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RESEARCH ARTICLE

Antioxidant and Antibacterial Property of Biosynthesised Silver Nanoparticles

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ABSTRACT

Objective(s): The present work shows the green synthesis of silver nanoparticles using C. roseus extract and its antioxidant, free radicals scavenging and antibacterial activities.

Methods: The C. roseus extract synthesized silver nanoparticles (CrAgNPs) were characterized by X-ray diffractometry, Scanning Electron Microscopy, Transmission Electron Microscopy and Fourier Transform Infra Red spectroscopy. The antioxidant, hydrogen peroxide scavenging, hydroxyl radicals scavenging, superoxide scavenging and reducing power activity of CrAgNPs were determined by DPPH, hydrogen peroxide scavenging, hydroxyl radicals scavenging, superoxide scavenging and reducing power assay methods. The antibacterial activity of CrAgNPs was analyzed by Agar dilution, Minimum Inhibitory Concentration methods.

Results: The CrAgNPs were synthesized by C. roseus extract and silvernitrate. The synthesis of silver nanoparticles was confirmed by color changes and UV-visible spectrophotometer analysis. The CrAgNPs were crystalline, variable size, elemental and spherical shape. The C. roseus extract and CrAgNPs have antioxidant, hydrogen peroxide scavenging, hydroxyl radicals scavenging, superoxide scavenging and reducing power activity. The zone of inhibition and MIC value of CrAgNPs confirmed the antibacterial activity. The CrAgNPs have greater antibacterial activity than C. roseus extract against the S. Typhi and P. vulgaris. The MIC results of CrAgNPs confirmed that CrAgNPs was highly effective against the S. Typhi and P. vulgaris bacteria.

Conclusions: Phenols and flavonoids of C. roseus extract reduced the silvernitrate into silver nanoparticles. The CrAgNPs were crystalline, spherical shape, variable particles size and elemental. The C. roseus extract and CrAgNPs have antioxidant, hydrogen peroxide scavenging, hydroxyl radicals scavenging, superoxide scavenging, reducing power activity and antibacterial activity.

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INTRODUCTION

Nanotechnology is an emerging field of scientific research that combines the chemistry, physics, biology and material sciences and finds wide applications in biotechnology, pharmaceuticals and nano-medicine [1][2]. The nanoparticles have the dimensions in the range of 1-100 nm [3]. Silver nanoparticles (agnps) are synthesized by physical, chemical and biological methods [4]. Various physico-chemical processes like laser ablation, sol-gel, pyrolysis etc. may be applied for the synthesis

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of agnps [5] but they involve high cost, low yield and highly toxic chemicals etc.[6] Therefore, the biogenic synthesis via fungi, bacteria, seaweeds, microalgae or plants has rapidly gained interest [7]. The green synthesis of agnps using plant materials embraces great advantages such as rapid single-step synthesis, eco-friendliness, easy availability, cost-effectiveness, mass production and negligible toxicity or pathogenicity [8] Moreover, unlike its other biosynthetic counterparts, there is no need for the maintenance of culture and environmental conditions and no microbial pathogenicity towards plants, animals or humans [9]. Various secondary metabolites like sapogenins, flavonoids and other phytocompounds act as reducing and stabilizing agents to synthesize agnps [10] Some reports have confirmed that synthesis of agnps using plant extracts such as *C. nocturnum* [4] *Jatropha curcas* [11] *Capsicum annuum* [12] *etc. Catharanthus roseus* plant belongs to the family Apocynaceae. This plant is an evergreen herbaceous plant which grows 1m tall. The white and pink flowered varieties are widely cultivated as an herbal medicine and ornamental plant [13]. It has been used as antibacterial, antifungal, wound healing, antiplasmodium, antioxidant and antiviral activities due to presence of vincristine, vinblastine and ajmalicine [14]. Due to the above said reasons this work focuses on the green synthesis of silver nanoparticles using *Catharanthus roseus* leaves and its antioxidant, free radicals scavenging, reducing power activity and antibacterial activity.

METHODS

Preparation of C. roseus extract

C. roseus leaves were collected from botanical garden of Banaras Hindu University, Varanasi India. *C. roseus* was dried at 40-45 ^{0}C in the oven for 7 days. Then *C. roseus* was powdered using the grinder. 2% extract was prepared by boiling 2 gram powder in 200 ml flasks containing 100 ml distilled water for 5 minutes. Then extract was filtered using Whatman No.1 filter paper and filtrates were stored in the refrigerator [15].

Green synthesis of Silver nanoparticles

10 ml *C. roseus* extract (2%) was mixed with 90 ml $AgNO_3$ (1mM) in 250 ml flask and observe the color of the solution. The color of solution was changed from light yellow to dark brown color. This color changes indicated the conversion of silvernitrate ($AgNO_3$) into silver nanoparticles (CrAgNPs). Then 2 ml of this solution was taken and absorbance was recorded at 200-600 nm using UV-visible spectrophotometer (Systronics; AU-2701). Then solution was centrifuged at 5,000 rpm for 15 minutes and silver pellet was collected. Further pellet was washed 3 times using 5 ml deionized water and centrifuged for 15 minutes. Then purified pellet was dried in hot air oven (80 ^{o}C) for 5 hours [4].

