

# Synthesis, Characterization and Antioxidant studies of Silver Nanoparticles using Hibiscus flower extract

<sup>[1]</sup> A.Eppi Queens, <sup>[2]</sup> J.Jeyapratha, <sup>[3]</sup> G.Kalarani, <sup>[4]</sup> J.Bharathi

<sup>[1][2][3][4]</sup> Department of Chemistry, Kamaraj College, Thoothukudi, Affiliated to M.S. University, Tirunelveli

**Abstract:** -- Silver nanoparticles have received attention due to their physical, chemical and biological properties and were thus applied in numerous biomedical applications. In this study we have done the green synthesized silver nanoparticles using Hibiscus flower extract. The prepared nanoparticles were characterized by using analytical techniques such as FT-IR, UV-visible, AFM. The metal nanoparticles were studied by UV-visible absorption spectroscopy studies. The stretching and bending vibrations of metal oxide are characterized by using FT-IR techniques. The particle sizes and morphology of nanoparticles were determined through Atomic force Microscope (AFM). The silver nanoparticles was found to be highly active towards antioxidant studies.

**Keywords:** -- Ag-NPs, Hibiscus, antioxidant, FT-IR, UV-visible and AFM

## I. INTRODUCTION

Metal nanoparticles using plant extract are a way ahead towards greener approach for application as drug delivery system. Silver nanoparticles have attracts for the wide range of applications in biomedicine. Silver nanoparticles (AgNPs) also possess antifungal, antioxidant, anti-inflammatory, antiviral, and antiplatelet activity.

Plants contain phytochemicals with various bioactivities including antioxidant, anti inflammatory and anticancer activities. Currently, about 25% of the active component was identified from plants that are used as prescribed medicines. Reactive oxygen species (ROS) exert oxidative damaging effects by reacting with nearly every molecules found in living cells including protein, lipid, amino acids and DNA, if excess ROS are not eliminated by antioxidant system. They play important roles in aging and in the pathogenesis of age related disorders such as cancer, hypertension, atherogenesis, Alzheimers disease and Parkinson disease. The natural antioxidants e.g. polyphenols, present in medicinal and dietary plants. Natural antioxidants increase the antioxidant capacity of the plasma and reduce the risk of diseases. Hibiscus rosa-sinensis or China rose (Malvaceae) is a beautiful, showy flower native to East Asia, mainly China. The flowers of Hibiscus rosa-sinensis are edible. The flower is additionally used in hair care.

Our study reports a simple, eco-friendly and economical method to biosynthesize silver nanoparticles using aqueous petal extracts obtained from hibiscus flower and to evaluate

their antibacterial and antioxidant activity in vitro.

## II. MATERIALS & METHODS

### 2.1 Collection of plant materials and Preparation of the aqueous flower extract:

The flowers were collected from a village in Tuticorin District. The petals were separated and dried under shadow. About 20g of Hibiscus rosa-sinensis flower was weighed and transferred into 500 ml beaker containing 300 ml of distilled water and boiled for 20 minutes. The extracts was then filtered thrice through Whatmann No.1 filter paper to remove particulate matter and to get clear solution and stored in dark place used for the further analysis.

### 2.2. Biosynthesis of Silver nanoparticles:

1mM aqueous solution of silver nitrate was prepared and used for the synthesis of silver nanoparticles. Sample and silver nitrate was added in 1:2 ratio kept at dark place at 72 hours inhibition time. The floral extract was added for reduction of silver ions into silver nanoparticle. In the mean time, color change of the mixture from pink color to dark brown.

### 2.3.Characterization of Silver nanoparticles:

The UV-Visible absorption spectra for the flower mediated synthesized AgNPs were recorded by the JASCO variant 630 spectrometer within a range of wavelength 100-900nm. The FTIR spectra was recorded using Thermo scientific Nicolet iS5ATR-iD1 Spectrometer. The FTIR was recorded in the range of 400-4000 cm<sup>-1</sup>. The morphology was investigated by Atomic Force Microscope (AFM) with

Nanosurf Easyscan 2 AFM.

