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Silver Nano Scaffold Formation by Flowers of Hibiscus *Rosa Sinensis*

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Hibiscus rosa *sinensis* grows for their showy flowers and are used as landscape shrubs. Hibiscus rosa *sinensis* also has too many medicinal properties. Flowers are rich in polyphenols, flavonoids and anthocyanins. In our study the flower extracts of Hibiscus rosa *sinensis* has been used for the synthesis of silver nano scaffold formation and its reduction by the flowers. The synthesis of silver nanoparticles occurred under the exposure of the flower extract to 1mM ($AgNO_3$), Silver nitrate aqueous solution. During this process the complete reduction of nanoparticles was observed nearly 48hours of reaction at 30°C under vigorous shaking conditions. The colour change is noted in the reaction mixture and observed during the process of incubation period, it may be due to the formation of the silver nanoparticles which are able to produce the colour in the reaction mixture may be due to their specific properties of surface Plasmon resonance. The colour change was made confirmed by visualization and the characterization by FT-IR, UV-Visible Spectroscopy. Thus we conclude that the reduction process and capping may be having occurred due to the presence of many flavonoids, terpenoids, anthocyanins, some aminoacids and proteins. In conclusion, Hibiscus *rosa sinensis* flower extract appears capable of forming silver nanoparticles. It can be concluded that, Hibiscus *rosa sinensis* flower extract was able to form the silver nano scaffold and may be probably due to its antioxidants potential, some flavonoids, terpenes etc. This study is a preliminary effort and requires further investigation at different levels.

Keyword: Hibiscus rosa *sinensis* flowers, Silver nano scaffold, UV-Visible Spectroscopy, FT-IR.

1. Introduction

Hibiscus rosa *sinensis*, a member of the Malvaceae family, is widely cultivated in the tropics as an ornamental plant. It is often planted as a fence or hedge plant, and has several forms of flowers with varying colours. It is also used in traditional medicine to induce abortion [1], ease menstrual cramps, assist in childbirth and relieve headache, fever and inflammation. Hibiscus *rosa sinensis* is a glabrous shrub widely cultivated in the tropics as an ornamental plant. Previous studies have showed that Hibiscus *rosa sinensis* possesses many biological activities such as anticomplementary, antidiarrhetic and antiphlogistic activity [2]. It has also been reported that the plant's flower possesses antispermatogenic, androgenic, antitumour and anticonvulsant properties in addition, the

leaves and flowers have been found to be hair growth promoters and aid in the healing of ulcers [3,4].The reported biological activities of Hibiscus *rosa sinensis* include antidiarrhetic [5,6], antioestrogenic, anti-implantation, abortifacient, antipyretic, antispasmodic, hypotensive, embryotoxic, antispermatogenic, insect attractant, analgesic, antifungal and anti-inflammatory properties. The objective of this study was to identify new potential plant antimicrobial agents from Hibiscus species that could be developed by the pharmaceutical industry, and also to promote the use of Hibiscus species in the treatment of various diseases.
Hibiscus *rosa sinensis* belongs to the family Malvaceae. The roots are cylindrical, 5-15 cm in length and 2cm in diameter, off white and with light brown transverse lenticles. The roots taste sweet and are mucilaginous. The leaves are simple ovate or ovatelancolate, and are entire at the base and coarsely toothed at the apex. The flowers are pedicillate, actinomorphic, pentamerous and complete. The corolla consists of 5petals, red coloured and about 8cm in diameter. Traditionally this plant is used for the control of dysfunctional uterine bleeding and as an oral contraceptive. Some of the chemical constituents isolated from this plant are cyanidin, quercetin, hentriacontane, calcium oxalate, thiamine, riboflavin, niacin and ascorbic acid. Flavonoids are also present. In hindi known as Gudhal, Japa in Sanskrit and in English Shoe flower.

