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DETERMINATION OF MEMBRANE STABILIZING AND ANTIOXIDANT EFFECTS OF SILVER NANOPARTICLES OF LEAVES EXTRACT OF *Crataeva nurvala*

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ABSTRACT

Plant-based synthesis of nanoparticles has gained high consideration due to its use in various sectors especially in pharmaceuticals. *Crataeva nurvala* is a moderate-sized deciduous tree of the Capparidaceae family cultivated throughout India. The present study explores membrane-stabilizing and antioxidant activities of Silver nanoparticles and ethanol extract of leaves of *Crataeva nurvala.* Plant-mediated synthesis of Silver nanoparticles (AgNPS) was done from ethanol extract of leaves and analyzed by Visual Change in Colour of the solution, UV Spectra, and SEM (Scanning Electron Microscope). The ethanol extract was analyzed for the presence of phytoconstituents. The hypotonicsolution-inducedhemolysis method was used to find out erythrocyte membrane stabilizing activity. Antioxidant activity was determined by the DPPH method. Ethanol extract showed the presence of all tested phytoconstituents. Ethanol extract and Silver nanoparticles showed the highest erythrocyte membrane-stabilizing and antioxidant effects at concentrations of 2000 and 1000 µg/ml respectively.

Keywords: *Crataeva nurvala*, AgNPs, Antioxidant, Membrane Stabilizing, Hypotonic solution.

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INTRODUCTION

Nanotechnology has great scientific interest for new drug design and development, and synthesis of various medicinal agents as their vast application in the area of medicinal and chemical sciences and being used throughout the globe.[1] Nanoparticles are synthesized from various metals that are diverse in chemical properties from their parent individual molecule or metals.[2] Various methods are used for the biosynthesis of nanoparticles which are more expensive and dangerous to nature. So there is a demand to synthesize nanoparticles that are non-toxic, safer, ecosystem-friendly, and easy to synthesize. Among various metals, silver metals are utilized by the entire world for the synthesis of nanoparticles. Silver nanoparticles (AgNPs) are biosynthesized by using various parts of the plant.[3]Phytoconstituents are present in whole plants and these phytoconstituents formAg+ ions into their nanoparticles by reducing them.[4]Various parts of plant are used to prepare plant extracts which are utilized for the biosynthesis of AgNPs for their application in numerous sectors especially in Pharmaceuticals.[5]*Crataeva nurvala.* Isfound throughout India and tropical regions of the world. *Crataeva nurvala* has been applied as folkloric medicine for the treatment of memory loss, breathing problems, fever, weak immune system, and blood purifier. The plant has reported many phytoconstituents like Betulinic acid, Catechin, Kaempferol, Rutin, Beta-sitosterol, Lupeol, etc. Various parts of the plant have been explored for their medicinal properties like Anti-inflammatory, Wound healing, Cardioprotective, Anti-pyretic, Anti-urolithic, Anxiolytic activities, Anti-diabetic, and Anti-cancer activities.[6] In the present study, an attempt has been made to synthesize AgNPs from ethanol extract of leaves of *Crataeva nurvala* and determination of membrane stabilizing and antioxidant activities.

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EXPERIMENTAL

Plant Samples

Leaves of *Crataeva nurvala* were collected from near Forest Research Institute, Dehradun, and were identified and authenticated by Dr. Vidit Tyagi, Botanist, Department of Botany, DIBNS, Dehradun.

Extraction of Leaves of *Crataeva Nurvala*

To remove other undesirable material, leaves were washed with water and dried under shade. The air-dried leaves (200 gm) of *Crataeva nurvala*were crushed. The crushed leaves were subjected to extraction by hot percolation with ethanol. The solvent was evaporated under reduced pressure till dryness to obtain residue.[7]

Phytochemical Analysis

The ethanol extract was investigated for the presence of phytochemicals such as carbohydrates, Steroids, alkaloids, saponin, protein, amino acids, and phenolics.[7]

Biosynthesis of Silver Nanoparticles (AgNPs)