#### 2.4. Phytochemical analysis:

The active phytochemicals present in the floral extract were determined by various tests.

##### *Test for alkaloids:*

A fraction of extract was treated with Wagners test reagent (1.27g of iodine and 2g of potassium iodide in 100mL of water) and observed the formation of reddish brown colour. It indicates presence of alkaloids.

##### *Test for Flavonoids:*

A small amount extract was treated with aqueous sodium hydroxide and hydrochloric acid and observed. No yellow orange color. It indicates absence of flavonoids.

##### *Test for Tannins:*

Few mL of extract was treated with 10% alcoholic ferric chloride solution and observed the formation of blue colour solution. It indicates presence of tannins.

##### *Test for Phenols:*

The fraction of extract treated with 5% ferric chloride and observed of deep blue colour. It indicates presence of Phenols.

##### *Test for Saponins:*

To the small amount of the extract, 10 mL of distilled water was added and shaken for about 30 seconds and observed the formation of foam, it indicates the presence of saponins.

##### *Test for anthocyanin:*

A small amount of extract was treated with sodium hydroxide and observed the formation of blue green. It indicates presence of anthocyanin.

##### *Test for Triterpenoids:*

To the small amount of the extract, few drops of concentrated sulphuric acid was added from the sides of the test tube. No greenish blue colour indicates the absence of triterpenoids.

##### *Test for glycosides:*

To 2mL of the extract, 1mL of pyridine and 1mL of sodium nitroprusside were added. The change in pink colour indicates the presence of glycosides.

#### 2.5. Antioxidant activity of AgNPs:

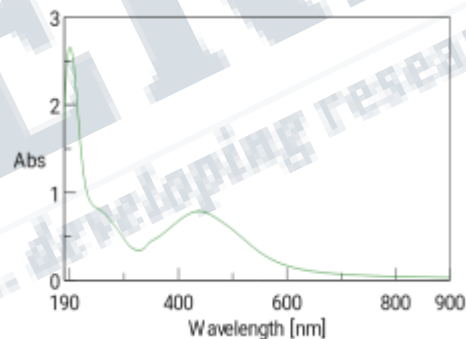
Phosphomolybdenum method: The total antioxidant activity was evaluated by phosphomolybdenum method

described by Prieto et al. 1.0 mL of the extract was mixed with 1.0 ml of the standard reagent solution (0.6M sulphuric acid, 28mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a thermal block at 95°C for 90 min. After cooling to room temperature, the absorbance was measured at 695 nm against a reagent blank. The total antioxidant capacity was expressed as milligram of Ascorbic Acid Equivalence (AAE) per gram of extract.

### III. RESULTS AND DISCUSSION

#### 3.1. UV-Visible spectroscopy of AgNPs:

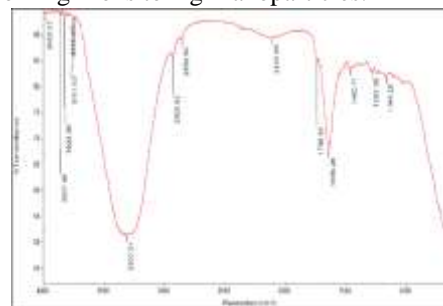
The characteristic brown color arises due to excitation of surface plasmon vibrations in the silver metal nanoparticles. This bioreduction of Silver nitrate ions was followed by UV-vis spectroscopy (Figure 2). The maximum absorbance was noticed at 429 nm which clearly shows the generation of AgNPs. It also confirmed that incubation time has direct correlation with size, shape dispersity and synthesis rate of AgNPs.



**Figure: 1 UV spectra of Silver nanoparticles synthesized from Hibiscus rosa-sinensis extract**

#### 3.2. FTIR spectroscopy of AgNPs

FT-IR spectroscopy analysis was accomplished to determine the functional groups responsible for the reduction of Ag<sup>+</sup> ions to Ag<sup>0</sup> nanoparticles.



