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GREEN SYNTHESIS OF SILVER NANOPARTICLES FROM *Catharanthus roseus* AND ESTIMATION OF ITS *IN VITRO* ANTI-OXIDANT, ANTI-DIABETIC AND ANTI-INFLAMMATORY ACTIVITY

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Abstract - The present study aims the green synthesis of silver nanoparticles (AgNPs) by using *Catharanthus roseus* as leaf extract and to analyze the biological properties by using *invitro* methodologies. These AgNPs were synthesized biologically by mixing aqueous leaf extract of *Catharanthus roseus* with silver nitrate($AgNO_3$) as precursor. The AgNPs were confirmed by Double beam UV-Visible spectroscopy in which high absorbance peak was obtained at 450 nm, generally they exhibit high absorbance at 350-600 nm. The synthesized AgNPs were characterized by Fourier Transform Infra-Red spectroscopy (FT-IR) in which the functional groups present were identified and scanning electron microscopy (SEM) in which three dimensional images of nanoparticles were obtained. The properties of biologically synthesized nanoparticles were evaluated by *in vitro* methodologies such as, DPPH and H_2O_2 scavenging assay for anti-oxidant activity, inhibition of protein denaturation assay for anti-inflammatory activity and α-glucosidase inhibition assay for anti-diabetic activity.

Key Words: Silver nanoparticles (AgNPs), *Catharanthus roseus* leaf extract, anti-oxidant activity, anti-inflammatory activity.

1.INTRODUCTION

Catharanthus roseus is popularly known as the Madagascar periwinkle or Sadabahar which belongs to the family of Apocynaceae. Two of the plant alkaloids (vincristine and vinblastin) are responsible for its anticancer property. In traditional chinese medicine, extracts from it have been used to cure various diseases like malaria, diabetes, leukemia and hodgkin's lymphoma. A compound called vinpocetine has been reported to have a variety of actions that is used in treating alzheimer's disease [1]. An Alkaloid called vindolicine from leaves of *Catharanthus roseus* is proven in reducing the blood glucose levels of patients with type 2 diabetes mellitus. The activity of vindolicine alkaloid against cells has shown to improve cells activity and induce insulin secretion thus preventing further hyperglycemia in these patients [2]. This plant parts are used to treat gastrointestinal aliments, reduce fever, pain and infectious diseases. They are mainly used to treat inflammation and have been source of COX inhibitors [3]. Silver nanoparticles appears yellow in solutions and when they aggregate, they appear in grey colour. The green synthesis of nanoparticles is a eco-friendly, cost effective and a rapidly developing technology which is proven to be more efficient than the chemically synthesized nanoparticles since the plant extract replaces the toxic chemicals [4]. Nanoparticles can be characterized by using several methods such as Scanning Electron Microscope (SEM) to observe the three dimensional images of the nanoparticles, Ultraviolet-Visible (UV-Vis) spectroscopy for characterization and identification of nanoparticles and Fourier Transform Infra-Red (FT-IR) spectroscopy to identify the functional groups present[5]. AgNPs have board range of applications in biomedical device coatings, drug delivery carriers, imaging probes, diagnostic and optoelectronic platforms [6]. AgNPs also function as a antibacterial agents which carry very less toxic effects and play an important role in pharmaceutical industries. AgNPs have been used in antimicrobial and also anticancer therapy, and also speeds up the process of wound repair, bone healing or as the vaccine adjuvant [7]. Different nanoparticles have been synthesized using plant extract in wide range of shapes and size which possess anti-inflammatory properties. When compared to their bulk counterparts, nanoparticles block inflammatory-enhancers like cytokines, and inflammatory-assisting enzymes in a better way. Nanoparticles have a great penetrating capacity in epithelial and inflammatory cells which leads to better effectiveness and improved persistence in the treatment. They also have a better

selectivity of target sites such as inflammatory cells or tissues [8]. Nano particles are also used as therapeutic delivery agents and most of them proved to have reduced side effects and toxicity and also increase a drug's bioavailability and effectiveness at the inflammation site [9]. The antioxidants have potential to minimize oxidative stress, which is created due to the imbalance between production of oxidants and the endogenous antioxidants to counteract. Novel metal nanoparticles (Au, Ag, Pt) are commonly used and tested for their antioxidant activity [10]. Hence in this study, an attempt was made to synthesize silver nanoparticles from *Catharanthus roseus*. The synthesized nanoparticles were characterized by three different methodologies (UV-vis spectroscopy, FTIR and SEM. The anti-oxidant and anti-inflammatory propertie and anti-diabetic property were estimated by DPPH, H_2O_2 radical scavenging assays, protein denaturation assay and α-glucosidase inhibition assay respectively.

