Comparative study of green synthesized silver nanoparticles using flower extract of *Rosa chinensis* and *Nelumbo nucifera* and its antimicrobial activity

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Abstract- **Biologically synthesized silver nanoparticles (AgNPs) are being widely used in the field of medicine. The biosynthesis method is the best eco-friendly. In the present study is to compare the biosynthesis of silver nanoparticles using the fresh flower extracts of Rosa chinensis and Nelumbo nucifera and its antimicrobial activity. The silver nanoparticles were characterized by using ultraviolet-visible(UV-Vis), FTIR spectroscopy techniques. The bioreduction of silver ions was observed by the colour changes from pink to brown . Fourier transform infrared spectroscopy (FTIR) analysis revealed that the polyphenoliccompounds , flavonoids and other secondary metabolites including in the aqueous extracts may act as capping agent for the nanoparticle synthesis. The biosynthesized AgNPs has been proven antimicrobial activity.**

Keywords: Rosa chinensis, Nelumbo nucifera, Silver nanoparticles, UV, FTIR, secondary metabolites , antimicrobial activity.

1. INTRODUCTION

Nanoparticles are particles that exist on a nanometre scale (i.e., below 100 nm in at least one dimension). Nanotechnology covered all branches of chemistry. Silver nanoparticles (AgNPs) have attracted and demandable research of interest in the field of nanotechnology.Nanosilver has many medical application [1]. A number of biological approches are already reported for the synthsis and stabilization of silver nano particles [2-5]. The green synthesis silver nano particles were evaluated for their synergistic antimicrobial activity as earlier[6-11]. They can possess physical properties and their potential application in optical properties [12,13].

The first flower Rosa chinensis commonly referred as "Rose" in English "Gulaab ka phool" in Hindi and ''Paneer rose'' in Tamil. Rose have been used for stomach problems, and are being investigated for controlling cancer growth. It is used as antiseptic, anti- oxidant and is a rich source of vitamin A, B3, C, D and E. The second flower Nelumbo nucifera commonly referred as "lotus" in English "Kamal" in Hindi and " Ambal" in Tamil. Lotus is used for worshipping and for flavoring the food recipes. The petals are also effective in reducing the symptoms of thirst and inflammations in the body. The flowers have high amounts of calcium and iron. Both the flowers has been reported to antimicrobial activity. The present study was to synthesize AgNPs by using the two floral extracts of Nelumbo nucifera and Rosa chinensis and to compare its phytochemical constituents and antimicrobial potentials[14].

2. MATERIALS AND METHODS:

2.1. Collection and Preparation of the aqueous flower extract:

Figure.1 Rosa chinensis

Figure.2 Nelumbo nucifera

The flowers taken for the present study were namely Nelumbo nucifera (lotus) and Rosa chinensis (rose). These two flowers were collected from Eral region, Thoothukudi District, Tamil Nadu, India.

Preparation of Flower extract:

About each 20g of Rosa chinensis and Nelumbo nucifera sample were weighed separately and transferred into 500 ml beaker containing 300 ml of distilled water and boiled for 20 minutes. The extracts were 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.

Figure.3 Rosa Chinenis flower extract

Figure.4 Nelumbo nucifera flower extract

2.2.Synthesis 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.

Figure.5 Synthesized AgNPs from R.Chinensis

Figure.6 Synthesized AgNPs from N.nucifera

2.3.Characterization of silver nanoparticles:

The UV-Visible absorption spectra for the two 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-1.

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 for the formation of reddish brown colour.

Test for Flavonoids:

A small amount extract was treated with aqueous sodium hydroxide and hydrochloric acid and observed for the formation of yellow orange color.

Test for Tannins:

Few ml of extract was treated with 10% alcoholic ferric chloride solution and observed for formation of blue colour solution.

Test for Phenols:

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

Test for Proteins :

The few ml of extract was treated with aqueous ninhydrin and observed the formation of purple colour, it indicates the presence of protein.

Test for anthocyanin:

A small amount of extract was treated with sodium hydroxide and observed for the formation of blue green.

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. Appearance of greenish blue colour indicates the presence 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.Antimicrobial activity of AgNPs :
The agar well diffusion method was employed for determination of the antimicrobial activity of silver nanoparticles. Four different bacterial suspension culture like Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Proteus vulgaris and 2 fungal cultures such as Candida albicans, Furasium solani was used for this study. The colony forming units (CFU/ml) of 0.1ml suspension of the post operative wound pathogens were adjusted to 5 ×105 CFU/ml. A suspension of test organism (0.1ml) was swabbed on the surface of Muller Hinton Agar medium (MHA) by using the sterile cotton swab. After that, a sterile cork borer (5 mm diameter) was used to made wells in the seeded Muller-Hinton agar. Then, concentration of nanoparticles (100µl, 125µl, 150µl and 200µl) were delivered into wells separately and allowed to diffuse at room temperature. The plates were incubated at 37°C for 24hours. The zone of inhibition was measured in millimeter (mm).