**Figure: 2 FT-IR spectra of AgNPs from Hibiscus rosa-sinensis**

Figure.2 shows intense bands at 3980, 3307, 2925, 1636, and 1281  $\text{cm}^{-1}$  respectively. The peak at 3751  $\text{cm}^{-1}$  corresponding mainly to OH stretching vibration of free alcoholic group. The peak at 3307  $\text{cm}^{-1}$  corresponding to N-H stretching frequency. The peak at 2925-2852  $\text{cm}^{-1}$  corresponding to C-H aldehydic stretching frequency. The peak at 1736  $\text{cm}^{-1}$  is corresponding to C=O aldehydic stretching frequency. The peak at 1636  $\text{cm}^{-1}$  corresponding to enol stretching frequency. The peak at 1282- 1164  $\text{cm}^{-1}$  C-OH stretching frequency. These stretching vibrations represents compounds like alkaloids, flavonoids and terpenoids.

#### Phytochemical Analysis:

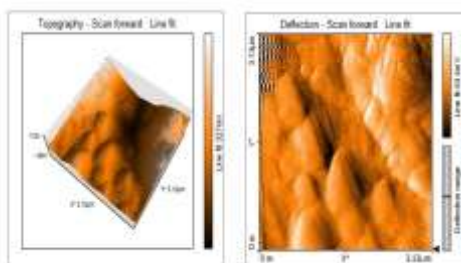
The phytochemical analysis of Hibiscus rosa-sinensis flower extract and tabulated as follows:

**Table: 1 Phytochemical analysis of flower extracts of Hibiscus rosa-sinensis**

Phytochemicals/Tests	Hibiscus rosa-sinensis flower extract
Alkaloids	Present
Flavonoids	Absent
Tannins	Present
Phenols	Present
Saponin	Present
Anthocyanin	Present
Triterpenoids/ steroids	Absent
Glycoside	Present

#### 3.4 Atomic Force Microscopy study:

The Hibiscus rosa-sinensis nano particles surface morphology got using Nano surf easy 2 scan. As figure representation this NPs are equal in size and their morphology also rough in nature so it will use medicine and many other application.



**Fig. 3. AFM image of Hibiscus rosa-sinensis nanoparticles.**

#### 3.4. Antioxidant activity of Ag-NPs:

The total antioxidant activity of the ethanol extract was evaluated by the phosphomolybdenum method according to the procedure described above. According to the results obtained from antioxidant capacity assays, the Hibiscus flower extract has shown a significant total antioxidant capacity. Biosynthesis of nanoparticles by a green method has more advantages over chemical and physical methods as well as it is cost effective and eco-friendly and does not require the use of pressure, energy, temperature, and toxic chemicals. The nanoparticles synthesis process has diverse applications in the field of healthcare, medicine, electronics, etc.

**Table 2 Antioxidant activity of Hibiscus rosa-sinensis nanoparticles**

Sample	Concentration $\mu\text{g/ml}$	OD at 695 nm	TAA mg/g equivalents of ascorbic acid
Standard Ascorbic acid	200	0.05	
	400	0.35	
	600	0.38	
	800	0.63	
	1000	0.74	
HFAGnp	200	0.17	2.266
	400	0.29	7.733
	600	0.46	18.4
	800	0.69	36.8
	1000	0.88	58.667

#### IV. CONCLUSION

The investigations of the study confirmed the green synthesis of stable Ag-NPs from the flower extract of Hibiscus rosa-sinensis by UV-Vis spectroscopy. Through FT-IR spectroscopy, the active functional groups which may involve in the bioreduction of silver ions were identified. The AFM images confirm nanoparticles shape in nanoscale. These plants contain active ingredients, so it is obvious and necessary to exploit the vast therapeutic potential. The developed method has potential for allowing large scale commercial production in biomedical application.

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