Characterization of green synthesized silver nanoparticles

CrAgNPs were characterized by X-ray diffractometry, Transmission Electron Microscopy, Scanning Electron Microscopy and Fourier Transform Infrared spectroscopy. The powder XRD (Bruker Advanced D8, Eco) technique was performed for phase identification of CrAgNPs. TEM (JEOL JEM 200 CX) was used to check the morphology of CrAgNPs. SEM (JEOL-MODEL 6390) was performed for size determination of CrAgNPs. FTIR (Varian Excalibur 3000, Palo Alto, CA) was performed to check the functional groups that was present in the *C. roseus* extract an on the surface of CrAgNPs.

Analysis of Antioxidant Activity of C. roseus and CrAgNPs

2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) Method

Antioxidant activity of CrAgNPs and *C. roseus* extract was analyzed by Keshari et al; 2018 method. 4 ml DPPH (0.004%; methanol) solution was mixed with 1ml *C. roseus* extract (10-100 µg/ml; ethanol) and kept at room temperature for 30 minutes in the dark. After incubation absorbance was recorded at 517nm using UV-Visible spectrophotometer (Systronics-AU 2700). Same procedure was followed for the preparation of CrAgNPs and Vitamin C [4]. Then percentage of antioxidant activity was calculated using the formula no 1:

Antioxidant Activity (%) = {OD(control)-OD(sample)}/(OD(control)}×100 (Formula no.1)

Note: OD (control); optical density of control, and OD (sample); optical density of the sample.

Analysis of Free Radicals Scavenging Activity of C. roseus extract and CrAgNPs

Analysis of Hydroxyl Radical Scavenging Activity

Hydroxyl radical scavenging activity (HRSA) of *C. roseus* extract, CrAgNPs and vitamin C were performed according to Keshari et al 2018. 0.075ml *C. roseus* extract (50-250µg/ml in methanol),

0.45ml sodium phosphate buffer (200mM, pH; 7), 0.150ml H_2O_2 (10mM), 0.150ml 2-deoxyribose (10mM), 0.150ml $FeSO_4$-EDTA (10mM) and 0.525ml deionized water were mixed and incubated at 37°C for 4hours. Then reaction was stopped by the addition of 0.750ml trichloroacetic acid (2.8%) and 0.750ml Thiobarbituric acid (TBA) (1% TBA in 50mM NaOH solution). Then placed in boiling waterbath for 10 minutes and absorbance was recorded at 520nm using UV-Vis spectrophotometer [4]. This procedure also followed for the preparation of CrAgNPs and vitamin C [15]. Then percentage of hydroxyl radical scavenging activity was calculated as;

HRSA (%) = (OD(control)-OD(sample))/ (OD(control))×100 (Formula no.2)

OD (control) = optical density of control, OD(sample) =optical density of sample

Analysis of Superoxide Scavenging Activity

Keshari et al; 2018 method was performed for the analysis of superoxide scavenging activity (SSA) of *C. roseus* extract, CrAgNPs and Vitamin C. 1000µl NBT (50µM), 200µl *C. roseus* extract (100-500µg/ml in methanol), 1000µl NADH (78µM), 1000µl Tris-HCl buffer (16mM, pH=8), and 1000µl PMS (10µM) solution were mixed. Then solution was kept at 25°C for 5 minutes than absorbance was recorded at 560nm using a UV-Vis spectrophotometer (Systronics, AU-2701) [4]. This procedure also followed for the preparation of CAgNPs and vitamin C. Then percentage of superoxide scavenging activity was calculated as:

SSA(%)=(OD(control)-OD(sample))/ (OD(control))×100 formula no 3.

OD(control): optical density of control, OD(sample): optical density of sample

Analysis of Hydrogen Peroxide Scavenging Activity

Hydrogen peroxide scavenging activity (HPSA) of *C. roseus* extract, CrAgNPs and Vitamin C were performed according to Keshari et al; method. 0.1ml *C. roseus* (25-250µg/ml in 50mM phosphate buffer, pH=7.4) was mixed with 0.3ml phosphate buffer (50mM, pH=7.4) and 0.6µl H_2O_2 solution (2mM in 50mM phosphate buffer). Then solution was kept at 10 minutes and absorbance was recorded at 230nm [4]. This procedure was followed for the CrAgNPs and vitamin C. Then percentage of hydrogen peroxide scavenging activity was calculated as:

HPSA (%) = (OD(control)OD(sample))/ (OD(control))×100 formula no 4.