2. Scope of Our Study

In this study the use of Hibiscus *rosa sinensis* is used for the medicinal purpose used in Ayurveda, Siddha, Yunani, locally in the treatment of various diseases and we examined for their green synthesis of silver nanoparticles and their characterization. The results of our studies conducted and herewith we report that Hibiscus *rosa sinensis* is useful in controlling the size of silver nanoparticles and also helpful in further strengthening and can also be used for their antimicrobial potential. Therefore, the present investigation is part of continuing programme related to the biochemical screening of local plants.

3. Materials and Methods

3.1 Chemicals

All the fine chemicals were purchased from Sigma Chemicals Co., USA. All other Chemicals used were of Good Quality and Analytical Grade.

4. Phytochemical Analysis

Qualitative analysis of phytonutrients:

4.1 Test for carbohydrates

A small quantity of extract was dissolved separately in 5ml of distilled water and filtered. The filtrate was tested to detect the presence of carbohydrates.

4.1.1 Molisch's test: To 2ml of extract, 2ml of Molisch's reagent was added. Then, 2ml of concentrated Sulphuric Acid was added along the sides of the test tubes. Disappearance in color on the addition of excess solution indicated the presence of carbohydrates.

4.1.2 Benedict's test: To 0.5ml of extract, 5ml of Benedict's reagent was added. The mixture is then boiled for 5mins. Presence of a bluish green precipitate indicated the presence of carbohydrates.

4.2 Test for Glycosides: To 2ml of extract 1ml of aqueous NaOH solution was added. The appearance of a yellow color indicated the presence of glycosides.

4.3 Test for Proteins and Amino acids

Ninhydrin test:

A small quantity extract solution was boiled with 0.2% solution of Ninhydrin. Purple

color indicated the presence of free amino acids.

4.4 Test for Phytosterols and Triterpenoids

Salkowski test:

To 2ml of the extract, 1ml of concentrated Sulphuric acid added. Chloroform was added along the sides of the test tube. A red color produced in the chloroform layer indicated the presence of Phytosterols or if it is yellow in color at the lower layer indicated the presence of triterpenoids.

4.5 Zinc hydrochloride reduction test:

The extract was treated with mixture of zinc dust and concentrated hydrochloric acid. Red color indicated the presence of flavanoids.

4.6 Test for Alkaloids

A small portion of the solvent free extract was stirred separately with a few drops of dilute hydrochloric acid and filtered. The filtrate was tested with Mayer's reagent (Potassium mercuric iodide solution). The cream precipitate indicates the presence of alkaloids. Dried powder of herb treated with 5% Ammoniacal Ethanol and the test carried out after 48 hours, the fraction was treated with Mayer's, Wagner's and Dragndroff's reagent. Exactly 0.5g of the plant extract was dissolved in 5ml of 1% HCL on steam bath. About 1ml of the filtrate was treated with few drops of Dragendorff's reagent. Turbidity or precipitation was taken as indicative of the presence of alkaloids.

4.7 Tests for Tannins

Gelatin test:

To 5ml of extract, few drops of 1% lead acetate were added. Absence of a yellow or red precipitate indicated the absence of tannins.

To 5gm of extract in 50ml water and boiled for 45min. in waterbath and 2% gelatin solution is added drop wise. A blue colouration resulting from the addition of ferric chloride reagent to the filtrate indicated the presence of tannins in the extract.

4.8 Tests for Saponins:

To 5ml of the extract, a drop of sodium bicarbonate was added. It is shaken vigorously and kept undisturbed for 3minutes. Appearance of a honey comb like froth indicated the presence of saponins.

4.9 Proanthocyanidin:

To 2ml of extract add 5ml of 2N HCl and kept in water bath for 30minutes. Then the mixture was cooled and shaken with amyl alcohol. The Total proanthocyanidin was determined based on the procedure of Sun *et al.* The mixture of 3ml of vannilin-methanol (4% v/v), 1.5ml of hydrochloric acid was added to 0.5ml of extract dissolved and vortexed.

4.10 Iridoids:

To 1ml of extract add 5ml of aqueous HCL and kept for 3-6 hours, 0.1ml of extract is decanted and macrate and treated with trim Hill reagent.

