Green Synthesis of Silver Nanoparticles from Hibiscus Syriacus Plant Species Extract and Identifying their Antioxidant, Antimicrobial and Photocatalytic Activity

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Research Article

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Green Synthesis of Silver Nanoparticles from *Hibiscus Syriacus* Plant Species Extract and Identifying their Antioxidant, Antimicrobial and Photocatalytic Activity

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Abstract

Hibiscus Syriacus is a plant species that is rich in phytochemicals according to various reports and has seen utilization in many forms of medicine, especially eastern traditional medicine. It displays "pharmacological effects and related properties like anti-aging, anticancer, anti-depressant, anti-fungal, anti-melanogenic, anti-oxidant, cytoprotective effects. In this study, we build a green and facile method for the synthesis of silver nanoparticles from *Hibiscus Syriacus* plant. The presence of silver nanoparticles were proved by UV-vis and FT-IR. Anti-oxidant properties of silver nanoparticles were identified by Folin-Ciocalteu assay. Antibacterial capabilites of silvernanoparticles were proved on *E.coli* and *S.aureus* cultures via Minimum Inhibitory Concentration (MIC) and agar well diffusion methods. Photocatalytic degradation activity of silver nanoparticles were tested on methylene blue, methyl red and methyl orange dyes.

Keywords: silver nanoparticles, *Hibiscus Syriacus*, anti-oxidant, antibacterial, photocatalyic degradation

1 Introduction

Silver nanoparticles (AgNPs) serve as efficient catalysts in chemical reactions, contribute to advancements in electronics through conductive inks and sensors, and enhance the performance of photovoltaic devices. Their antimicrobial characteristics also extend to textiles and food packaging, ensuring safety and longevity. Additionally, silver nanoparticles play a role in healthcare, water purification, cosmetics, and coatings for their unique properties. Ongoing research focuses on their potential toxicity, ensuring responsible application across diverse fields.

The green synthesis of AgNPs is a novel idea that has seen extensive research in recent times, aiming to explore different sources and methods for establishing an optimum synthesis process and product. The synthesis of AgNPs through green technology epitomizes an ecologically conscious methodology wherein natural entities, such as plant extracts and microorganisms, function as both reducing and stabilizing agents during nanoparticle formation.

This sustainable approach obviates the use of deleterious chemicals and energy-intensive methodologies inherent in conventional synthesis protocols. Various botanical extracts, including those sourced from *Aloe vera*, green tea, and neem, have been scrutinized for their innate reducing attributes. The resultant AgNPs manifest heightened biocompatibility, thereby holding considerable promise for multifaceted applications in medicine, catalysis, and sensing domains. Ongoing scholarly endeavors seek to optimize the green synthesis protocol, diversify the repertoire of natural sources, and elucidate the determinants influencing the physicochemical attributes of the synthesized silver nanoparticles. This paradigm aligns seamlessly with the escalating imperative for sustainable practices within the domain of nanotechnology.

Hibiscus Syriacus is a plant species that's native to southern China territories, but also has been introduced to numerous regions, including the city of Istanbul, where it can be found in various public areas albeit rarely. It is rich in phytochemicals. It is characterized as a small tree, similar to a shrub trees, growing up to around 4 meters in length. Its leaves are usually triple bladed, up to 2 cm in length and its flower comes in various colors such as white, purple and pink, but what makes them distinct is the middle inner region of its corolla being dark red in color. This species has been utilized mostly for ornamental purposes, but it also has had it has been used in traditional medicine in the eastern regions of the world, for treatment of "diseases such as abdominal pain, ascariasis, colitis, diarrhea, dysentery, dyspepsia, gastro-intestinal dysfunctions. Its flower and leaves contain a suitable number of phytochemicals and flavonoids which help in the reduction and capping process, this in theory makes it a valid choice for synthesizing AgNPs with good properties.

In this study, our aim is to synthesis the silver nanoparticles using the plant species *Hibiscus Syriacus* otherwise known as rose of Sharon, through its leaves and flowers extract. Silver nanoparticles have emerged as versatile materials with a broad spectrum of applications. Renowned for their antimicrobial properties, these nanoparticles find use in medical applications such as wound dressings, drug delivery systems, and diagnostic imaging.

2 Materials and Methods

2.1 Materials

2.1.1 *Hibiscus Syriacus* Plant

The sources for *Hibiscus Syriacus* plant flowers and leaves were several when it came to field sampling, and in botanical gardens was almost always present. Three of the plant species were brought, possessing different flower colors; purple, white and pink from botanical gardens in Istanbul; with authorized taxonomists confirming the species. For field sampling, the species was found grown in a couple of areas in and around Uskudar's region in Istanbul, Turkey. After confirming its taxonomy and getting authorization from the concerned parties for collecting samples, both leave and flower samples of the three different colors were secured.