OD (control) = optical density of control, OD(sample) = optical density of sample

Analysis of Reducing Power Assay

Keshari et al; 2018 method was performed for the determination of reducing activity of *C. roseus* extract, CrAgNPs and Vitamin C. 1000µl *C. roseus* extract (100-500µg/ml) was mixed with 2500µl phosphate buffer (0.2M, pH=6.6) and 2.5ml $K_3Fe(CN)_6$ (1%). Then solution was placed at 50°C for 20minutes. Then 2500µl trichloroacetic acid (10%) was added and centrifuged at 3,000 rpm for 10 minutes. Further 2500µl of this solution was mixed with 500µl $FeCl_3$ and 2500µl deionized water and absorbance was recorded at 700nm. The reducing power activity of sample was calculated as vitamin C equivalent per 100gm of dry sample [4]. This procedure also followed for the preparation of CrAgNPs and vitamin C. The reducing power activity increases by increasing the absorbance of sample.

Antibacterial Assay

Analysis of antibacterial activity

Antibacterial activity of *C. roseus* extract and CrAgNPs was determined by Keshari et al; 2018 method. 10 µl *C. roseus* extract and CrAgNPs was dropped on the agar plates that were swabbed by S. Typhi and *P. vulgaris* bacteria. Then plates were placed in the incubator at 37 ^{0}C for 24 hours. Then inhibition zone was measured using ordinary scale [4].

Analysis of Minimum Inhibitory Concentration

Minimum inhibitory concentration value of *C. roseus* extract and CrAgNPs was determined using Keshari et al; 2018 and 2020 method. 50 µl McFarland's (0.5) bacterial suspensions and 50 µl Luria Bertini (LB) media was poured in microtiter plates. Then 50 µl *C. roseus* extract and CrAgNPs (0.5, 1, 2, 4, 8, 16, 32, 64, 128, 256, 512 and 1024 µg/ml) was used against the S. Typhi and *P. vulgaris* bacteria. Further bacterial strains were incubated with *C. roseus* extract and CrAgNPs by two folds serial dilution and placed at 37 ^{0}C for 24 hours [4,17].

RESULTS

Green synthesis of silver nanoparticles

10 ml *C. roseus* extract (2%) was added to 90 ml $AgNO_3$ (1mM), this extract responsible for the formation of CrAgNPs. The synthesis of CrAgNPs

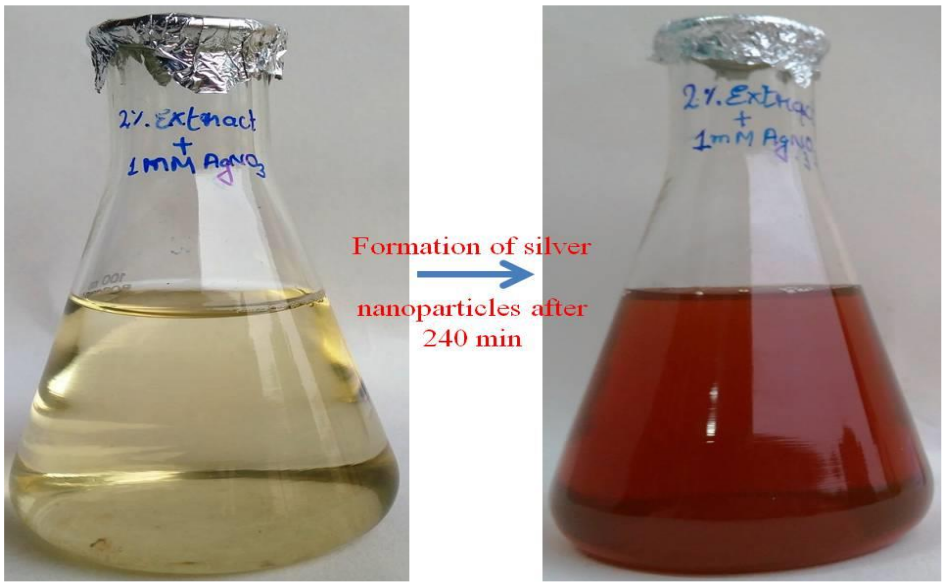

Fig. 1. The image represents the visible synthesis of silver nanoparticles

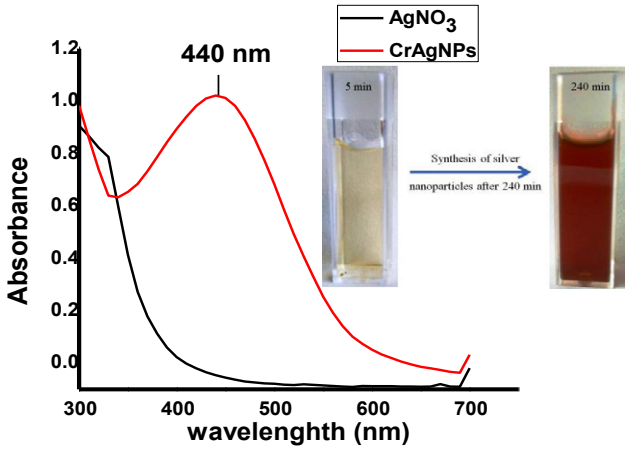

Fig. 2. The image shows the formation of silver nanoparticles

was started after 5 minutes of the extract addition which was observed visually by the development of reddish brown color (**Fig. 1**). The spectral analysis confirmed that synthesis of CrAgNPs occurs due to the presence of peak at 440nm. The synthesis of CrAgNPs was completed after 240 minutes of reaction that was confirmed spectrophotometer (**Fig. 2**).