2.MATERIALS AND METHODS

2.1 Sample Collection and processing

The fresh leaves of *Catharanthus roseus* were collected during early morning from Surapet (13.1445° N, 80.1838° E) Chennai. The collected leaves were washed twice with distilled water to remove the dust particles. Washed leaves were shade dried for a period of two weeks and powdered using a commercial blender. The obtained dry powder was about 50 g and stored in an air tight container at 4˚C until further use. The extraction method was standard Soxhlet method and water was the solvent used for extraction,

2.2 Synthesis of nanoparticles

The biological synthesis of nanoparticle was performed by using *Catharanthus roseus* leaves extract as a reducing agent. The bioactive components present in the extract helps in reducing the precursor into a nanoparticle. The chosen precursor for the biosynthesis of Silver nanoparticle was Silver nitrate($AgNO_3$).The plant extract of 10 ml was taken in a clean and sterile conical flask, 90 ml of 1 millimolar aqueous $AgNO_3$ solution was prepared in separate conical flask and was added and heated at 80˚C for 15 mins. The synthesized nanoparticles were then characterized by UV-Vis spectroscopy, Scanning Electron Microscopy and Fourier Transform Infra red spectroscopy.

2.3 Anti-oxidant activity:

The free radical scavenging activity of the AgNPs of *C.roseus* was measured in terms of hydrogen donating or radical-scavenging ability using the stable radical DPPH .Solution of DPPH in ethanol (0.1 mM) was prepared and 1.0 ml of this solution was added to 2.0 ml of synthesized AgNPs of *C. roseus* extract at different concentrations (1-5mg/ml). After, thirty minutes, the absorbance was measured at 517nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity .

The hydrogen peroxide scavenging activity was determined. A solution of H_2O_2 was prepared in phosphate buffer(0.1mM) and their concentration was determined spectrophotometrically from the absorption at 230 nm. Various concentrations of synthesized AgNPs of *C.roseus*((1-5mg/ml)were added to I ml of H_2O_2 and incubated for 10 min. The absorbance at 230 nm was determined against a blank containing phosphate buffer without H_2O_2 . The percentage scavenging of DPPH and H_2O_2 was calculated by

Inhibition(%) = [1-(Abs sample/Abs control)] x 100

Where Abs sample is absorbance of the test sample AgNPs of *C.roseus* extract, Abs control is absorbance of the control.

2.4 Anti-inflammatory activity:

This method of in vitro analysis of anti-inflammatory activity has a base concept of preventing the protein denaturation. A volume of 1 ml of extracts of synthesized AgNPs of *C.roseus* at different concentrations (100, 200, 500, and 1000 µg/ml) was homogenized with 1 ml of aqueous solution of bovine serum albumin (5%) and incubated at 27°C for 15 minutes. The BSA and distilled water mixture constituted the control tube. The proteins were denatured by placing the mixture in a water bath for 10 minutes at 70°C. The mixture was cooled at ambient room temperature, and the activity each mixture was measured at 660 nm . The inhibition percentages were calculated.

2.5 Anti-diabetic activity:

α-Glucosidase inhibition assay

α-Glucosidase is a main enzyme involved in carbohydrate metabolism by catalyzing the cleavage of oligosaccharides and disaccharides into monosaccharides. The inhibition α-glucosidase helps in delaying the digestion and absorption of carbohydrates and thus helps in reducing the blood glucose level.

The α-glucosidase inhibitory activity was determined using pNPG as substrate. 20 µl of varying concentrations of AgNPs synthesized from C.roseus extract (0.2,0.4,0.6,0.8and 1.0 mg/ml) were taken in a 96 well microtitre plate and (acarbose) was used as positive control. α-glucosidase (50µl) and phosphate buffer (50µl, pH 7.0) were mixed to the samples and control and pre-incubated at 37 °C for 10 min. Then pNPGsubstrate(50 µl, 20 mM) was added to start the reaction. After incubation at 37 °C for 30 min, the absorbance was measured at 405 nm and the inhibition percentages were calculated.

3. RESULTS

3.1 Nanoparticle synthesis

After mixing the required volume of precursor and plant extract, a colour change from pale yellow to reddish brown was observed which indicates the synthesis of silver nanoparticle (Figure: 1).