4.11 Flavonoids:

To 1ml of extract add 10ml of 95% ethanol and kept in boiling water bath for 15minutes and after filtration magnesium ribbon were added along with 2-3 drops of HCL. The occurrence of a red or orange colour was indicative of flavonoids. The Aluminium chloride colorimetric method was used for flavonoids determination. One milliliter (1ml) of sample was mixed with 3ml of methanol, 0.2ml of 10% aluminium chloride, 0.2ml of 1M potassium acetate and 5.6ml of distilled water and remains at room temperature for 30min.

4.12 Steroids:

To 1ml of extract was extracted with methanol for 15minutes and then Libermann Burchard reagent was added drop wise. Red coloration was observed which is indicative for the presence of steroids [7,8].

4.13 Determination of total phenols

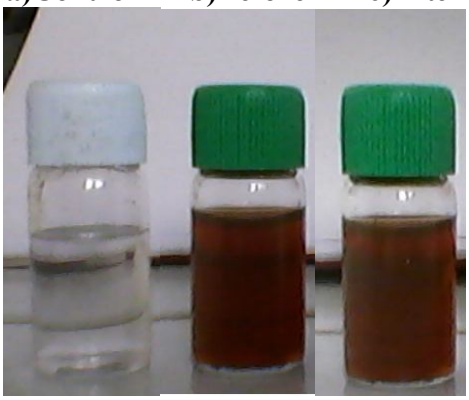
The total phenol content of the extract was determined with Folin Ciocalteau reagent by the method of Spanos GA, *et al*., To 2.5ml of 10% Folin Ciocalteu reagent and 2ml of Na_2CO_3 (2% w/v) was added to 0.5ml of each sample (3 replicates) of plant extract solution (1mg/ml). The resulting mixture was incubated at 45°C with shaking for 15min.

5. Preparation of Flower Extract

The Hibiscus *rosa sinensis* flowers were collected from our College Campus, Islamiah College (Autonomous) Vaniyambadi, Tamilnadu. It was authenticated by our Botany Professor. In our research, the flowers of Hibiscus *rosa sinensis* were involved in the synthesis of silver nanoscaffold. Young and Fresh flowers samples of Hibiscus *rosa sinensis* were collected washed thoroughly with sterile double distilled and sterilized with 0.1%$HgCl_2$ for 2-5min under the hood of laminar air flow. About 25g of Hibiscus *rosa sinensis* flowers were taken and cut into small pieces. Finely cut flowers were placed in a 500ml Erlenmeyer flask containing 100ml of Double Distilled water. The mixture is boiled for 3-5min and filtered through Whatmann No.1 filter paper and stored it at 4°C in a refrigerator for the further study. Then 5ml of the flower extract is taken in 100ml of conical flask and add 1mM $AgNO_3$ solution and is kept in a shaker for 48hours and the colour change is noted and observed. Similarly 1mM Silver nitrate alone without the flower extract alone was also taken and it was the control in our study.

6. Characterization

a)Control b)Before c)After



HIBISCUS *ROSA SINENSIS*

Fig 1: Biosynthesis of Silver Nanoscaffold using Hibiscus rosa *sinensis* Flower Extract. (a) 1M $AgNO_3$ (b) Initial point (c) Final point of time.