X-Ray diffractometry

The XRD peaks of CrAgNPs at 2θ values of 38.29, 44.55, 64.81, and 77.43 represent the planes of silver at 111, 200, 220 and 311 respectively. These planes are matched by standard powder diffraction card of JCPDS, silver file No. 04-0783. The XRD results confirmed the formation of crystalline CrAgNPs which was matched by silver planes **(Fig. 3)**.

Transmission Electron Microscopy

TEM results confirmed the synthesis of spherical shaped CrAgNPs **(Fig. 4A)**. The diffraction pattern of CrAgNPs was confirmed the synthesis of metallic silver nanoparticles. The clear diffraction rings (111, 200, 220, 311 and 222) proved that CrAgNPs was polycrystalline **(Fig. 4B)**. The histogram of CrAgNPs confirmed that presence of 20 nm size particles was maximum in number

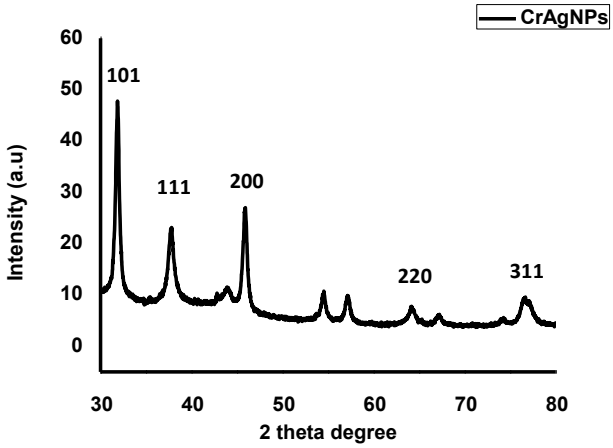

Fig. 3. The image represents the synthesis of crystalline silver nanoparticles

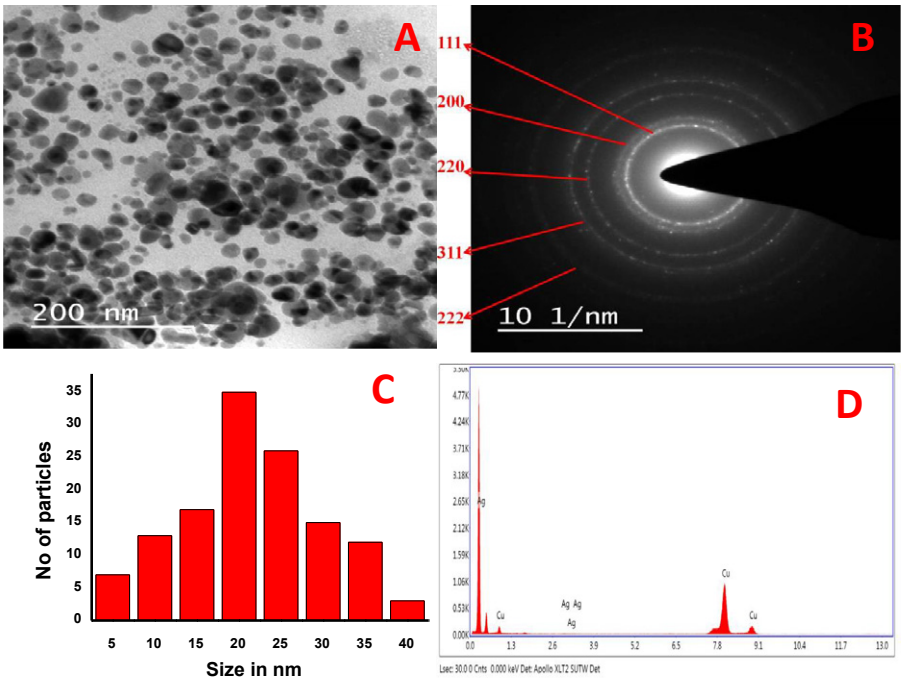

Fig. 4. The images represent the spherical (A), polycrystalline (B), variable size (C) and elemental (D) silver nanoparticles

but *C. roseus* extract synthesizes variable size CRAgNPs **(Fig. 4C)**. EDAX result confirmed the formation of elemental silver which was confirmed by intense peak at 3 KeV. This result determined the major constituents were elemental silver with small amount of copper and oxygen signals **(Fig. 4D)**.

