

“Green synthesis of silver nanoparticles from flower extract of *Hibiscus rosa sinensis* and its influence on growth parameters of silkworm (*Bombyx mori*)”

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Research Article

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Abstract

This study presents the sustainable green synthesis of silver nanoparticles (AgNPs) utilizing Hibiscus flower extract as a dual-functioning reducing and capping agent. Green synthesis was performed using an aqueous solution of (*Hibiscus rosa sinensis*) petal extract and AgNO₃(1). Comprehensive physicochemical characterization via X-ray diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR), and SEM-EDS confirmed the crystalline nature, nanoscale size, and organic phytocompound capping of the AgNPs.(2) Key FTIR absorption bands indicated the successful integration of phenolic and carbohydrate residues on nanoparticle surfaces, while XRD patterns revealed face-centered cubic crystalline domains.(3) To evaluate the biological efficacy of these biogenic nanoparticles in sericulture, silkworm larvae were fed with three distinct diets: (1) deionized water-treated mulberry leaves, (2) Hibiscus extract-treated leaves, and Hibiscus-AgNP-treated leaves. Quantitative analysis revealed that Hb-AgNP supplementation at optimal concentrations significantly enhanced larval survivability (up to 96.7%), larval and cocoon weights, and shell yield when compared to control and conventional extract groups. (4)These improvements were achieved without imparting any observable toxicological effects at effective dosages. (5) This research demonstrates not only the scientific and agronomic value of phytochemical-driven, green nanoparticle synthesis but also its potential to sustainably bolster productivity in silk farming, providing an environmentally responsible alternative to conventional feed additives.Silkworms (*Bombyx mori*) are an important economic insect and a tool to convert leaf protein into silk protein. Due to its evocative physical and chemical properties, silver was of particular interest for this process.(6) A fixed ratio of metal ions to petal extract was prepared, and the color change observed demonstrated nanoparticle formation. The nanoparticles were characterized using FTIR, XRD, SEM, and a UV-visible spectrophotometer. Biogenic silver nanoparticles (AgNPs) significantly influence the physiological parameters of silkworms (*Bombyx mori*). Additionally, they can improve larval weight and cocoon quality at moderate doses, but excessive exposure has detrimental effects.

INTRODUCTION

The synthesis of metal and biological nanoparticles represents a rapid expanding research domain within 'Nanotechnology', which has emerged as a pivotal field in both material sciences and biological sciences. (7)Nanotechnology offers revolutionary solutions for global challenges while addressing environmental concerns through sustainable approaches.(8) Modern material science has increasingly focused on creating metal nanoparticles that are both sustainable and effective in reducing the environmental impact associated with traditional chemical synthesis techniques.(9)

Silver nanoparticles (AgNPs) have achieved significant attention due to their exceptional properties, including effective catalysis, high conductivity, unique plasmon resonance optical properties, and clinical efficacy in antibacterial and antiviral applications(10).(11) Their superior bioavailability and role as effective carriers in targeted drug delivery systems make AgNPs particularly promising for overcoming existing challenges in clinical applications.(12)

Compared to conventional synthesis methods, green synthesis offers remarkable benefits including a 30% reduction in energy consumption, cost savings of up to 40%, and a 50% increase in production output.(13) The method is promising than chemical and physical approaches because it is cost effective, more bio-sustainable, and easier to scale up for large-scale synthesis.(14)

The phytochemical-mediated reduction mechanism offers precise control over nanoparticle properties while ensuring environmental compatibility. (15)As research continues to address current limitations and expand applications, green synthesis will undoubtedly play an increasingly important role in the global transition toward sustainable manufacturing and environmental remediation technologies.(16)

Plant flower extracts such as *Hibiscus rosa sinensis* (China rose) which belong to *Malvaceae* family contain diverse bioactive compounds that serve dual functions as reducing agents and stabilizing agents and holds medicinal properties which aids in nanoparticle formation.(17)

METHODS AND MATERIAL

2.1) Preparation of flower extract

The flowers of Hibiscus rosa Sinensis, (China rose) a small herbaceous plant belonging to the Malvaceae family were collected from fields of Jammikunta village, Telangana state were shade dried to remove moisture, and grinded into a fine powder and sieved, 1% (w/v) of the powdered flower extract is mixed with 100ml of sterile deionized water and centrifuged at 5000rpm at room temperature for equal suspension, and collected supernatant is filtered with a muslin cloth to make the aqueous solution of flower extract used for further analysis.(18)

2.2) PREPARATION OF SILVER NANOPARTICLES USING FLOWER EXTRACT

i). **OPTIMISATION OF AgNPs through SILVER NITRATE SOLUTION:** Stock solutions of each 100ml using standard concentrations (1mM-5mM) were prepared adding the fine crystals of Silver nitrate(AgNO_3) weighed in (w/v) and made their volume using distilled water. (figure 2). (19)

ii). **SYNTHESIS OF AgNP-FLOWER EXTRACT SOLUTION:** Add AgNO_3 solution optimized in given

concentrations to the filtrate in a 4:1 ratio (i.e, 75ml of Silver nitrate solution added to 300ml of flower filtrate), Prepare 300ml of each concentration and incubate it for 24hrs and notice the color change.

Look For the color change(figure2.) and noted the absorbance using a UV-visible spectrophotometer.

To Proceeded with further analysis dry the reaction mixture of the AgNP-aqueous Petal extracts using ROTA-VAPOUR or a Hot air oven between (75°C and 90°C) for characterization studies.(20)

2.2) CHARACTERISATION OF HIBISCUS - SILVER NANOPARTI :

i.UV-VISIBLE-ANALYSIS:

The UV-visible spectrophotometer (Perkin-Elmer, Lambda 35, Germany) was used to measure the optical characteristics of the AgNPs synthesized in the reaction mixture. The spectrum was collected between 300 and 800 nm after AgNO₃ was added to the plant extract. After adding AgNO₃ to the aqueous extract for 24 hours, the spectra are obtained. Consider the concentration of the reaction mixture that produces a spectral peak between 400 and 450 nm, indicating the formation of silver nanoparticles, and examine the graph plotted against OD (y) and wavelength (x) for each concentration of silver nitrate. (Figure 3.) (10)

ii. FTIR ANALYSIS : (KBr Pellet method) :

Analyzed the chemical makeup of the produced silver nanoparticles. using a FTIR spectrometer Perkin-Elmer L^S-55-Luminescence.