3. RESULTS AND DICUSSION:

3.1.UV-Visible spectroscopy of AgNPs synthesized from two floral extract:
According to literature studies silver nanoparticle solution has dark brown or dark reddish in colour. In Rosa chinensis and Nelumbo nucifera before the addition of AgNO3 its colour was pale pink but after its treatment with AgNO3 its colour changed to dark brown which indicates the formation of AgNPs. (Fig.5&6) These colour changes were due to the property of quantum confinement which is a size dependent property of nanoparticles which affects the optical property of the nanoparticles. The spectrum displayed the characteristic surface plasmon resonance band of silver nanoparticles, synthesized from Rosa chinensis at 429 nm (Fig.7). Similarly spectrum showed a peak at 435 nm (Fig.8) for silver nanoparticles synthesized using Nelumbo nucifera.

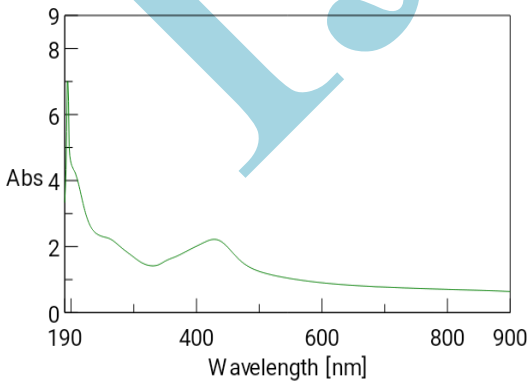

Figure: 7 UV spectra of Silver nanoparticles synthesized from Rosa chinensis extract

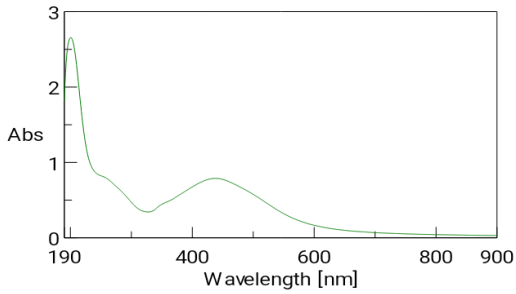

Figure: 8 UV spectra of Silver nanoparticles synthesized from Nelumbo nucifera extract

3.2. FTIR spectroscopy of AgNPs synthesized from two flower extract:
The functional groups of N. nucifera and R.chinensis responsible for the bio-reduction of AgNO3 into Ag nanoparticles can be explained from FTIR analysis.

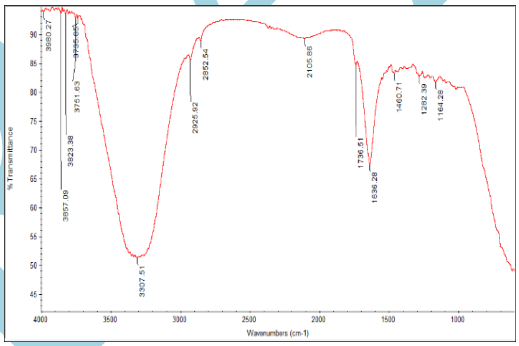

Figure 9: FTIR spectra of AgNPs from Rosa Chinensis

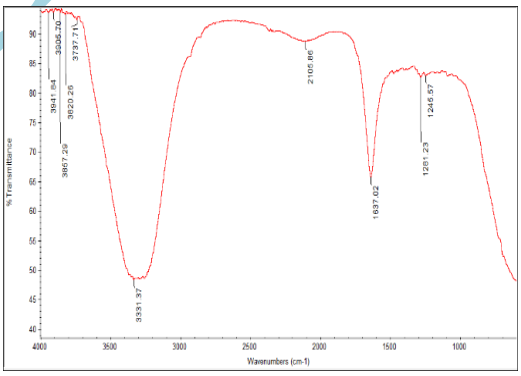

Figure 10: FTIR spectra of AgNPs from Nelumbo nucifera

In figure 9,10 prominent bands of absorption observed at around 3980,3307,2925,1636,and 1281cm-1.The peak at 3751cm-1 corresponding mainly to OH stretching vibration of free alcoholic group. The peak at 3307 cm-1 corresponding to N-H stretching frequency. The peak at 2925-2852 cm-1 corresponding to C-H aldehydic stretching frequency. The peak at 1736 cm-1 is corresponding to C=O aldehydic stretching frequency .The peak at 1636 cm-1 corresponding to enol stretching frequency. The peak at 1282- 1164 cm-1 C-OH stretching fequency. These stretching vibrations represents compounds like alkaloids, flavonoids and terpenoids.