Scanning Electron Microscopy

SEM results confirmed the formation of spherical and variable size CrAgNPs (**Fig. 5**).

Fourier Transform Infrared

FTIR results of *C. roseus* extract and CrAgNPs clearly indicated the absorption peaks location on 3414, 2924, 2846, 1747, 1635, 1488, 1018 and 605 cm^{-1}. The band at 3414 cm-1 indicated the formation of OH stretch that corresponds to alcohol and phenols. Band at 2924 and 2846 cm-1 indicated the formation of medium C-H stretch for alkane. Band at 1747 cm-1 indicated the formation of strong C=O stretch for carbonyl

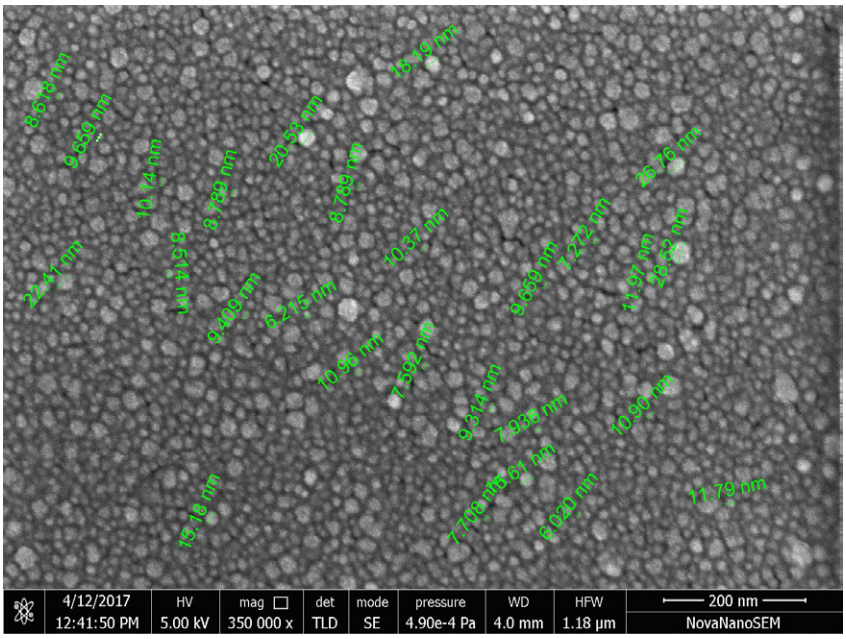

Fig. 5. The image shows the spherical and variable size silver nanoparticles

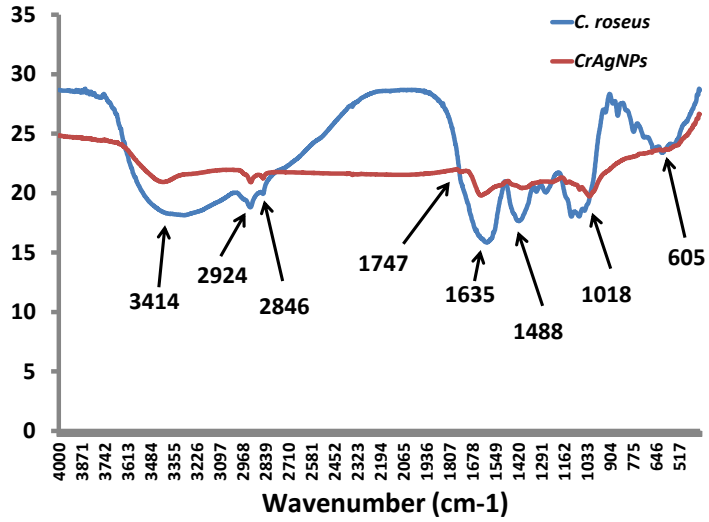

Fig. 6. The image represents the presence of various functional groups in the *C. roseus* extract and on the CrAgNPs

group. Band at 1635 cm-1 represented the N-H bend for primary amines. Band at 1488 cm-1 represented that medium C-C stretch for aromatics. Band at 1018 cm-1 represented the medium C-N stretch for aliphatic amines. Band at 609 cm-1 represented that strong and broad $-C\equiv C-H{:}C-H$ stretch for alkynes. FTIR results confirmed that hydroxyl (–OH), carboxyl (–C=O), and amine (N-H) groups of *C. roseus* extract are mainly involved in reduction of Ag+ ions to Ag^0 nanoparticles. Protein present in *C. roseus* extract acts as stabilizing agents that prevents agglomeration. The carbonyl group of amino acid residues is responsible for a layer covering on CrAgNPs and acting as a stabilizing agent to prevent agglomeration in the aqueous medium [16]. All these observations confirmed the presence of phenols, tannins, steroids, alkaloids and proteins etc. in the *Catharanthus roseus* extract. All the functional groups present on the surface AgNPs that provides its stability and compressed into nano size (**Fig. 6**).