Fig.1 After formation of Ag nanoparticles

3.2. UV-Vis Spectroscopy of *C.roseus* silver nanoparticles:

The obtained nanoparticles were characterized by using UV-VIS double beam spectrophotometer. It was scanned under a wavelength scanning range of 300 nm to 600nm. The highest peak was obtained at 450 nm . Generally, Ag nanoparticles exhibit highest peak at a range of 350- 600 nm . Thus this confirms the presence of Ag nanoparticles in the synthesized *C.roseus* extract.

3.3. Scanning electron microscopy of *C.roseus* silver nanoparticles:

The Scanning Electron Microscopy (SEM) of Ag nanoparticles was performed at Anna University, Chennai, India. The three-dimensional images of

the nanoparticles were obtained at nanoscale. The nanoparticles were identified and marked in a magnification of 41.0kx at an amplification 30 kV in a micrometer scale to nanometer scale (Figure:2). At this magnification different sized nanoparticles such as 54.53nm, 54.73nm, 69.23.38nm, 66.77 , 73.41 and 89.42nm was observed.

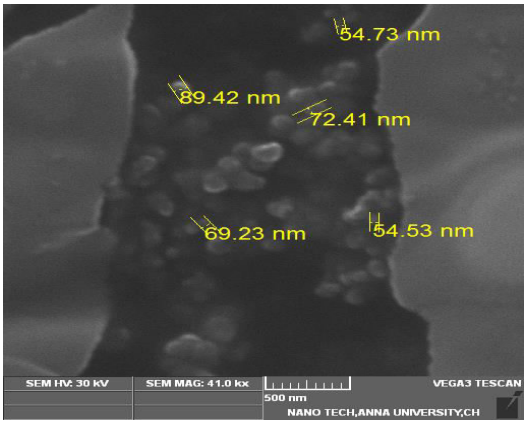

Fig.2 SEM Result

3.4 Fourier Transform Ifra-Red Spectroscopy (FT-IR) Of *C.roseus* AgNPs

The Fourier Transform Infra-Red (FT-IR) Spectroscopy was performed to characterize the biologically synthesized nanoparticle. It was carried out at Avinashlingam Institute, Coimbatore. The different functional groups present in the surface of the nanoparticles were identified at various transmittance peaks (Fig 3).

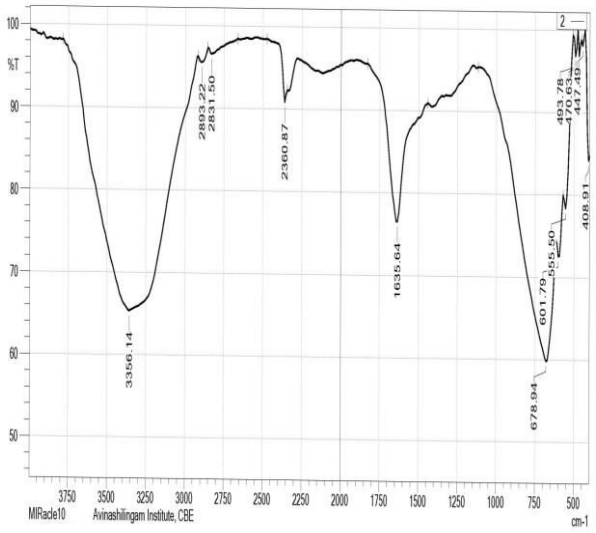

Fig.3 FT-IR absorbance plot of *C.roseus* Ag nanoparticles

The peak values were compared with the standard IR chart (Appendix 1) and different functional groups were identified (Table 1). In this absorbance peak the absorbance value corresponding to 555.50 cm^{1} represents the vibration caused by Agand other peak values such as 1635.64 corresponds to C=C stretching, 2360.87 corresponds to S-H stretching, 2831.50 corresponds to C-O stretching and 3356.14 corresponds to N-H stretching

Table 1 FT-IR_ Functional group present in Ag nanoparticles

ABSORBANCE PEAK CM-1	FUNCTIONAL GROUP	COMPOUND CLASS	APPEARANCE
555.50	C-I STRETCHING	HALO COMPOUND	STRONG
601.79	C-I STRETCHING	HALO COMPOUND	STRONG
678.94	C-BR STRETCHING	HALO COMPOUND	STRONG
1635.64	C=C STRETCHING	CONJUGATED ALKENE	MEDIUM
2360.87	S-H STRETCHING	THIOL	WEAK
2831.50	C-H STRETCHING	ALDEHYDE	MEDIUM
2893.22	OH STRETCHING	CARBOXYLIC ACID	WEAK, BROAD
3356.14	N-H STRETCHING	ALIPHATIC PRIMARY AMINE	MEDIUM

3.5 ANTIOXIDANT ACTIVITY

DPPH ASSAY

The free radical scavenging activity is determined from the ability of the silver nanoparticles to scavenge DPPH ions. The absorbance was measured at 517nm as shown in table 4.3 and the percentage inhibition was calculated. Silver nanoparticles synthesized from aqueous extract of *Catharanthus roseus* have exhibited (37-62%) of antioxidant activity for 1,2,3,4,5mg/ml of nano particles concentration respectively.