The solutions were dried at 75 degrees, and using the KBr pellet technique, the dried powders were characterized in the 4000-400 cm⁻¹ range. (Figure 4)

Pellet technique for KBr: Measure equal amounts of 0.50gms each of KBr and the solid sample using micro spatula. In a mortar, fully grind them while using a pestle. Before adding KBr, grind the sample separately if it shows larger crystal remains. Put just enough samples in the pellet die cell to fill the bottom. press and apply 5000–100,000 psi of pressure.(21)

iii.XRD ANALYSIS: X-ray diffraction spectroscopy (PANalytical) was used to determine the phase variety and grain size of the produced silver nanoparticles. The produced silver nanoparticles were analyzed using CuK α radiation at a scan rate of 0.03 /s. voltage of 30 kV, and current of 20 MA. The different phases present in the synthesized samples were recognized by the X'pert high score program used a search and match function to identify the various phases found in the synthesized samples. Using Scherrer's equation, the particle size of the generated samples was calculated as follows: $D = 0.97 / \beta \cos \theta$. Determine the size of the produced nanoparticles using the XRD calculator using the graph's peak position, X-ray wavelengths, and FWHM_{2 θ} (Figure 5) (22)

iv.SEM ANALYSIS: To describe the sample morphology, SEM analysis was performed using the JEOL Jsm-6480 LV. An auto fine coater was used to apply a platinum coating after the samples had been spread out on a slide.After then, the material was examined. (Figure 6,7)(23)

Feeding HbAgNps to the silkworm–(*Bombyx mori*):

Silkworm rearing: The silkworm rearing unit in Vangapahad village, Warangal dist, Telangana. Operated by sericulture farmer M.Raju was chosen to perform our work.

National Silk Production Policy (NSPP) and Central Silk Board (CSB) approved eggs of an Indian multivoltine hybrid silkworm from the Sericulture complex were used by the farmer at Elkathurthy, Warangal, Telangana.

These eggs After hatching larvae were separated from stock culture nearly 50-100 larvae were taken , for the Hb-AgNPs study. they were placed in a plastic incubator with an ambient temperature of 25 to 29°C and a relative humidity of 70 to 80%. The larvae were raised in 22 x 15 x 6 cm plastic tray boxes that were put in an iron platform with ant wells and covered with a nylon net. (24)

To determine the effect of Hb-AgNPs in the feeding patterns and the weights of the cocoons the larvae are distributed into 3 groups and they were given 3 distinguished feeding stock of mulberry leaves sprayed with our test solutions, All the leaves stock were soaked over night in the test solutions.

1. T1 (HB-Stock solution) where Leaves soaked in Hibiscus petal extract solution
2. T2 (HB-AgNps solution) where Leaves soaked in synthesised Hibiscus Silver Nano particles(Hb-AgNPs) extract solution.

100. T3 where leaves were soaked in normal deionised water

The silkworm larvae were feeded with the similar patterns from their first to fifth instars of the growth till they form the cocoon. (25)

Determination of the Weights of the larvae and the cocoon:

The weights of the larvae at the second instars were taken and considered as the initial weights and at the fifth instar considered the final weights which is approximately between 18-22 days and the growth index is calculated for the three groups. (26)

Growth Index:

$$\frac{\text{Final weight of larva (g)} - \text{Initial weight of larva (g)}}{\text{Initial weight of larva (g)}}$$

RESULTS AND DISCUSSION

1)OBSERVATION OF SILVER NANOPARTICLES SYNTHESISED:

For the freshly collected, shade-dried flowers when grind into fine powder we obtained 25gm of dried petal extract powder which is further filtrated.

After mixing and incubating the hibiscus flower extract solution with aqueous silver nitrate for 24 hours, we saw that the solution's color changed from thick red to dark brown, (figure 2) indicating that the phytocompound's oxidation and reduction reaction created the silver nanoparticles.

When aqueous petal extract was added, the silver nitrate solution was seen to turn dark brown, indicating the creation of Ag-NPs. In contrast, the control group showed no color change. In the absence

of aqueous petal extract. (27)

2)UV-VISIBLE ABSORPTION STUDIES DATA: UV-vis spectrophotometry is performed for the aqueous 5mM Hb Ag-NPs extract. Using absorption spectra at a wavelength range of 300-480 nm, using systronic UV-VIS spectrophotometer machine and analysed the peak. (Figure. 3). (28)

The 5mM solution of Hb-AgNPs has shown a peak at 410-414 nm, A single, broad, and powerful Surface Plasmon Resonance (SPR) peak at 413.6 nm was visible in the absorption spectra of a 5 mM solution in the UV-Vis region, confirming the synthesis of Ag-NPs. (10) literature suggested that an SPR peak located between 410-450 nm confirmed the synthesis of Ag- NPs and attributed to spherical nanoparticles

3) FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR): The FTIR measurements were carried out for the presence of various functional groups in the biomolecule responsible for the bioreduction of Ag⁺ and capping/stabilization of Ag-NPs. The functional groups were determined by comparing the observed intensity bands to standard values. The FTIR spectra revealed absorption bands at 3383, 2924, 1620, 1383, 1043, 503, 443, 433, 426 & 419 cm⁻¹, indicating the presence of phytochemicals in addition to the silver nanoparticle. (29)

The lower wavenumber region, with peaks at 503, 443, 433, 426, and 419 cm⁻¹, is assigned to metal-oxygen (Ag-O) vibrations, confirming the successful synthesis and stabilization of silver nanoparticles by phytochemicals. These absorption peaks to specific functional groups follows previously reported interpretations for FTIR spectra of silver nanoparticles synthesized using plant extracts. The presence of both phytochemical-related and metal-oxygen bands confirms that phytochemicals from Hibiscus extract are involved in both the reduction of silver ions and the capping/stabilization of the resulting nanoparticles, as supported by literature and standard FTIR reference values (21) (figure 4)

