biosynthesis of silver nanoparticles increases significantly
- ✓ Examining the optimal **pH values** for this biosynthesis concluded that acidic environments are suitable, and the most optimal oH value is 4.
- ✓ By examining this reaction at different concentrations of $AgNO_3$, we concluded that it is best to do this synthesis with an $AgNO_3$ concentration of 5 mM.

- ✓ **The best conditions for the highest LSA-AgNPs yield production were a 5 mM concentration of $AgNO_3$, a reaction temperature of 25 ^{0}C, pH 4, and a reaction time of 3h for synthesis.**

Acknowledgments

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