Figure 2.1: a) White colored double flowering *Hibiscus Syriacus* tree, brought from a botanical garden in Istanbul, Türkiye for experimentation. b) Pink *Hibiscus Syriacus* flowers plucked from a small garden next to Uskudar University, its taxonomy confirmed by a professional botanic. c) *Hibiscus Syriacus* leaves collected and washed thoroughly in preparation for processing its extract

It is important to note that it is a seasonal plant, generally blooming from mid/late-summer to fall and leans towards a dry weather for it to effectively grow. As such this does present a limiting factor due to the climate change affecting the average effect on the weather of a season's cycle; specifically, in Istanbul, whereby as we attempted

performing field and botanical surveys the blooming season was late by about a month, as opposed to the prediction given by various botanical professionals based on the calendrical cycle in relation to seasons. As of Nov- Dec of 2023, the tree sample we had at our disposal has all but withered. Thus, it is important to put into consideration the effect of the intensity of a climate change when growing this species.

2.2 Methods

Different methods were attempted for the production of the AgNPs listed by more than one academic source[3][4]; which utilized different plant extraction methods. The sources for *Hibiscus Syriacus* plant flowers and leaves were several when it came to field sampling, and in botanical gardens was almost always present. Three of the plant species were brought, possessing different flower colors; purple, white and pink from botanical gardens in Istanbul; with authorized taxonomists confirming the species. For field sampling, the species was found grown in a couple of areas in and around Uskudar's region in Istanbul, Turkey. After confirming its taxonomy and getting authorization from the concerned parties for collecting samples, both leave and flower samples of the three different colors were secured.

2.2.1 Preparation of *Hibiscus Syriacus* flower extract

After collecting enough flowers (about 30, measuring 7.245 g in weight); the petals were plucked of the flowers and washed thoroughly with tap water and then three times with distilled water. The petals were then cut to pieces and placed into two vials each, pouring 36ml distilled water into each, after balancing their weight, they were placed in the centrifuge at 4200 rpm, for 24hrs at 25^{o}C. After the 24 hours, the samples were taken out and the extract was filtered into a volumetric flask, left overnight for the process to finish, and resulting in a 42.5 ml extract.

Another method attempted was through drying a purple colored flower in the oven and then centrifuging its grounded powder. Started by washing the sample flower thoroughly, then positioning the samples on a filter paper and inserting them into a dark oven to dry for two days at 40^{o}C. After 42 hours, the dried petals were taken out of the oven and grounded into fine powder to get 1.6 g. They were then placed into two centrifuge tubes equally and 33 ml distilled water was added to each, centrifuging at a given set period of 24 hours, at 4200rpm and at 40^{o}C according to a research article[3] done on the synthesis of AgNPs through walnut extract. The extract was later taken and filtered overnight into a volumetric flask inside a fume hood.

Started by washing 30 of our flower samples thoroughly with tap water first and then distilled water three times. Then the petals were placed on a filter paper and placed in the oven to dry at 60^{o}C for 42 hours. After the drying process the leaves where grounded with a pestle and mortar into a fine powder weighing 4 g. 2 g were taken and added it to 300 ml distilled water, mixing them thoroughly and heating it till boils for 10 minutes, to prepare the flower extract. After that we double filtered our extract into a volumetric flask.

2.2.2 Preparation of *Hibiscus Syriacus* leaves extract

For the leaves, two different methods were attempted, that are similar to the ones performed using the flower extract; the results given by the leaves showed slightly different results and properties. For the first method, an experiment similar to the first method that implemented centrifugation was attempted on the flower samples. Starting by washing thoroughly our samples and then placing them in a dark place to dry at 50^{o}C for overnight. After 24 hours we grounded the leaves into fine powder that was a total of 2.8 g, then it was mixed 100 ml distilled water, separated into two vials for balance and centrifuged overnight at 4200 rpm, for 24 hrs at 25^{o}C. After 24 hours the extract was filtered. The second method we attempted was the most efficient and successful. According to second method; after washing the leaves thoroughly and drying them in the oven at 80^{o}C for 24 hours, they were grounded into fine powder, netting us a total weight of 3.6 g. Taking 2 g, 300 ml distilled water was added to it and mixed it thoroughly for 10 mins while it boils. We then filtered our extract into a volumetric flask in a fume-hood overnight, and after that further filtered it again after 24hrs.