4) XRD ANALYSIS: The crystalline nature of the synthesized silver nanoparticles (AgNPs) was confirmed by X-ray diffraction (XRD) analysis, as shown in the corresponding XRD pattern (Figure 5). The diffraction intensities were recorded over a 2θ range from 0° to 100°, revealing four distinct and sharp Bragg reflection peaks at 38.26°, 44.38°, 64.56°, and 77.48°. These peaks correspond to the crystallographic planes (111), (200), (220), and (311) respectively, which are characteristic of the face-centered cubic (FCC) structure of metallic silver. The presence of these well-defined peaks verifies the successful formation of crystalline silver nanoparticles. (20)

To quantitatively estimate the average crystallite size, the Debye-Scherrer equation was applied:

$$D = k\lambda / \beta \cos \theta$$

where D represents the average crystallite size, k is the shape factor (commonly taken as 0.9), λ is the wavelength of the X-ray source, β is the full width at half maximum (FWHM) of the diffraction peak in radians, and θ is the Bragg diffraction angle. Using this formula, the average crystalline size of the AgNPs synthesized at a 2 mM silver precursor concentration was estimated to be approximately 13 nm. (30)

5) SCANNING ELECTRON MICROSCOPE STUDIES:

Elemental Composition Analysis of Synthesized HbAg-NPs:

The elemental composition of the synthesized (15)silver nanoparticles (Ag-NPs) was determined using SEM-EDS analysis. The results confirmed the presence of silver along with various elements originating from the plant extract matrix and the synthesis process. Two representative EDS spectra were selected, with the corresponding quantitative data summarized in figure 7.

Table 1. Elemental composition of HbAg-NPsbased on EDS analysis.

Element	Weight % (Spectrum 1)	Atomic % (Spectrum 1)	Weight % (Spectrum 2)	Atomic % (Spectrum 2)
C	--	--	35.58	46.03
N	--	--	0.00	0.00
O	57.76	79.67	54.02	52.47
Na	4.28	4.11	--	--
Mg	1.60	1.45	--	--
Cl	4.53	2.82	--	--
K	12.94	7.30	--	--
Ca	2.27	1.25	--	--
Ag	16.63	3.40	10.40	1.50
Total	100.00	--	100.00	--

The EDS spectra revealed prominent signals for silver (Ag), confirming the presence of Ag-NPs in the sample. Peaks corresponding to oxygen (O), carbon (C), and other elements such as sodium (Na), magnesium (Mg), chlorine (Cl), potassium (K), and calcium (Ca) were also detected. These elements likely originate from residual phytochemicals and minerals in the plant extract used for nanoparticle synthesis. The relatively high weight percentages of oxygen and carbon suggest the presence of organic moieties capping the silver nanoparticles, further supporting the role of the plant extract in both reduction and stabilization. The atomic percentage of silver was observed to be 3.40% in spectrum 1 and 1.50% in spectrum 2, while its respective weight percentages were 16.63% and 10.40%. (23) (Table 1.)

6)IMPACT OF BIOGENIC SILVER NANOPARTICLES ON WEIGHTS OF SILKWORM (*Bombyx mori*) LARVAE AND COCOON:

The impact of dietary supplementation of Hb-AgNPs on silkworm development was assessed by comparing the survivability and weights of larvae, cocoons, after fifth instar and cocoon formation across three treatment groups: deionized water (control), Hibiscus flower extract (Hb-FE), and synthesized Hibiscus flower extract silver nanoparticle (Hb-AgNPs) solution. Each group initially consisted of 30 larvae, and results represent averages from replicate batches.(31)(figure 8)

Larval survivability: The parameter of the survivability of the larvae is notably the number of surviving larvae compared to the initial number of larvae, We noticed that that the rate of survival differed notably among the groups. The control group exhibited the lowest survivability, ranging from 80.0% to 86.7% across replicates, with an average around 83.3%. Larvae fed with Hibiscus flower extract (Hb-FE) demonstrated a similar survivability rate from control (80.0–86.7%, mean $\approx$ 83.3%). The highest survivability was consistently observed in the - group treated with Hibiscus Flower extract AgNP with values between 90.0% and 95.7% (mean $\approx$ 93.3%) (figure9). This improved survivability in the AgNP group was paralleled by enhancements in growth and silk production metrics.(31)

LENGTH & Weight Parameters:

The larval length increased from an average range of 240mm in control group, 245mm in Hibiscus flower extract (Hb-FE) and increased upto more than 300mm in the group treated with the Hibiscus flower extract silver nanaoparticles (Hb-AgNPs) (figure 10,11)and the average cocoon shell length increased from the control group (mean $\approx$ 3cm), to the Hibiscus extract group (mean $\approx$ 3.5 cm), and was highest in the Hibiscus-AgNP group (mean $\approx$ 4.25 cm).(26)(figure 11)

Similarly, the weights increased sequentially from the control group (mean $\approx$ 2.4 g), to the Hibiscus extract group (mean $\approx$ 2.5 g), and was highest in the Hibiscus-AgNP group (mean $\approx$ 3.1 g). Similar trends were noted for cocoon shell weights: cocoon weights ranged from approximately 0.3 g (control) to 0.35 g (Hibiscus extract) and reached up to 0.42 g in the Hb-AgNP group.(32) (figure13,14),

Statistical comparison among groups (e.g., using ANOVA or t-test) would likely confirm that both Hibiscus extract and especially Hibiscus-AgNP supplementation significantly improved silkworm survival and productivity compared to the control.(31) These results suggest that Hibiscus-mediated silver nanoparticles, when administered at optimal concentrations, not only enhance silkworm survivability but also promote greater larval biomass and cocoon yield, likely due to synergistic effects of phytochemicals and nanosilver on silkworm physiology.(33)

CONCLUSION

This study demonstrates a highly sustainable approach to the synthesis of silver nanoparticles (AgNPs) using Hibiscus flower extract as both a reducing and stabilizing agent. Employing a green chemistry protocol, the biosynthesis route reduces the release of the toxic reagents and minimizes environmental

impact, further leveraging the natural phytochemicals inherent in Hibiscus for nanoparticle stabilization. (12)(Fig. 1,2,3)