Ethanol extract of *Crataeva nurvala*was used for the biosynthesis of AgNPs. Silver nitrate ($AgNO_3$) of 1mM aq.The Solution was made. 500 mg of ethanol extract was taken and dissolved in a minimum amount of ethanol and diluted up to 100 ml with water. 10ml of silver nitrate solution was mixed with 60 ml ethanol extract solution and stirred for 2 hrs. And then kept at room temperature to react.[8]

Characterization

The biosynthesized AgNPs were authenticated by an Ultraviolet Spectrophotometer to find absorption maximum from 200–700 nm in a Thermo Scientific UV spectrophotometer. Further, synthesized AgNPs were analysed by Scanning Electron Microscope (SEM), and images of AgNPs were examined using SEM (JEOL, Model JFC-1600).

Membrane Stabilizing and Antioxidant Activities of Ethanol Extract and AgNPs

Ethanol extract and AgNPs were subjected to evaluation of membrane stabilizing and antioxidant effects.

Membrane Stabilizing Activity

The membrane stabilizing activity was studied on goat erythrocytes (whole blood) by the method of Shinde *et. al.*[9-12]

Preparation of Erythrocyte Suspension

Goat whole blood was mixed with NIH-ACD solution to prevent clotting. The 0.9 % saline was centrifuged with whole blood and prepared as 40 % (v/v) suspension with an isotonic buffer solution of pH 7.4. Isotonic buffer solution of pH 7.4was prepared by weighing Sodium dihydrogen phosphate 0.26 g, Disodiumhydrogen phosphate 1.15 g, and sodium chloride 9 g (10 mM sodium phosphate buffer) and dissolved in 100 ml distilled water.

Hypotonic Solution-Induced Haemolysis

The experiment was carried out with a hypotonic solution. 5 ml of the hypotonic solution containing test samples (ethanol extract and AgNPs) at concentrations of 1000 and 2000 µg/ml was mixed with 30 µl of Stock erythrocyte suspension while control was taken without drug and incubated for 10 min at room temperature after that it was centrifuged for 10 mins at 3000 g. The supernatant was taken for absorbance at 560 nm and compared with acetyl-salicylic acid as the reference standard.

Calculation

Inhibition of hemolysis was determined by the following formula:

$$\% \text{ inhibition of haemolysis} = \frac{\text{Abs of control - Abs. of Test}}{\text{Abs of control}} \text{ X } 100$$

Antioxidant Activity

DPPH Radical Scavenging Method

The free radical scavenging ability of ethanol extract and AgNPs was tested by DPPH radical scavenging assay as described by Molyneux P.[13] and Williams W.B.*et.al.*[14]250, 500, and 1000 µg/ml of ethanol

extract and AgNPs were prepared. Ascorbic acid was taken as the reference standard.100 µg/ml solution of ascorbic acid was prepared. The DPPH Solution was prepared by weighing 20 mg of DPPH and dissolved in 100 ml of methanol. 1 ml of DPPH and 3 ml of each concentration of control (drug-free), test, and standard samples were mixed and incubated for 30 minutes then absorbance was taken at 517 nm in a UV spectrophotometer. Antioxidant activity was determined by the following formula:

$$\% \text{ antioxidant activity} = \frac{\text{Abs. of Control - Abs. of Test}}{\text{Abs. of Control}} \text{ X } 100$$

RESULTS AND DISCUSSION

Phytochemical analysis results showed that carbohydrates, alkaloids, phenolics, protein and amino acids, saponins, steroids, and terpenoids were present in ethanol extract.

Synthesis of AgNPs and Observation of Colour Change

Biosynthesis of AgNPs of ethanol extract was done which was identified by solution color changed from yellow to brown.This results from the Ag+ ion being reduced to AgNP form in an aqueous solution.[15]The presence of some phytochemicals like carbohydrates, alkaloids, Phenolics, saponins, steroids, etc acts as a reducing agent Fig.-1.[16-17]