2.2.3 Preparation of silver nanoparticles from *Hibiscus Syriacus* flower extract

For the white flower extract, a 1 M silver nitrate solution was prepared by adding 100ml distilled water to 0.017 g AgNO3 and covering its beaker to avoid an interaction with light. After preparing the AgNO3 solution, 4 ml of the petal extract was added to it (the solution was transparent and colorless), the solution was then placed on a magnetic stirrer at 70^{o}C, 500rpm for 5 hours. After 5 hrs, the color of the solution has turned dark yellow in color, indicating that a reduction process did occur. After preparing the purple colour extract, a 1 M AgNO3 solution was prepared, then 4 ml of the flower extract was added to it and heated at 90^{o}C for 40 mins. After preparing the pink colour extract, a 1 M AgNO3 was prepared, and 10 ml of the flower extract was added into the 100 ml AgNO3 solution and boiled for 5 minutes while mixing thoroughly. During the synthesis process, it is observed that the colour of the solution turned from colorless transparent form into dark orange colour, indicating the formation of silver nanoparticles.

2.2.4 Preparation of silver nanoparticles from *Hibiscus Syriacus* leaves extract

A 1 M 100ml AgNO3 solution and added to it 10 ml of the leaves extract, and boiled the solution for 5 mins while stirring thoroughly. A colour change was observed from a transparent solution into dark brown colour.

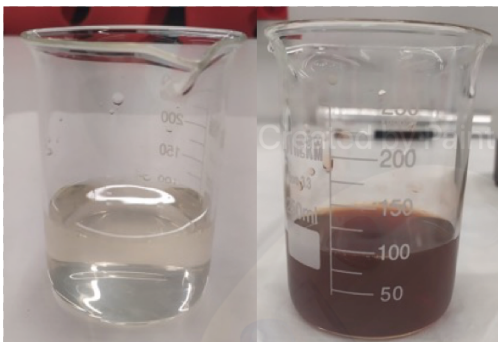

Figure 2.2: a) AgNO3 and leave extract solution colorless at the beginning of the process. b) AgNPs formed, solution became dark brown

2.2.5 Photocatalytic degradation assay

For the photocatalytic degradation of methylene blue (MB), both AgNPs synthesized from flowers and leaves analyzed. For both AgNPs, a solution of 0.033ml of MB mixed with 10ml distilled water, 2mg of NaBH4 mixed with 10ml distilled water, and 50l AgNPs were prepared. 2ml of the MB solution was taken into a cuvette, and had 0.95ml NaBH4 and 50l AgNPs added to it. After mixing in the dark for 30 mins, T0 was taken giving a peak at 660nm. The solution was then exposed to a light source for every 20 minutes to get T1 , T2 , T3 , T4 , T5. The absorption from the peaking 660nm kept decreasing the more the solution was exposed to light; with a total of 100 mins of light exposure. The resulting OD reading suggests the progressive degradation of the dye and the dark blue color, was lighter in tint at the end of the 100 mins mark. This procedure was repeated with methyl orange and methyl red dyes.

2.2.6 Folin-Ciocalteu Phenolic reagent assay

Started by preparing our solutions, first; a GA stock solution by measuring 500mg of its powder into a beaker, mixing it with 100ml distilled water + ethanol solution (9:1). Different concentration of gallic acid were taken by adding "0 ml, 0.1 ml, 0.2 ml, 0.3 ml, 0.5 ml, 1 ml" of gallic acid stock solution into 6 different tubes and filled with 10ml with distilled water ethanol solution (9:1). The 0 ml GA tube serves as a blank sample, and from samples s2 to s6 GA with increased concentration are prepared to measure the increase in it phenolic content. Then F-CR was prepared by adding 18ml distilled water to 2 ml F-CR in an amber colored bottle to avoid photochemical reaction. Na2CO3 was preparing by measuring 7.5 g and adding 100 ml distilled water to it. From each concentration of the 6 prepared GA tubes 0.5 ml was taken into 6 different tubes (0.5 distilled water for the blank sample), and a seventh tube was introduced with 0.5 ml of the *Hibiscus Syriacus* leave extract's' synthesized AgNPs added to it. Then, 2.5 ml of F-CR and 2ml of Na2CO3 were added to each of the seven tubes. The tubes were left to settle in the dark for 30 minutes, then their optical density was measured in UV-vis spectra at 760 nm wavelength (Fig. 2.3).