Phytochemical Analysis:
The phytochemical analysis tests were done using the floral extracts of Rosa chinensis and Nelumbo nucifera and tabulated as follows:

Table: 1 Phytochemical analysis of flower extracts of Rosa chinensis and Nelumbo nucifera

Phytochemicals/Tests	***Rosa chinensis* flower extract**	***Nelumbo nucifera* flower extract**
Alkaloids-Wagner's Test	Present	Present
Flavonoids-Sodium hydroxide Test	Present	Present
Tannins-Braymers Test	Present	Present
Phenols-Ferric Chloride	Present	Present
Protein-Ninhydrin Test	Present	Present
Anthocyanin - sodium hydroxide test	Present	Present
Triterpenoids/steroids-Salkowaski Test	Present	Present
Glycosides-Legal Test	Present	Present

3.4. Antimicrobial activity of AgNPs:
Biological synthesis of nanoparticles by plant extracts is at present under exploitation as some researchers worked on it [15] and testing for antimicrobial activity [16] .Similarly in the present work, the SNPs of Rosa chinensis and Nelumbo nucifera extract of 200µl showed highest antimicrobial activity against microorganisms(bacteria and fungi) [17], reported that the growth of microorganisms was inhibited by the green synthesized SNPs showed variation in the inhibition of growth of microorganisms may be due to the presence of peptidoglycan, which is a complex structure and after contains teichoic acids or lipoteichoic acids which have a strong negative charge. This charge may contribute to the sequestration of free silver ions. Thus gram positive bacteria may allow less silver to reach the cytoplasmic membrane than the gram negative bacteria [18] reported that silver nanoparticles synthesized using oak bark extracts demonstrated approximately 1.5 times stronger inhibition to reference culture of B.cereus ATCC 11778 than against the cultures of B. cereus isolated from food products. The high bactericidal activity is certainly due to big changes in the membrane structure of bacteria as a result of the interaction with silver cations lead to the increased membrane permeability of the bacteria [19]. The ionic silver strongly interacts with thiol group of vital enzymes and inactivate the enzyme activity [20]

The Antimicrobial activities of synthesized AgNPs were determined against bacterial suspension such as Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Proteus vulgaris and fungal suspension like Candida albicans,Furasium solani using agar diffusion method. The results were recorded by measuring the zones of growth inhibition. The flower extract of Rosa chinensis and Nelumbo nucifera which showed a promising antibacterial activity against all the pathogens (Table 2 and Table 3).

Table:2 Antibacterial and antifungal activity of Rosa chinensis

Bacterial pathogens	**Concentration of AgNPs**			
	Zone of inhibition (mm)			
	100µl	125µl	150µl	200µl
Escherichia coli	9	10	11	12
Staphylococcus aureus	11	12	14	15
Bacillus subtilis	10	12	13	15
Proteus vulgaris	10	12	13	14
Fungal pathogens				
Candida albicans	5	6	8	10
Furasium solani	6	7	9	11

Table:2 Antibacterial and antifungal activity of Nelumbo nucifera

Bacterial pathogens	**Concentration of AgNPs**			
	Zone of inhibition (mm)			
	100µl	125µl	150µl	175µl
Escherichia coli	16	18	20	24
Staphylococcus aureus	9	11	12	14
Bacillus subtilis	18	20	24	26
Proteus vulgaris	18	20	22	26
Fungal pathogens				
Candida albicans	6	8	9	11
Furasium solani	5	7	8	10

4. CONCLUSION:

Rosa chinensis and Nelumbo nucifera showed great capability to synthesis AgNPs at optimum temperature conditions. The UV absorption peak at 429nm and 435nm clearly indicates the synthesis of AgNPs. FTIR studies confirmed the bio fabrication of the AgNPs by the action of different phytochemicals with its different functional groups present in the extract solution. The AgNPs have great antimicrobial activity against bacteria such as Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Proteus vulgaris and 2 fungal cultures such as Candida albicans, Furasium solani. The formulations of flower extract with silver nanoparticle worked out in this project to find the antimicrobial activity would be useful in various fields of biotechnology.

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