Characterization of the Hibiscus-mediated AgNPs confirms their crystalline nature and nanoscale dimensions, as evidenced by the UV Visible spectral sharp peak at 410-413nm reflecting the metallic silver emission spectra (Fig. 2) and the sharp Bragg reflections in XRD patterns corresponding to the (111), (200), (220), and (311) planes, a hallmark of face-centered cubic silver (Fig. 5). FTIR analysis elucidates the critical capping role of phytochemicals, with functional groups such as O–H, C–H, C = O, and Ag–O identified. SEM-EDS results provide further validation, confirming elemental silver's presence alongside organic moieties derived from the plant matrix. (Fig. 6) (29),(30)

Functionally, the application of these biogenic Hb-AgNPs in sericulture yields significant enhancements in silkworm physiological growth indices and cocoon production. When administered at optimal concentrations, Hibiscus-AgNP supplementation increases larval and pupal survivability by 93.3% (Fig. 9) and identified average larval and cocoon weights increased are upto 3.1gm and 0.42gm respectively, and the feed supplementation also improved the larval and cocoon length identified to be 300mm and 4.25cm respectively compared to controls, the shell yield compared to both traditional feeds and feeds supplemented with non-nanoparticle extracts also increased (Fig. 13,14)(32). This is achieved without imposing toxicological stress, as seen in normal development and high survival rates, underscoring the nanoparticles' compatibility and the potential for dose optimization.(34)

Collectively, these findings not only reinforce the scientific validity of green nanoparticle synthesis but also highlight the substantive agronomic and environmental co-benefits, such as improved silkworm health, increased silk yield, reduced reliance on chemical feed additives, and lower ecological footprints. The integration of plant-based nanotechnology into sericultural practice thereby represents a promising and environmentally compatible strategy for enhancing silk industry sustainability.(35),(36)

We believe the future research should focus on long-term field trials, mechanistic studies of nanoparticle-biomolecule interactions in silkworms, and broader ecological assessments to further consolidate the role of green-synthesized nanoparticles in sustainable agriculture and biotechnological innovation.

Declarations

All experiments involving silkworms were conducted following institutional and national guidelines for the care and use of animals in research. Permissions were taken from the Vangapahad village sericulture rearing unit authorities, and the farmer "Raju" from waranagl district, Telangana.India. (figure15.)

V. FUNDING

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Figures

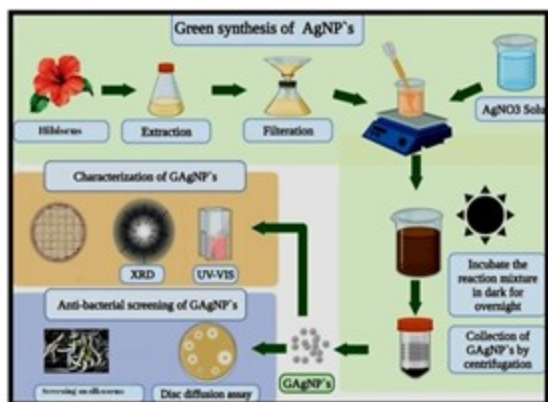


Figure 1

illustration of overall methodology

and goal of green synthesis of AgNps in this experiment



Freshly prepared Aqueous solution of Petal Extract



**Aqueous Petal Extract with AgNO₃ solution addition
After 24hrs**

Figure 2

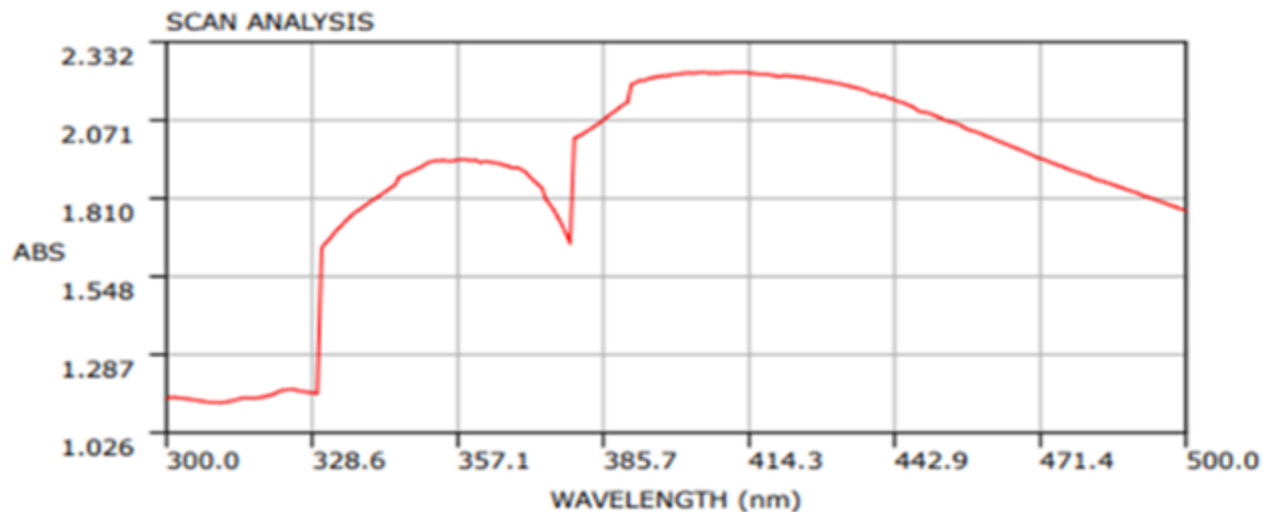
showing the color change from Red to brown due to the Phytochemical reduction of AgNO₃ into Silver nano particles. (AgNPs)

**SYSTRONICS
UV-VIS SPECTROPHOTOMETER - 117**

Date : 28-03-2023

Time : 02-36-20 PM

Name : **Systronics**
Sample ID :



LEGENDS:

— vs1

PEAK PICK

Wavelength	ABS
354.4	1.938
392.8	2.205

VALLEY PICK

Wavelength	ABS
379.2	1.660
438.4	2.159

POINT PICK

Wavelength	ABS
411.2	2.232
412.0	2.230
413.6	2.231
410.4	2.231
409.6	2.230

Figure 3

showing UV-spectrum for 5mM AgNps peak at 413nm determining the absorption of Ag ions in Hb-AgNPs.