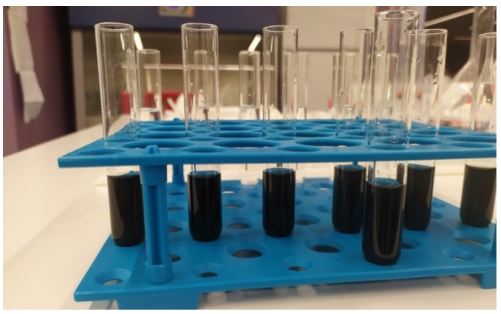

Figure 2.3: F-CR experiment tubes prepared. From the left to the right s1 to s6, with the frontal tube containing s7 the AgNPs added solution. (Covered with aluminum foil after preparation) After placing in the dark for 30 mins to settle and mix, the UV-vis readings were taken

2.2.7 Antibacterial activity assays

Preparing for liquid bacteria cultures, minimum inhibition concentration (MIC) method was applied. The procedure 14 tubes along with its stand and a number of tools such as spoon, toothpicks and tweezers were sterilized in an autoclave at 121^{o}C for 15 minutes. The liquid media was prepared by adding 2.5 g of LB-Broth to two different bottles and had 100 ml distilled water poured into them, then mixed thoroughly and placed in autoclave for sterilization. After letting the bottles settle for cooling. Following sterile technique; beads of *E.coli* and *S.aureus* were added to the two sterilized LB-Broth media bottles respectively, and each labeled. The bottles were then placed in an incubator for growing at 37^{o}C for 18 hours. When the incubation process was done, bio-safety cabinet was turned on and area of work was wiped with ethanol for sterilization before use, then each of the two liquid cultures were poured into the 6 tubes respectively.

The 6 tubes had the following measurements poured; Sample(s); s1 "C": control contains 4ml of *E.coli* and *S.aureus* liquid bacteria respectively, s2: contains 3.8ml of the respective bacteria + 0.2ml AgNPs, s3: 2.6ml + 0.4ml AgNPs, s4: 3.4ml + 0.6ml AgNPs, s5: 3.2ml + 0.6ml AgNPs, s6: 3.0ml + 1ml AgNPs.

After preparing each of the tubes and its contents, before the incubation T0 the UV-vis optical density was measured. And after an hour of incubation at 37^{o}C; T1 was taken, then T2, T3 till the next day at T24 , where the final reading was taken.

Preparing for solid bacteria cultures, agar well diffusion method was applied. A LB-broth was prepared by adding 2.5 g LB-broth with 1.5 g agar and pouring 100 ml distilled water into it, then it was mixed thoroughly before placing for autoclave at 121^{o}C for 15 mins. When the bottle was safe to handle, inside a biosafety cabinet the solid broth was poured into two different petri dishes then allowed to settle for 30 minutes before placing into an airtight bag upside down inside a fridge for later use. After the bacterial liquid stock cultures mentioned above were prepared, the petri dishes were taken out and allowed to settle for a bit, all while following aseptic technique in handling the tools and biosafety cabinet workplace.

Using a micropipette, 50 l of each bacteria where added to their respective plates, then they were spread evenly throughout the petri using a triangular inoculum. Then wells were made in three regions of the dish using a sterile pipette tip and care was taken to remove agar debris of the wells without damaging the overall structure of the media. The three wells then had our samples poured into them, where:

C: Control well was filled with 70 l distilled water, 1: AgNPs well was filled with 70 l, 2: Ampicillin well was filled with 70 l (320mg/ml).

Both *E.coli* and *S.aureus* petri culture dishes were then placed inside and incubated at 37ºC for 24 hours.

3 Results

3.1 UV-vis spectroscopy results

The UV-vis spectroscopy results of flower extract of *Hibiscus Syriacus* was presented in Fig. 3.1.

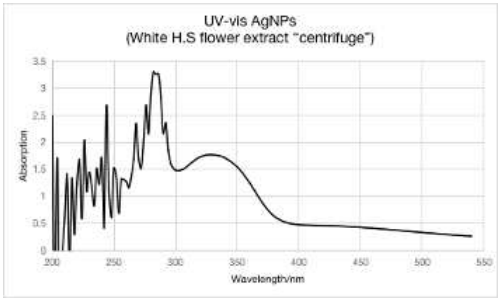

Fig 3.1: UV-Vis spectra of the AgNPs synthesized from a white *Hibiscus Syriacus* flower extract that was prepared through centrifugation

Taking the sample into a cuvette we attempted to get a UV-vis spectrum reading, which gave us a wavelength at 330nm.

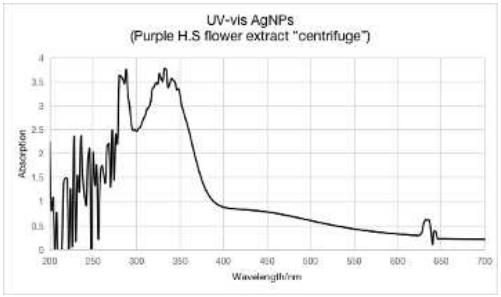

Fig 3.2: UV-Vis Spectra of the AgNPs synthesis attempt using purple *Hibiscus Syriacus* flower extract via drying and centrifuging

The resulting absorption showed a very miniscule peak around 425nm wavelength.