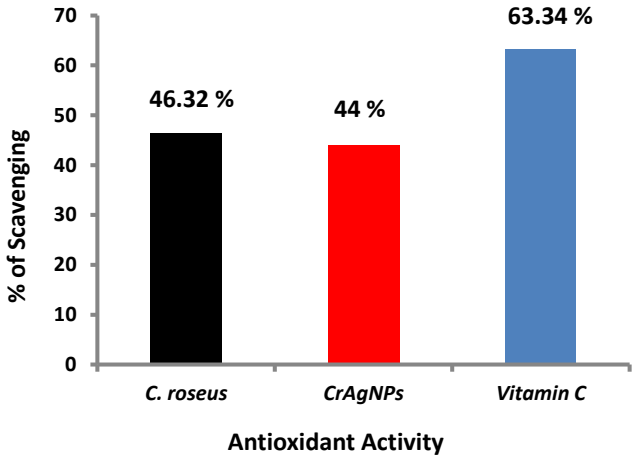

Fig. 7. The image shows the antioxidant activity of *C. roseus*, CrAgNPs and Vitamin C

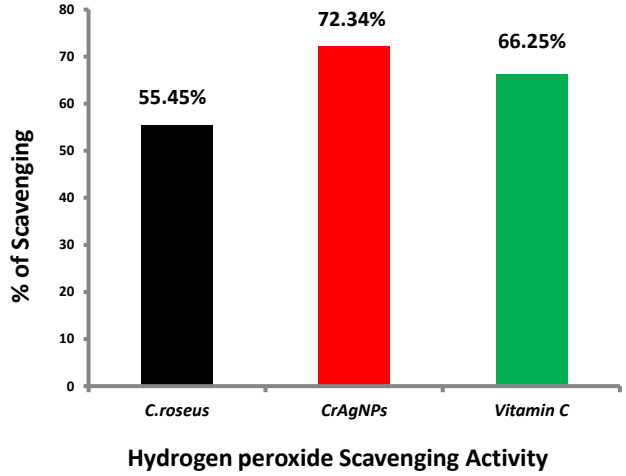

Fig. 8. The image shows the hydrogen peroxide scavenging activity of *C. roseus* extract, CrAgNPs and Vitamin C

Determination of Antioxidant Activity of C. roseus extract and CrAgNPs

2, 2-Diphenyl-1–picrylhydrazyl (DPPH) method

The antioxidant activity of *C. roseus* extract, CrAgNPs and Vitamin C were determined by DPPH method. The results confirmed that the *C. roseus* extract, CrAgNPs and Vitamin C have 46.32%, 44% and 63.34% antioxidant activity (Fig. 7).

Determination of Hydrogen Peroxide Scavenging Activity

Hydrogen peroxide scavenging activity of *C. roseus* extract, CrAgNPs and Vitamin C results confirmed that *C. roseus* extract, CrAgNPs and Vitamin C have 55.45%, 72.34% and 66.25% hydrogen peroxide scavenging activity (Fig. 8).

Determination of Hydroxyl radicals scavenging activity

The hydroxyl radicals scavenging activity of *C. roseus* extract, CrAgNPs and Vitamin C results confirmed that *C. roseus* extract, CrAgNPs and Vitamin C have 67.45%, 73.20% and 70.25% hydroxyl radicals scavenging activity (Fig. 9).

Determination of Superoxide Scavenging Activity

The superoxide scavenging activity of *C. roseus* extract, CrAgNPs and Vitamin C results confirmed that *C. roseus* extract, CrAgNPs and Vitamin C have 41.45%, 70.42% and 76.25% superoxide scavenging activity (Fig. 10).

Determination of Reducing Power Assay

The reducing activity of *C. roseus* extract,

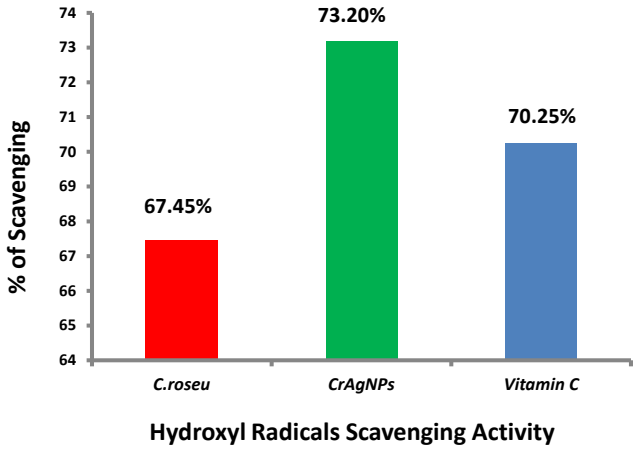

Fig. 9. The image represents the hydroxyl radicals scavenging activity of *C. roseus* extract, CrAgNPs and Vitamin C