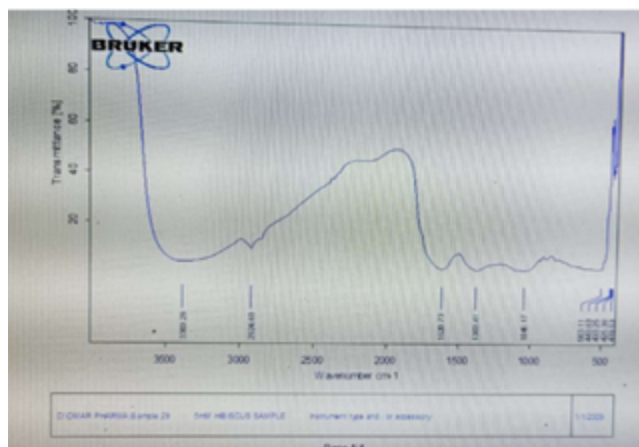


Figure 4

showing stretching vibration peaks of the Hb-AgNps.

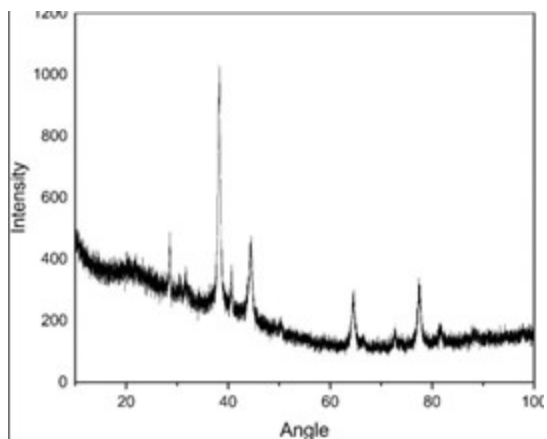


Figure 5

showing X-ray diffraction peaks for 5mM

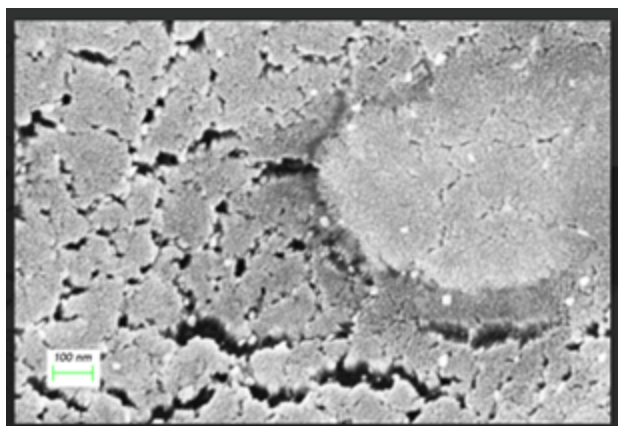


Figure 6

showing size of the particles around 100 nm

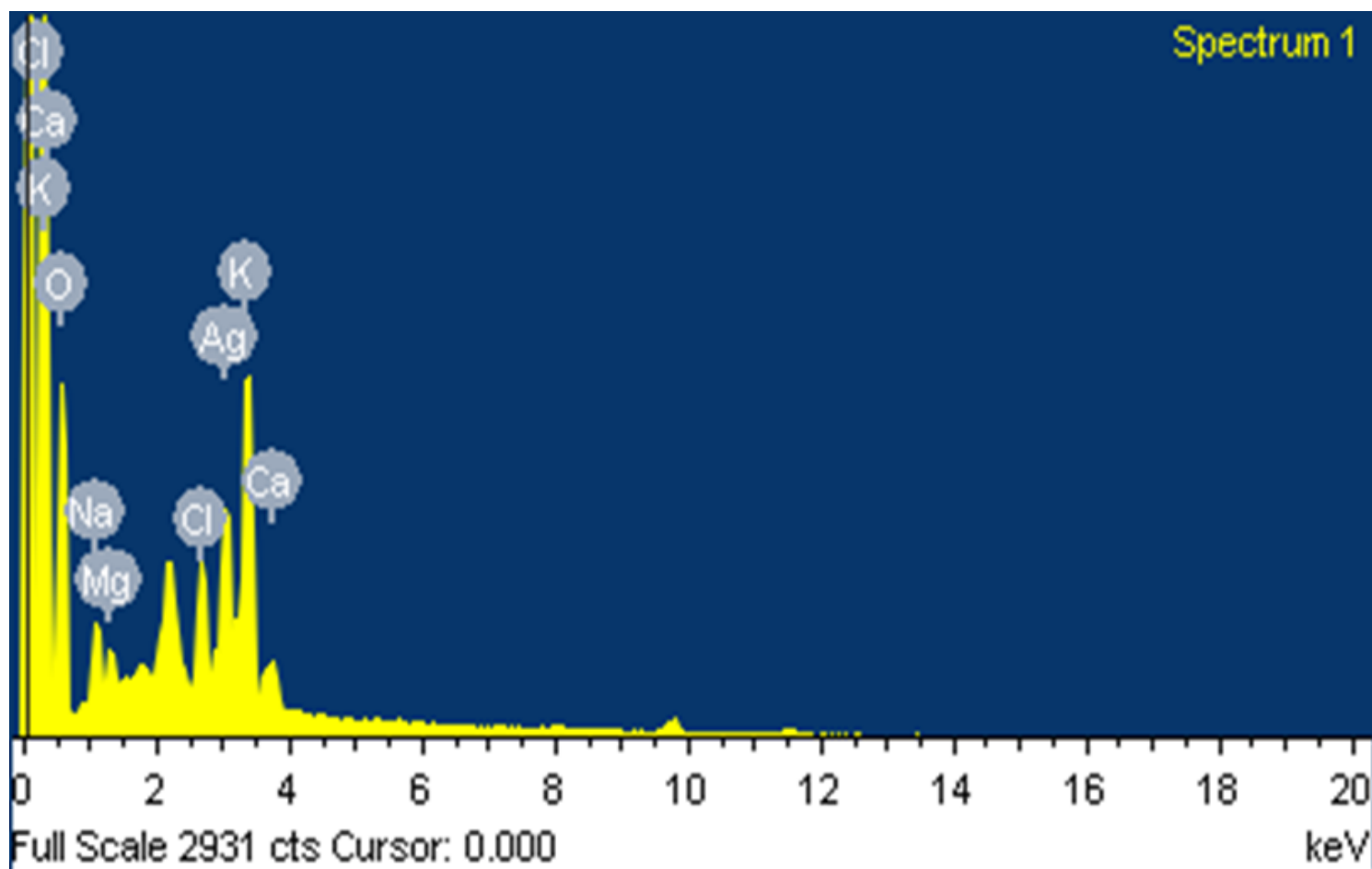


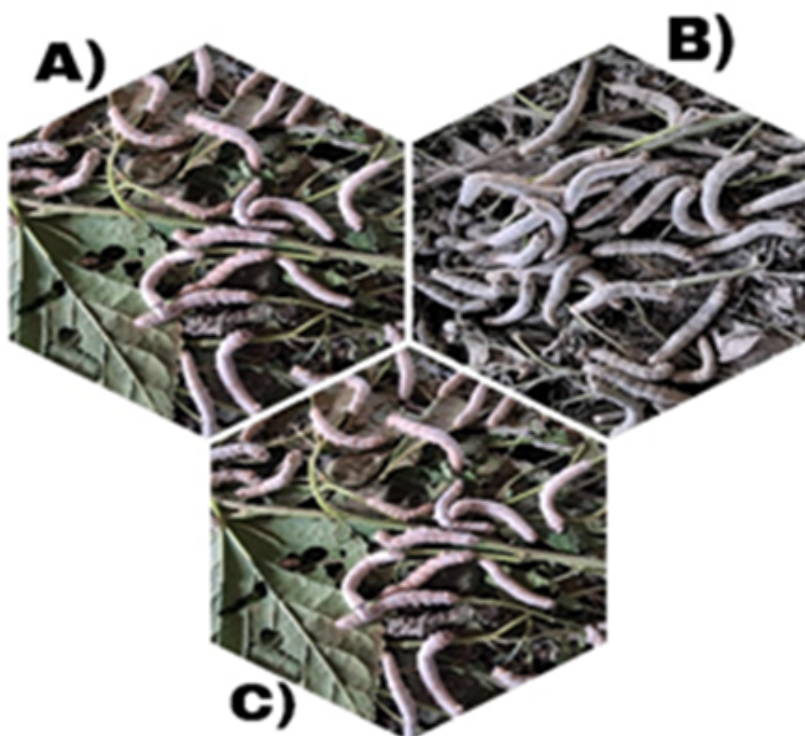
Figure 7