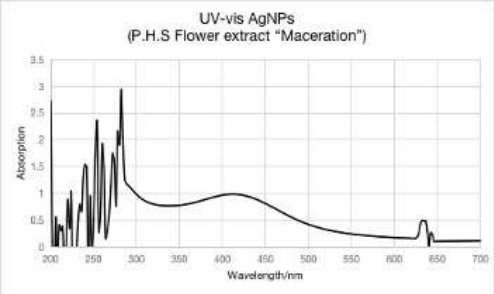

Fig 3.3: UV-vis spectra of the AgNPs synthesized through purple *Hibiscus Syriacus* extract via maceration

The absorption peaked at 410 nm indicating the successful formation of AgNPs. The experiments were carried out more than once to test the validity of the results and compare the effect of the color of the plant of the resulting extract and AgNPs. The UV-vis where always constant when following a specific protocol, and the colors of the flowers did not seem to affect the synthesis process.

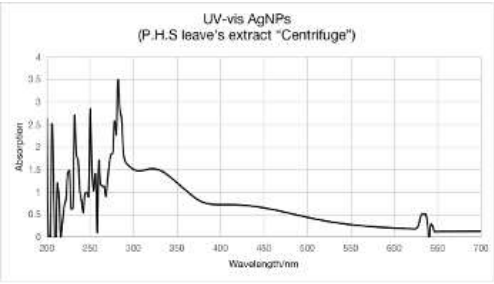

Fig 3.4: UV-vis spectra of AgNPs synthesis attempt using purple *Hibiscus Syriacus* leaves extract via centrifugation

The two peaks show the initiation of formation of AgNPs, which could indicate that the threshold wasn't reached for the process of capping and reducing AgNO3.

The result we got was a high peak at 330 nm and a miniscule insignificant peak at 430nm and the color change was observable from colorless transparent solution to dark orange.

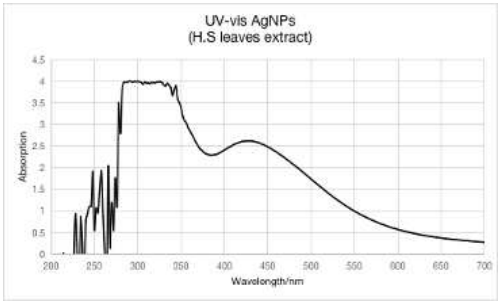

Fig 3.5: UV-vis spectra of AgNPs synthesis attempt using *Hibiscus Syriacus* leaves extract via maceration

It shows a very high absorption peak at wavelength 424 nm, indicating successful formation of AgNPs.

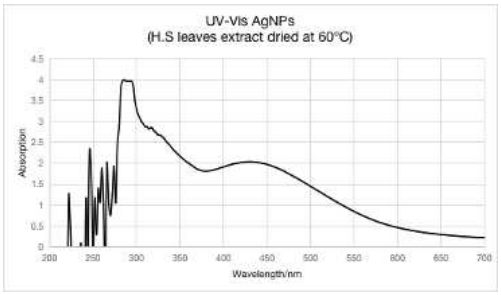

Fig 3.6: UV-Vis absorption spectra of AgNPs synthesis attempt using *Hibiscus Syriacus* leaves extract via maceration

The leaves were dried at 60^{o}C for 24 hours. The result shows a high absorption peak at wavelength 430 nm, indicating successful formation of AgNPs.

3.2 FT-IR spectroscopy results

The FT-IR analysis of the synthesized silver nanoparticles revealed prominent peaks at 3305 cm-1 and 1635 cm-1 (Fig. 3.7), providing insight into the composition and functional groups present in the sample.

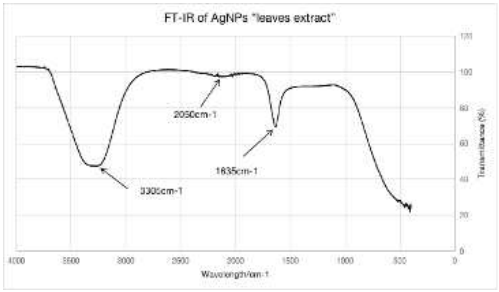

Fig. 3.7: FT-IR spectra of AgNPs synthesized through *Hibiscus Syriacus* extract, displaying peaks at 1635cm-1, 2050cm-1 and 3305cm-1

The peak at 3305 cm-1 suggests the presence of hydroxyl groups (-OH), which may be attributed to the organic molecules from the leaves used in the green synthesis. The hydroxyl groups are commonly found in biomolecules such as polyphenols, flavonoids, or other plant-derived compounds. These functional groups can act as capping or stabilizing agents, contributing to the stability and dispersibility of the silver nanoparticles.