Table 2 DPPH Scavenging activity of AgNPs synthesized using *Cathranthus roseus*

S.NO	CONCENTRATION (MG/ML)	OD AT 517NM	DPPH INHIBITION (%)
1	1	0.587	38.66
2	2	0.523	45.35
3	3	0.49	48.69
4	4	0.435	54.54
5	5	0.356	62.80

H_2O_2 SCAVENGING ASSAY

The hydrogen peroxide scavenging activity was determined from the ability of the silver nanoparticles to oxidize H_2O_2. The absorbance was measured at 230nm and the percentage inhibition was calculated as shown in table 3. Silver nanoparticles synthesized from aqueous extract of *Catharanthus roseus* have exhibited (34-72%) of antioxidant activity for 1,2,3,4,5 mg/ml of nanoparticles concentration respectively

Table 3 H_2O_2 scavenging activity of AgNPs synthesized using *Catharnthus roseus*

S.NO	CONCENTRATION (MG/ML)	OD AT 230NM	H_2O_2 INHIBITION (%)
1	1	0.467	34.68
2	2	0.315	55.94
3	3	0.276	61.39
4	4	0.238	66.71
5	5	0.197	72.44

3.7 ANTI-INFLAMMATORY ASSAY

The anti-inflammatory assay was performed by the inhibition of protein(BSA). The absorbance value was measured at 660nm and the percentage inhibition of the protein was calculated (Table 4).

Silver nanoparticles synthesized from aqueous extract of *Catharanthus roseus* have exhibited (50-74%) of anti-inflammatory activity for 0.2,0.4,0.6,0.8,1.0 mg/ml of nanoparticle concentration respectively

Table 4 Anti-inflammatory activity of AgNPs synthesized using *Catharnthus roseus*

S.NO	CONCENTRATION (MG/ML)	OD AT 660NM	PROTEIN INHIBITION (%)
1	0.2	0.471	51.54
2	0.4	0.437	55.04
3	0.6	0.395	59.36
4	0.8	0.312	67.90
5	1.0	0.245	74.79

3.8 ANTI-DIABETIC ACTIVITY

α-GLUCOSIDASE INHIBITION ASSAY

The α-glucosidase inhibition assay was carried out and the absorbance were measured at 405nmusing UV-VIS spec. The percentage inhibition of the α-glucosidase enzyme was calculated

(Table 5). The inhibition of α-glucosidase delays digestion and absorption of carbohydrates and can reduce the blood glucose level. The silver nanoparticles synthesized from aqueous extract of *Catharanthus roseus* have exhibited (30-63%) of α-glucosidase inhibitory activity for 0.2,0.4,0.6,0.8,1.0 mg/ml of nanoparticle concentration respectively.

Table 5 α-glucosidase inhibition assay of AgNPs synthesized using *Catharanthus roseus*

S.NO	CONCENTRATION (mg/ml)	OD AT 405NM	α-GLUCOSIDASE INHIBITION (%)
1	0.2	0.502	30.85
2	0.4	0.455	37.32
3	0.6	0.393	45.86
4	0.8	0.337	53.58
5	1.0	0.263	63.77

4. CONCLUSION

In this study we have biologically synthesized Ag nanoparticles using *Catharanthus roseus* plant source and examined their characteristics and their biological properties. The properties such as anti-oxidant activity, anti-inflammatory activity and anti-diabetic activity were analysed by *in vitro* methodologies. The high absorbance rate, three dimensional images and functional groups present were obtained by UV-Vis spectroscopy, Scanning Electron microscopy (SEM), Fourier Transform Infra-Red (FT- IR) spectroscopy characterization methods respectively. The reducing and stabilizing property of *C.roseus* Ag nanoparticles were studied and it was found that they have anti-oxidant activity. The anti-inflammatory property was confirmed by protein denaturation assay. The synthesized silver nanoparticles showed a considerable anti-diabetic activity by inhibiting the carbohydrate metabolizing enzyme α-glucosidase.

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