showing the presence of the Ag Silver ions in the EDS analysis.



Figure 8

) control, b) HBFE, c) HB+AgNps



Survivability of larvae before forming Cocoon

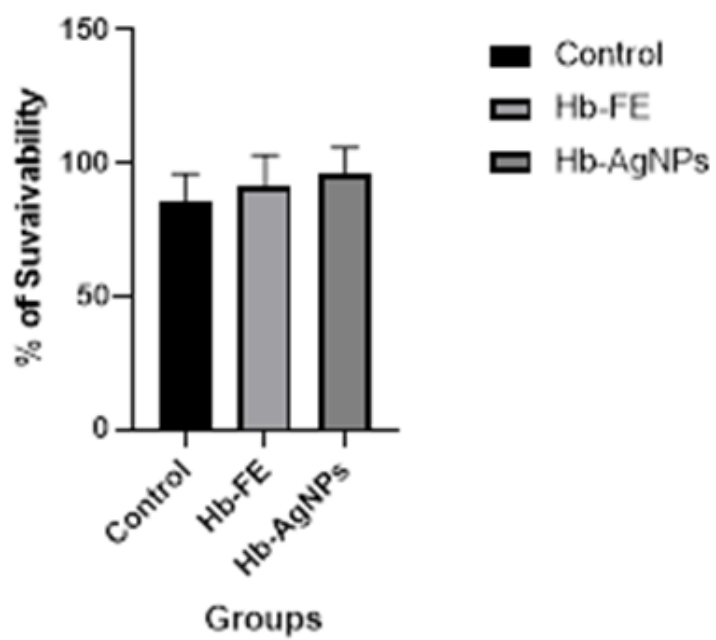


Figure 9

Showing larval survivability among three treatment groups

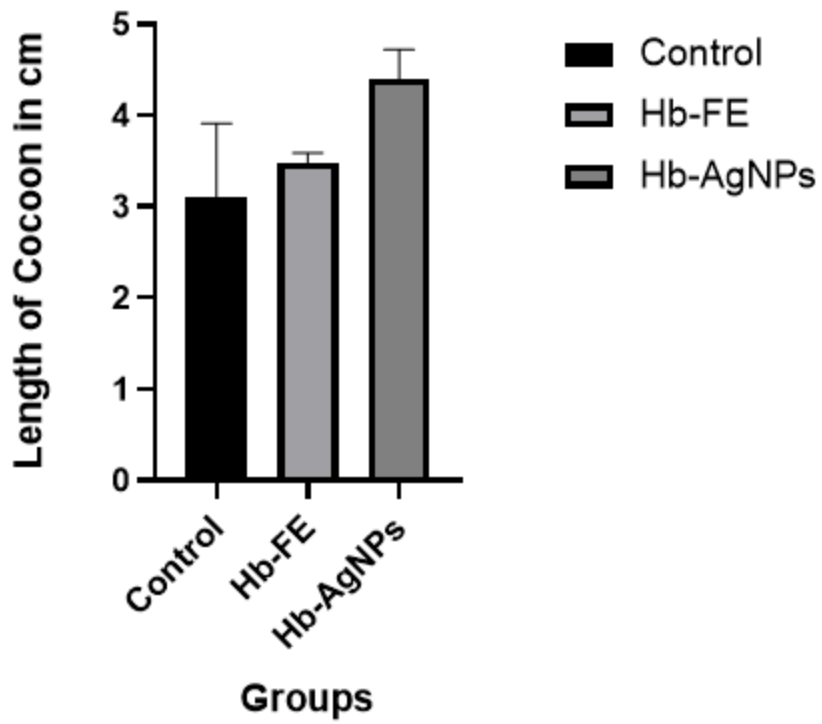


Figure 10

Graph showing average length of cocoons among three treated groups of larvae.