The peak at 1635 cm-1 is indicative of the presence of amide groups, specifically Amide I bands. This suggests the involvement of proteins or peptides derived from the plant extract in the synthesis process. Proteins can serve as effective capping agents and play a crucial role in the nucleation and stabilization of nanoparticles. The presence of these biomolecules not only influences the size and shape of the nanoparticles but also imparts unique bio-functionalities, making them potentially suitable for various applications, such as in medicine or catalysis.

A minor signal at 2050 cm-1 indicates the presence of carbon-carbon triple bonds (CC), typically associated with alkyne groups. This finding suggests the involvement of specific organic compounds, possibly derived from the plant extract used in the green synthesis.

In summary, the observed peaks at 3305 cm-1 and 1635 cm-1 in the FT-IR spectrum of the synthesized silver nanoparticles point towards the involvement of organic molecules, likely from the plant extract, and highlight the potential role of hydroxyl and amide groups in the green synthesis process. Further characterization and complementary analyses would be beneficial to gain a comprehensive understanding of the nanoparticle composition and structure. There was no notable difference between the FT-IR readings of the leave and flower synthesized AgNPs.

3.3 Photocatalytic degradation results

A series of tests were performed on different dyes to investigate the potential of AgNPs produced by both the flower and leave extracts of *Hibiscus Syriacus* in photocatalytic degradation activity on methylene blue, methyl orange and methyl red. The results were summarized at below.

3.3.1 Methylene blue degradation test results

For the photocatalytic degradation of methylene blue (MB), both AgNPs synthesized from flowers and leaves analyzed. The absorption from the peaking 660 nm kept decreasing the more the solution was exposed to light; with a total of 100 mins of light exposure. The resulting OD reading suggests the progressive degradation of the dye and the dark blue color, was lighter in tint at the end of the 100 mins mark.

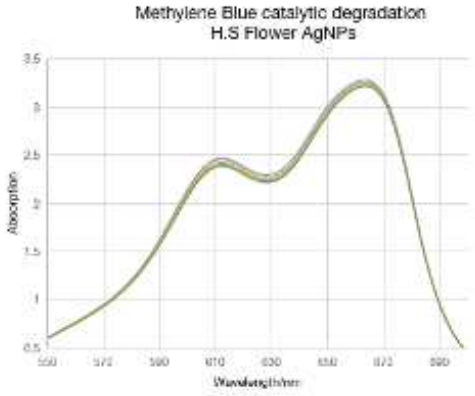

Fig 3.8: MB catalytic degradation using flower extract synthesized AgNPs, from T0 to T5, reading was taken every 20 minutes

The graph indicates the degradation of the dye as time passes.

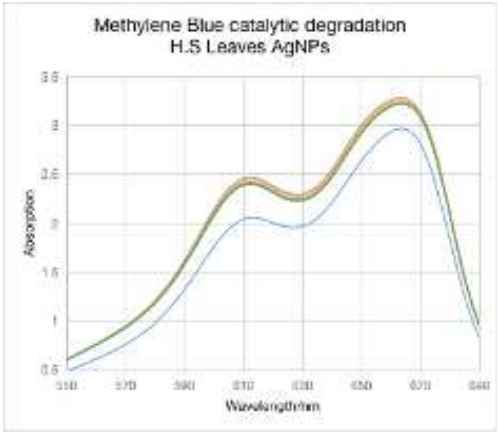

Fig 3.9: MB catalytic degradation using leaves extract synthesized AgNPs, from T0 to T5, reading was taken every 20 minutes

The UV-vis measurements indicate that the dyes were progressively being degraded. Various parameters do affect the rate at which the catalytic reaction

occurred, specially amount of NaBH4, were a slight increase of it would significantly boost the speed of the degradation speed; which indicate the success of AgNPs as a photocatalyst. Further trials performed suggest that leaving the cuvette overnight exposed to a light source would eventually fully degrade the dye.

3.3.2 Methyl red degradation test results

We preformed methyl red (MR) analysis for both flower and leave synthesized AgNPs. The optimum parameters required to degrade methyl red are not the same as methylene blue, as we attempted the same concentrations used above with only MB and MR being different and the degradation was extremely slow and almost no result was observable except through OD measurement. NaBH4 specifically had a great effect in boosting the degradation process depending on the administered concentration. Several trials showed the decolorization of the dye from dark red to colorless.

3.3.3 Methyl orange degradation test results

The analysis was done on the AgNPs synthesized through the leaves extract. For the methyl orange (MO) dye the effects of the AgNPs degrading it were almost similar in every aspect to MB, however the degradation was much slower when using similar parameters to the MB test done. The more diluted the methyl orange was, the faster was the degradation process was. NaBH4 also played a significant role in affecting the speed of the degradation in proportion to its concentration, care was always taken in keeping the conc. Tested constant across all trials.