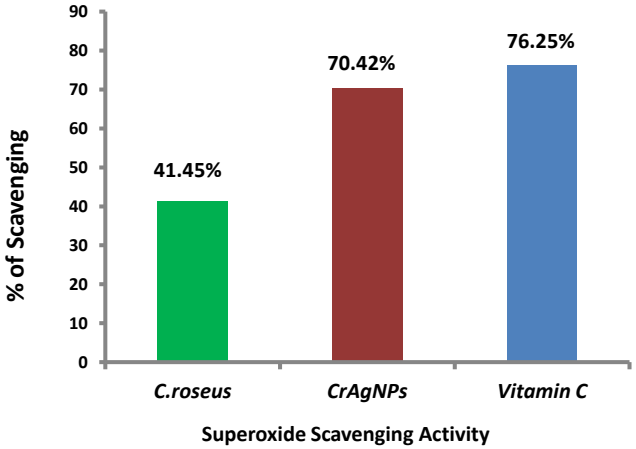

Fig. 10. The image shows the superoxide scavenging activity *C. roseus* extract, CrAgNPs and Vitamin C

CrAgNPs and Vitamin C were evaluated by reducing power assay method. The results confirmed that the *C. roseus* extract, CrAgNPs and Vitamin C have 0.04, 0.23 and 0.06 absorbance. As we know that greater the absorbance higher will be the reducing power activity. The CrAgNPs has greater reducing power activity than *C.roseus* extract and vitamin C (Fig. 11).

Determination of Antibacterial Activity

The antibacterial activity of *C. roseus* and CrAgNPs was analyzed against the S. Typhi and *P. vulgaris* using agar dilution method. The results confirmed that antibacterial activity was present in *C. roseus* (1) and CrAgNPs (2) while no antibacterial activity was recorded in the deionized water (3) against the S. Typhi and *P. vulgaris* bacteria. The CrAgNPs have greater antibacterial activity as compared with *C. roseus* extract which was confirmed by visible zone of inhibition on agar plates (Fig. 12).

Determination of Minimum Inhibitory Concentration

The minimum inhibitory concentration of *C. roseus* and CrAgNPs were analyzed by MIC method. The MIC result confirmed that CrAgNPs have greater MIC value as compared with *C. roseus* against the S. Typhi and *P. vulgaris* bacteria. The MIC value of *C. roseus* was 32 µg/ml against the S. Typhi and *P. vulgaris* bacteria (1) while CrAgNPs was showed 4 µg/ml (S. Typhi*)* and 8 µg/ml (*P. vulgaris)* (2). The greater MIC value of C. roseus and CrAgNPs higher will be the antibacterial activity. These results confirmed that CrAgNPs have greater antibacterial

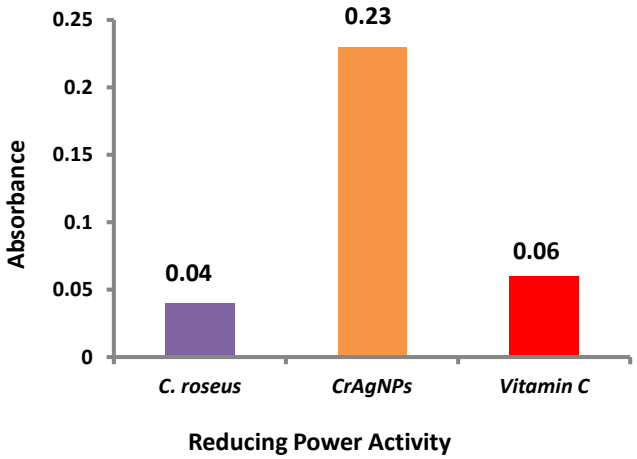

Fig. 11. The image represents the reducing power activity of *C. roseus* extract, CrAgNPs and Vitamin C