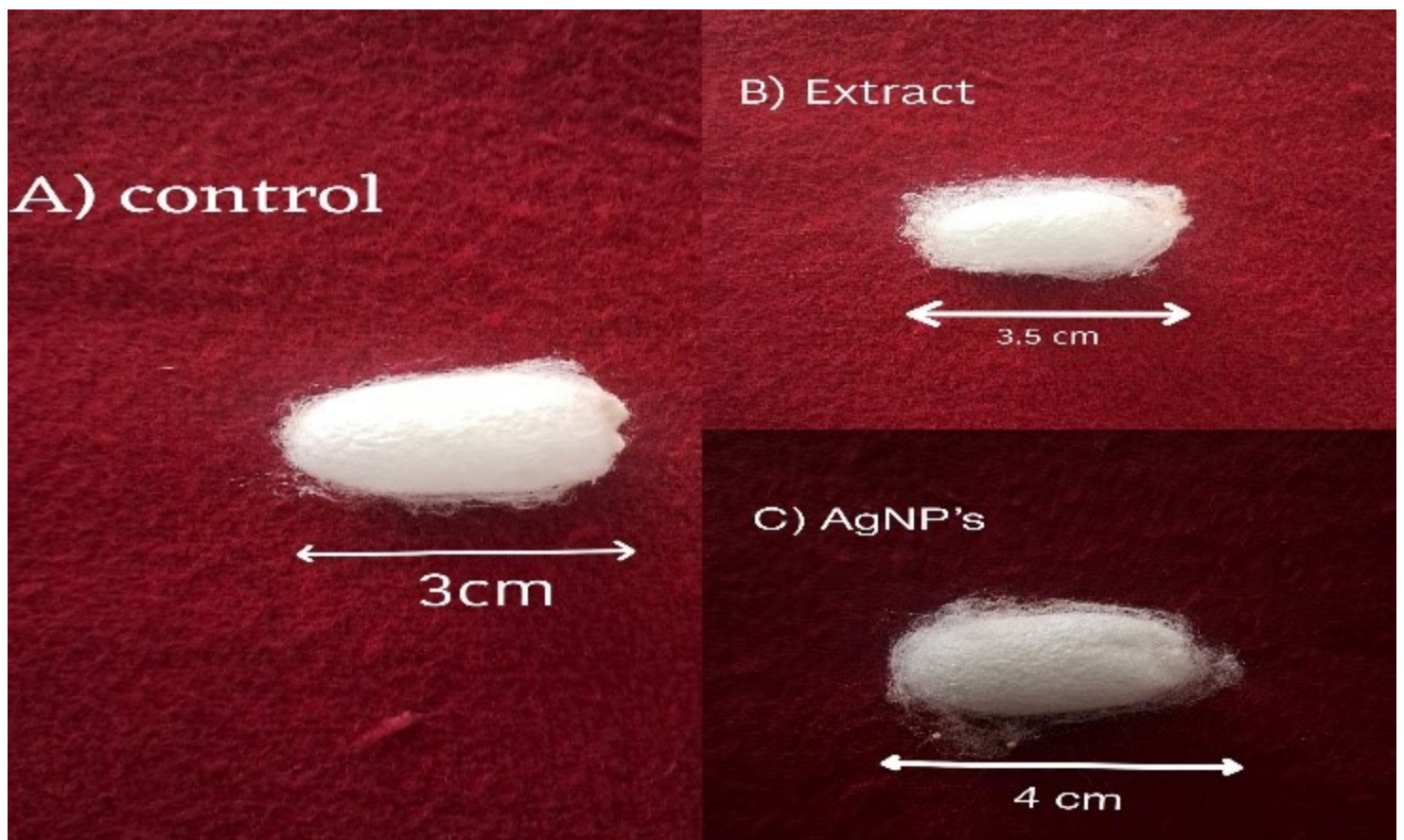


Figure 11

showing size of cocoon shell after treatments among various groups.

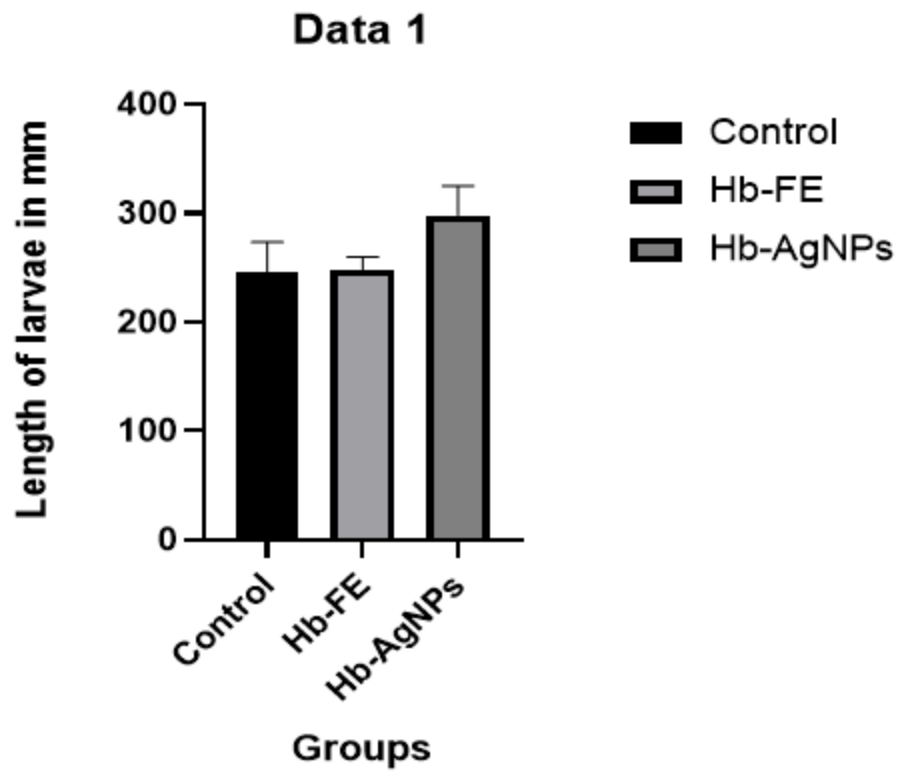


Figure 12

Graph showing the length of larvae in 3 different groups treated with control, Hibiscus flower extract, and Hibiscus flower extract silver nano particles.