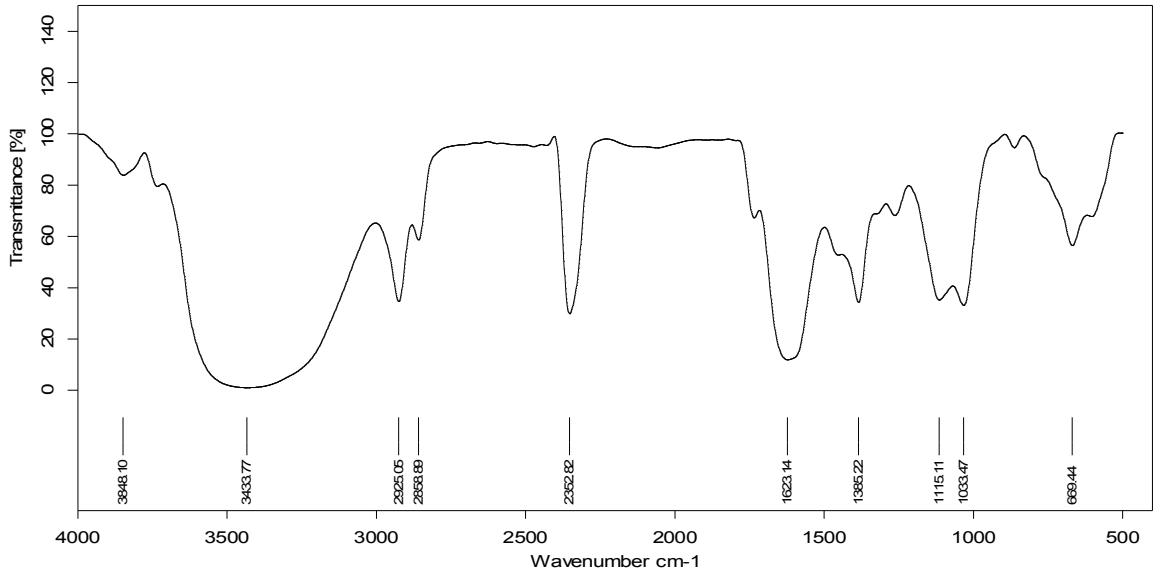
6.1 UV-Visible Spectroscopy Analysis

The colour change in the reaction mixture (ie. Silver metal solution + flower extract) was recorded through the visual observations. The bioreduction process of silver ions in aqueous solution was monitored by periodic sampling of aliquots (1ml) and subsequently measuring the UV-Visible Spectra of the solution. UV-Vis Spectra of these samples were monitored as

a function of time of reaction on Elico UV-Vis Spectrophotometer (model S3-159) operated at a resolution of 1nm [9].

6.2 FT-IR Measurement

FT-IR measurement of sample was performed using FT-IR spectrophotometer in a diffuse reflectance mode at a resolution of 4cm^{-1} in (KBr) Potassium bromide pellets.



Graph 1: UV-Vis Spectroscopy analysis

7. Results and Discussion

UV-Vis Spectroscopy analysis showed that the SPR absorbance band of silver nanoparticles synthesized using the flower extract of Hibiscus *rosa sinensis* flower extract centered at 440nm and steadily increased in intensity as a function of time reaction without any shift in their peaks wave length, frequency, width of the SPR absorption depends on the size and shape of the metal nanoparticles as well as on the dielectric constant of the metal itself and the surrounding medium. FT-IR analysis revealed that the carbonyl groups from the aminoacids residues and from the proteins extracts of the flower had a strong ability to bind metal indicating that the proteins could possibly form the layer covering the metal nanoparticles and prevent agglomeration and thereby stabilize the medium. Our experiment reveals that the biomolecules may possibly have dual formation and stabilization of the silver nanoparticles in the aqueous medium.

8. Conclusion

Aqueous extract of silver nitrate which, consist of silver ions exposed to the Hibiscus *rosa sinensis* flower extract for the synthesis of silver nano particles were made confirmed by the change of colour of the flower extract. More over further it was confirmed by UV-Vis Spectroscopy, FT-IR Spectra. In our present study we found out that the flowers of Hibiscus *rosa sinensis* were good sources for the synthesis of silver nanoparticles and they may have more advantages and the process can be scaled of, in obtaining the nano particles size and its viability.

The synthesis of silve nano scaffold and silver nanoparticles preparations with the herbal extracts will be the new alternative medicines for synthetic drugs. It is

suggested that using the extracts are effective and economic, herbal in drugs may be prepared for pathogenic infections too. Further study and research is need in this new arena and should search for further bioactive compounds can be made evaluated and being continued for the future studies.

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