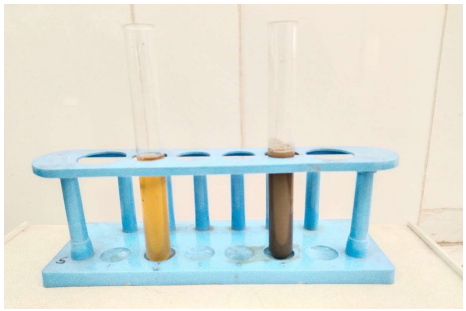

Fig.-1: Change in Color of the Solution after Synthesis of AgNPs

UV-Visible Spectral Analysis

The synthesized AgNPs were analyzed by UV-visible spectrophotometer and scanned peaks appeared at 450-500 nm.[18-19]Nanoparticles exhibit optical properties due to characteristics of surface plasmon resonance which depends on the shape and size of particles and their refractive index. The reduced AgNPs resulted in surface plasmon vibrations that were analyzed by UV spectrum which indicates the synthesis of AgNPs (Fig.-2).[20]

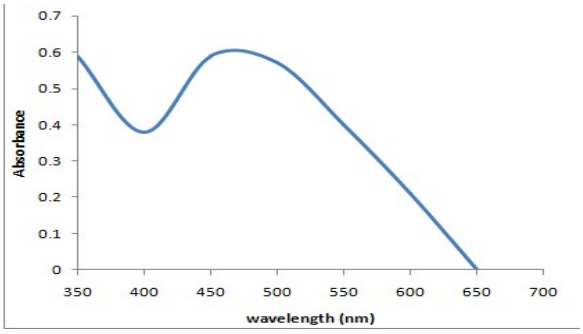

Fig.-2: UV- Spectra of AgNPs

SEM Analysis

The synthesized AgNPs were analyzed by Scanning Electron Microscope **(**SEM). It showed high-density AgNPs. SEM study showed that the diameter of synthesized AgNPs ranged from 5 nm to 100 nm. The SEM results indicated the interaction between phytoconstituents bound to AgNPs. SEM images of AgNPS exhibit morphology of the surface of the nanoparticle. It indicated that AgNPs were rough in shape and consistently distributed and agglomeration was found in AgNPs as shown in Fig.-3 and 4.

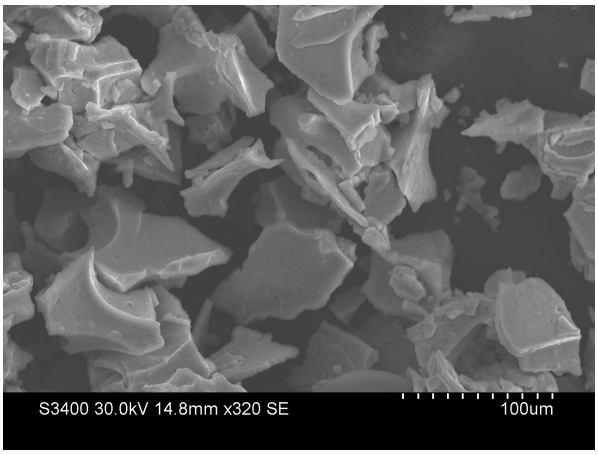

Fig.-3: SEM Analysis of Synthesized AgNPs

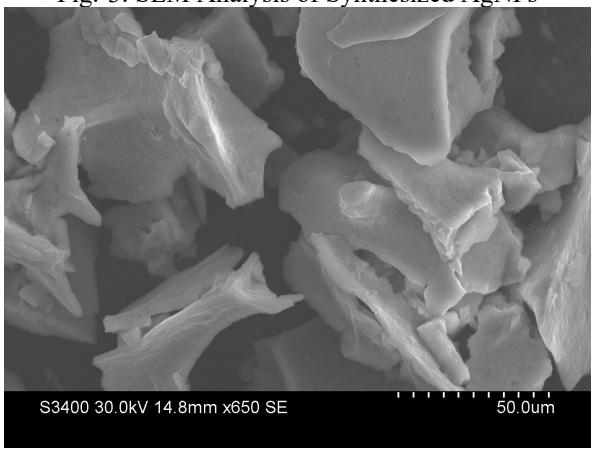

Fig.-4: SEM Analysis of Synthesized AgNPs

Membrane Stabilizing Activity

The membrane stabilizing activity of ethanol extract and AgNPS of *Crataeva nurvala*were compared with Aspirin. The results indicate that the concentration at 2000 µg/ml of ethanol extract showed significant membrane stabilization activity of 75.10±1.96% and AgNPs showed 84.37±1.53% activity at a concentration of 2000 µg/ml. Aspirin showed a 62.26±1.95% effect at a concentration of 200 µg/ml(Table- 1 and Fig.-5).