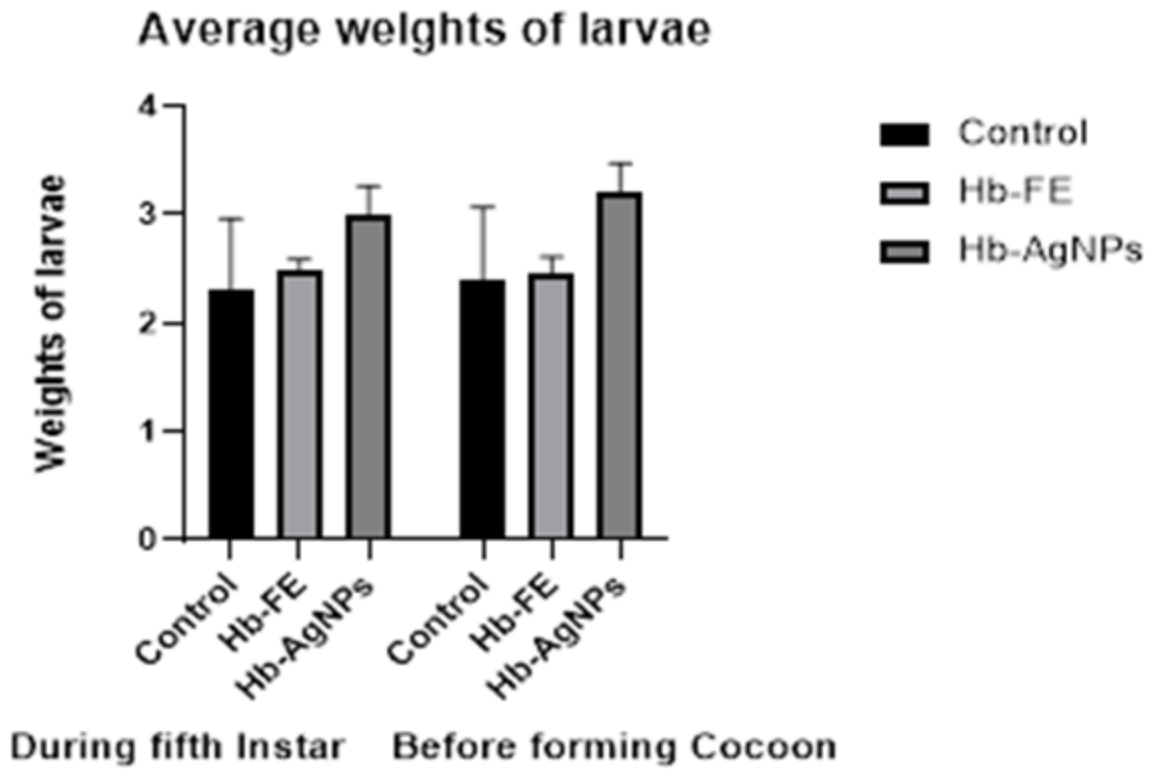


Figure 13

Showing average weights of larvae in 3 different groups during fifth instar and before formation of cocoons.

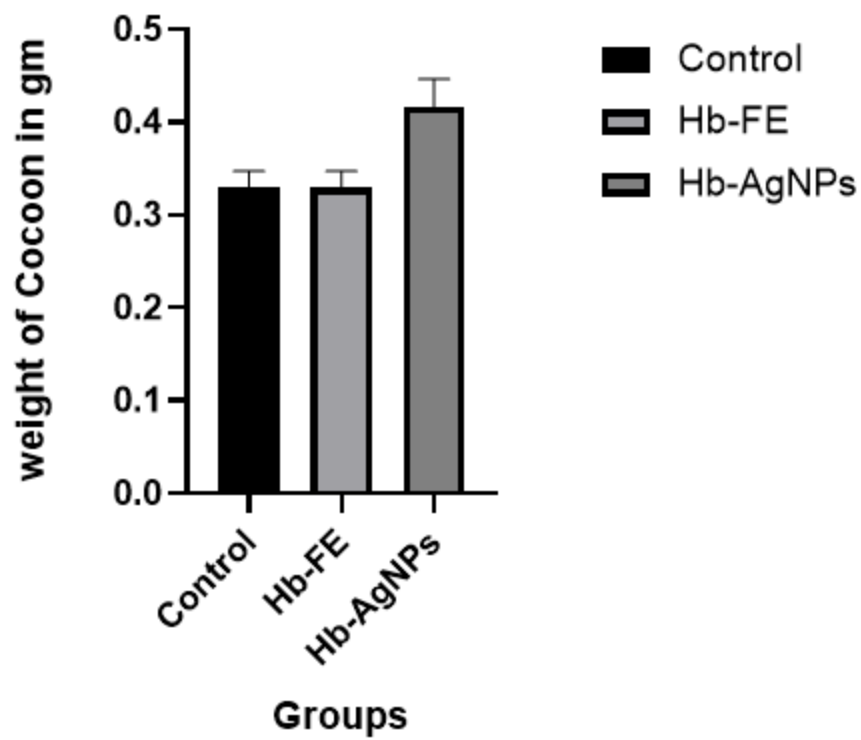


Figure 14

Showing average weights of larvae in 3 different groups during fifth instar and before formation of cocoons.