3.4 Folin-Ciocalteu phenolic reagent assay results

A Folin-Ciocalteu reagent (F-CR) experiment was performed on AgNPs synthesized through *Hibiscus Syriacus* "leaves" to determine the amount of phenolic content present within the AgNPs solution in relation to gallic acid (GA).

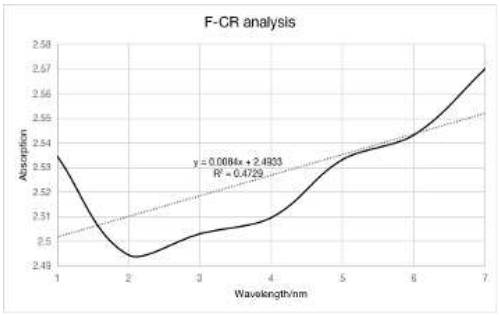

Fig 3.10: F-CR Plotted graph of the optimal density taken in UV-vis spectroscopy at wavelength 760 nm

The linear line displays the trend along the plotted points, indicating the presence of phenols, increasing as the concentration of the phenols within the sample increases.

s1: Blank, s2 to s6: 0.5ml of GA of increasing concentration, sx: 0.5ml of AgNPs synthesized through *Hibiscus Syriacus* leaves.

3.5 Anti-bacterial assay results

Two different experiments were done to analyze the anti-bacterial properties of the *Hibiscus Syriacus* "leaves extract" synthesized AgNPs. The anti-bacterial effect the synthesized AgNPs were analyzed and observed in *E. Coli* and *S.aureus* via on Minimum Inhibitory Concentration (MIC) and Agar well diffusion techniques.

3.5.1 Minimum Inhibitory Concentration (MIC) results

For *E.coli* the following where the resulting optical density readings (OD) measurements given by the UV-vis:

Table 3.1: UV-vis spectrophotometric readings of different samples at a controlled set of time intervals, showing the effect of the synthesized AgNPs on *E. Coli*

E. Coli – AgNPs Conc./hr	S1	S2	S3	S4	S5	S6
T0	0.8348	0.8456	0.8187	0.7944	0.7752	0.7417
T1	0.8455	0.8174	0.8265	0.7647	0.7776	0.7539
T2	0.8338	0.8322	0.8499	0.7886	0.7925	0.7677
T3	0.8639	0.8286	0.8512	0.8067	0.8288	0.7928
T24	1.0722	0.904	0.9263	0.9563	0.9483	0.7576

The results of the OD measurements (Table 3.1) on *E. Coli* suggest the effective inhibition and bactericidal properties indicated by the drastic contrast between s6 and control after 24 hours.

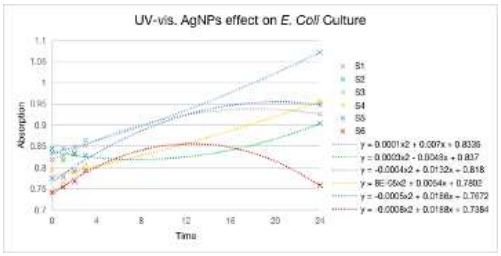

Fig 3.11: A polynomial graph plot of the 2nd degree showing the relationship and effectiveness of AgNPs on *E. Coli* across 6 sample during a set interval of time.

The polynomial graph (Fig. 3.11) displays the relation between the increased concentration of AgNPs and its effect over time on *E. Coli* liquid culture.

As the concentration of the AgNPs increases the effects as well on the growth of the culture increases. Interpreting the data provided by the polynomial 2nd degree graph suggests that the antibacterial efficacy of the AgNPs seems to pick up around T12. This cannot be attributed to media depletion as the C sample displayed continues growth through the 24hrs interval; meanwhile sample 6 does not only get inhibited in growth, but the concentration of the culture itself seems to fall of in number, indicating bactericidal efficiency; this is also observable with s3 and 5.

Worthy of note that if not for the abnormal reading at the beginning of s4, that the polynomial plot would have highly likely been similar in effect to s3,s5 and s6. s2 also displays an interesting effect even though the concentration of AgNPs in it is quite low, where the polynomial plot shows a sharp deviation from the control sample s1.

For *S.aureus* the following were the resulting OD measurements given by the UV-vis:

Table 3.2: UV-vis spectrophotometric readings of different samples at a controlled set of time intervals, showing the effect of AgNPs on *S.aureus*

S. aureus – AgNPs Conc./hr	S1 (Control)	S1	S2	S3	S4	S5
T0	1.238	1.191	1.213	1.244	1.266	1.283
T1	1.345	1.187	1.216	1.279	1.313	1.330
T2	1.358	1.208	1.237	1.311	1.346	1.357
T3	1.365	1.225	1.260	1.332	1.359	1.385
T24	1.437	1.358	1.360	1.375	1.396	1.394

The UV-vis spectrophotometric readings (Table 3.2) of *S.aureus* indicate that by T24 the bacterial growth is inhibited and bactericide have taken place, evident by the date present the control sample had a higher concentration of bacteria concentration throughout the time intervals.