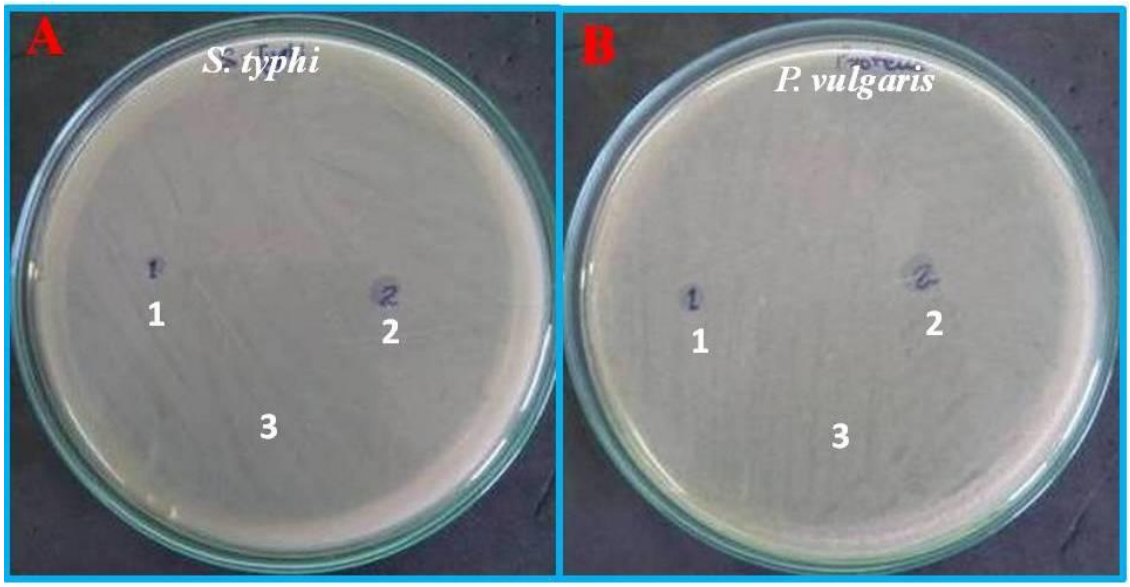

Fig. 12. The image represents the antibacterial activity of *C. roseus* (1) and CrAgNPs (2)

activity than *C. roseus* extract.

DISCUSSION

The present research work focuses on the green synthesis of silver nanoparticles using the *Catharanthus roseus* extract. Phenols and flavonoids are responsible for the reduction of silvernitrate into silver nanoparticles (Fig. 1). CrAgNPs was synthesized by the addition of plant extract in the $AgNO_3$ solution which was confirmed by the color changes of the solution (dark yellowish to brown color). CrAgNPs was crystalline, spherical, variable particles size and elemental in nature (Figs. 3-5). XRD spectrum confirmed the formation crystalline silver nanoparticles. The data analysis of TEM and SEM confirmed the synthesis of variable size (5-40 nm) and spherical shape silver nanoparticles (Figs. 4 & 5). The location of absorption peaks in FTIR clearly indicated the formation of O-H stretching corresponding to carboxylic acids, C=C stretching corresponding to alkenes and N-O stretching corresponding to nitro compounds. These functional groups are acting as reducing or capping agents (Fig. 6). *C. roseus* extract have greater antioxidant activity than CrAgNPs (Fig. 7) while CrAgNPs have greater

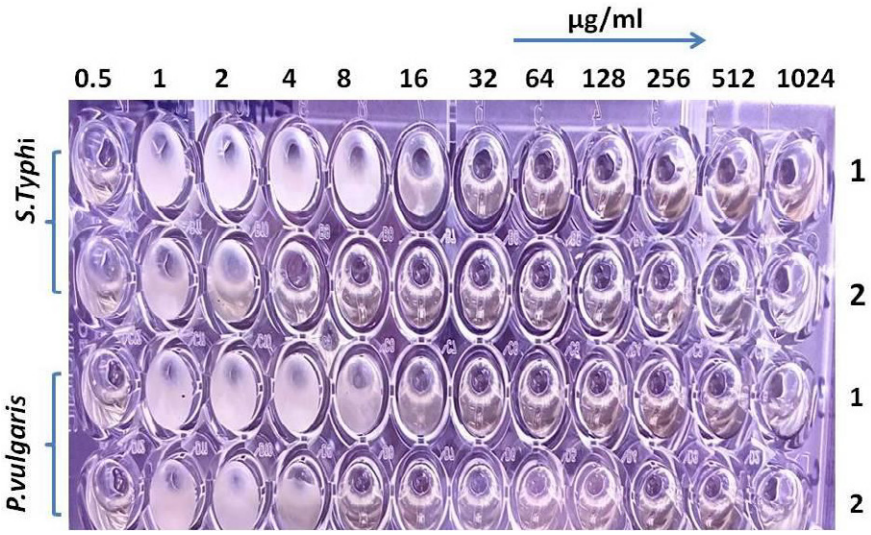

Fig. 13. The image shows the minimum inhibitory concentration of *C. roseus* and CrAgNPs

hydrogen peroxide scavenging, hydroxyl radicals scavenging and superoxide scavenging activity than *C. roseus* extract (Figs. 8-10). CrAgNPs have greater reducing power activity and antibacterial activity than *C. roseus* extract (Fig. 11).

CONCLUSION

This work describes the green synthesis of silver nanoparticles using *C. roseus* extract and silver nitrates. The green synthesis method is very simple, cheap, fast and environmental friendly. *C. roseus* extract contains phenols and flavonoids which are responsible for the reduction of silvernitrate into silver nanoparticles. Biosynthesized silver nanoparticles by *C. roseus* have greater antioxidant, hydrogen peroxide scavenging, hydroxyl radicals scavenging, superoxide scavenging and reducing power activity. Biosynthesized silver nanoparticles have greater antibacterial activity than *C. roseus* against the S. Typhi and *P. vulgaris* bacteria.

CONFLICT OF INTEREST

The authors of this have declared there is no conflict of interest.

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