Table-1: Membrane Stabilizing Activity of *Crataeva nurvala*

Concentration (µg/ml)	% Membrane Stabilizing activity			
	Ethanol extract	AgNPs	Standard Drug	
			Acetyl Salicylic acid	Concentration (µg/ml)
1000	66.55±1.82	80.19±1.47	62.26±1.95	200
2000	75.10±1.96	84.37±1.53		

Results are expressed as mean values ± standard error (n = 3)

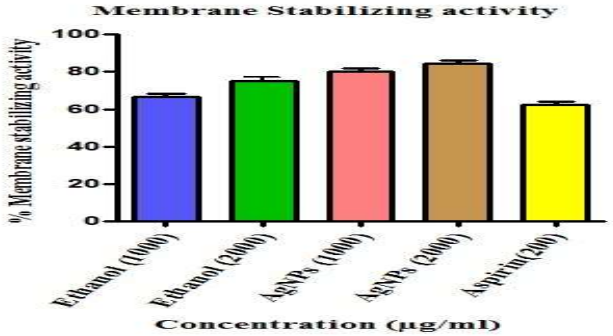

Fig.-5: Results of Membrane Stabilizing Activity of Ethanol Extract and AgNPs

Antioxidant Activity

Antioxidant activity of ethanol extract of leaves of *Crataeva nurvala*and AgNPs were compared with standard drug Ascorbic acid. Ethanol extract showed 77.98±1.22% antioxidant activity and AgNPs Showed 84.52±1.44 % antioxidant activity at a concentration of 1000 µg/ml. Standard drug Ascorbic acid showed 93.47±1.62% antioxidant activity at a concentration of 100 µg/ml (Table-2 and Fig.-6).

Table-2: Antioxidant Activity of *Crataeva nurvala*

Concentration (µg/ml)	% Antioxidant activity			
	Ethanol extract	AgNPs	Standard Drug	
			Ascorbic Acid	Concentration (µg/ml)
250	40.93±0.94	58.44±0.56	93.47±1.62	100
500	56.44±1.53	60.93±1.98		
1000	77.98±1.22	84.52±1.44		

Results are expressed as mean values ± standard error (n = 3)

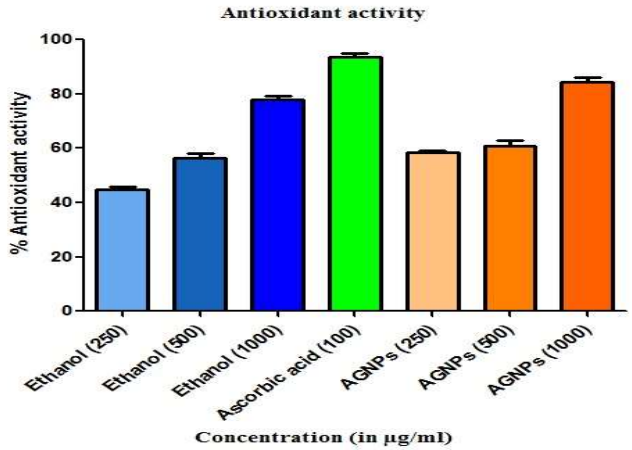

Fig.-6: % Antioxidant Activity of Ethanol Extract, AgNPs and Ascorbic Acid

CONCLUSION

We have synthesized one-step biosynthesis for AgNPs using ethanol extract fromthe leaves of the *Crataeva nurvala* plant. The biosynthesis process is very simple, rapid, reproducible, and environmentally friendly. Fabrication of nanoparticles was analyzed by observation of color change of solution, UV-visible, and SEM. The ethanol extract and AGNPs showed remarkable membrane-stabilizing and antioxidant effects. These biosynthesized AGNPs can be applicable in various pharmaceutical applications such as tablets, creams, ointments, gels, coatings, and so forth.

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CONFLICT OF INTERESTS

All authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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