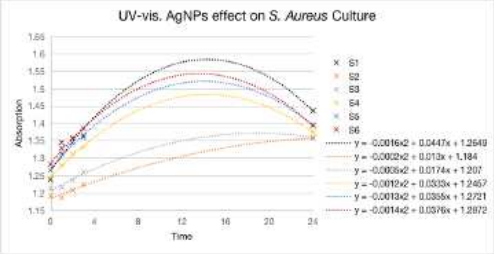

Fig 3.12: A polynomial graph plot of the 2nd degree showing the relation and effectiveness of AgNPs on *S.aureus* across 6 sample during a set interval of time

Around T13, the bacteria seem to be affected by the presence of the AgNPs in a different way than it did with *E. Coli*. S3 and S4 seem to display some abnormality in their reading at T0 which could be the reason why the polynomial plot does not seem to align with the pattern of the rest of the samples.

3.5.2 Agar well diffusion assay results

The results of agar well diffusion assay of *E. Coli* and *S. Aureus*, which were presented Fig. 3.13 and 3.14, showed an observable zone of inhibition. This indicates that the efficiency of antibacterial properties possessed by the AgNPs.

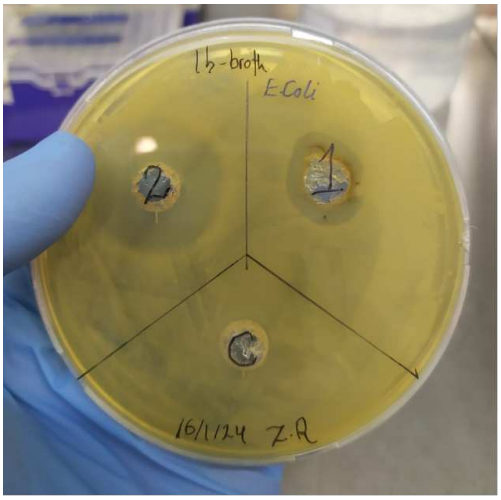

Fig: 3.13: AgNPs zone of inhibition on *E.coli*

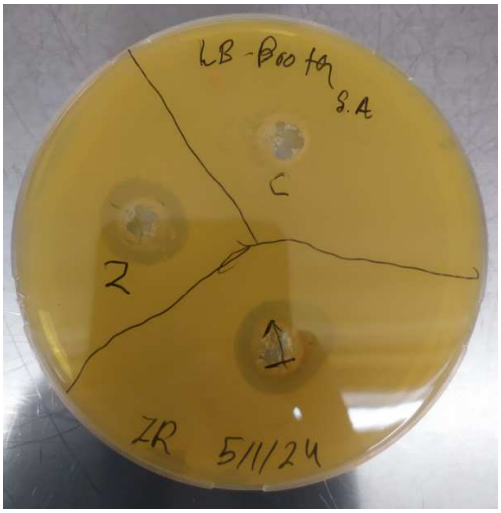

Fig: 3.14: AgNPs zone of inhibition on *S.aureus*

Ampicillin was used as a reference drug for its specific antibacterial capability. The areas of inhibition and effective bactericidal range are mentioned below in Table 3.3.

Table 3.3: Diameter of the zone of inhibition of AgNPs (1) in comparision to control and Ampicillin (2) on *E. coli*

Zone of inhibition Diameter	Control	AgNPs	Ampicilin
E. coli	0	1.7	2.7
S. aureus	0	1.5	1.7

4 Conclusion

In conclusion, throughout this research we were able to synthesize AgNPs using the extract of both the flower and leaves of *Hibiscus syriacus*; where maceration was a simple, quick and effective process for preparing the samples extract. The preparation of the samples before the extraction process seems to play a very big role in the end result of the synthesis process, as it was observed that pre-treating the samples with heat for drying it at a certain given set of time before grinding them into a fine power had a significant effect on the end UV-vis measurement regardless of the color change of the AgNO3 reduction process. The UV-vis result when following an optimal protocol gave us the reading 410 nm and 424 nm for the flower and plant extract synthesized AgNPs respectively, yet different levels of absorption were observed.

The analysis done on the AgNPs indicate that they possess the potential for use in various medical applications. As they were able to inhibit the growth of bacteria and even displayed bactericidal properties. Since, the plant has seen historically use in traditional medicine for niche objectives; the species extract could be researched further more on methods to enhance specific phytochemicals within it for utilization along with silver nanoparticles when synthesizing them, as the nature of the extract did indicate a degree of difference while performing the experiments on the flower and leave sample, in which both are different in chemical constitution.

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