# -GREEN SYNTHESIS OF GOLD NANOPARTICLES USING *CUSCUTA REFLEXA* STEM EXTRACT AND THEIR POTENTIAL ANTIBACTERIAL AND ANTICANCER ACTIVITIES

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**Abstract**

In the present study a simple and eco-friendly synthesis of AuNPs using stem extract of *C.reflexa* has been carried out. The colour changed from light yellow to purple colour which indicates the synthesis of AuNPs. The synthesised NPs showed SPR at 562 nm. SEM and TEM analysis confirmed that the particles were spherical in shape and mean particle size was 48nm. Hydrodynamic radius of NPs was estimated to be 76 nm with -31.6 mV zeta potential when the colloidal solution was analysed by DLS. FTIR spectroscopy confirmed various biomolecules such as proteins, flavonoids, and phenolics were responsible for capping and stabilizing the AuNPs. Antibacterial activity of AuNPs investigated using disc diffusion method revealed significant inhibitory activity against *E.coli* and *S.aureus*. The nanoparticles also showed potential anticancer activity against HeLa (human cervical) cell line. It is an interesting study showing the stem extract of *C.reflexa* acting as an efficient reducing agent in the formation of AuNPs with significant activity against human pathogenic bacteria and cytotoxic effect on HeLa cell line.

**Keywords:** Gold NPs, *Cuscuta reflexa*, antibacterial activity, cytotoxicity, green synthesis.

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**DOI:**- DOI: 10.48047/ecb/2023.12.si5a.084

## Introduction

Nanoscience is an emerging research field with numerous applications in pharmaceutical and biological science. Owing to its large surface area to volume ratio, NPs exhibit unique physical, chemical, and biological properties. Different methods such as physical, chemical have been used previously for NPs synthesis but they have their own limitations. Physical methods needs high energy consumption and chemical method where chemicals act as reducing agents can cause toxicity to human health and the environment. [1-5] So the only replacement to the physical and chemical method is biological method for the synthesis of metal NPs which is done by using bacteria, fungi, algae, and plants etc. [6,7] Biological method using plants is more preferable as it is nontoxic, highly stable, contain large amount of phytoconstituents, and can easily be scaled up. The bio-actives present in the plant extract act as reducing agent which reduces the metal ion into metal NPs with specific shape, size and biological activities. The mechanism behind the synthesis of metallic NPs includes the reduction of metal salt using phytoconstituents present in the plant extract, nucleation and further growth of NPs and in the end the biomolecules act as stabilizing and capping agent for the metallic NPs. [8-10]

The morphological nature of the biosynthesized metallic nanoparticles could be altered by several parameters like amount of plant extract, quantity of precursor used, pH, temperature, and reaction time. These factors should be considered and monitored while preparing metallic nanoparticles as they play a significant role in formation of NPs with distinctive properties and promising applications which can be easily scaled up. [11,12] Gold NPs have gained significant attention among different metallic NPs because of its easy fabrication, low toxicity, cost effective, high stability, optical properties and their varied application in different fields such as catalyst, drug delivery, photo thermal therapy, gene and drug delivery, optics, antimicrobial, anticancer, bio sensing and in cosmetics. The gold NPs displays the properties that are different from the bulk gold solution and thus can be synthesised into different shapes such as nanorods, nanospheres, and nanoprism. The small size, large surface area, and different shapes of gold NPs have drastically modified its chemical and biological properties and also has increased its applications in field of medicine. [13-16]

Various studies have been reported where researchers have utilised extracts of *Plumbago zeylanica, Alpinia nigra, Tribulus terrestris, Nerium oleander, Nepenthes khasiana, Zingiber officinale* for antibacterial and anticancer activities. [17-22]

*Cuscuta reflexa* is a perennial, parasitic herb of Convolulaceae family commonly known as Dodder or Akash Bel. It has been reported *C. reflexa* contains different phytochemicals such as cuscutin, kaempferol, quercetin, coumarin, stigmasterol, amarbelin, proteins, tannins, carbohydrates and possess antimicrobial, antihypertensive, anticancer, antioxidant, antiviral, antifungal, and anticonvulsant properties. [23-25] Silver and zinc oxide NPs using *C.reflexa* have already been reported but till now there is no report on gold NPs using *C.reflexa* has been published. [26, 27] So this study was aimed to synthesize gold NPs from aqueous extract of fresh stem of *C.reflexa* and evaluating their antibacterial and anticancer activities.

## Material and Methods

### Material

Fresh stems were collected from Dehradun, during the month of October 2022. Authentication was done by Dr Sunita Garg at (NISCAIR), National Institute of Science Communication and Information Resources (Ref. No NISCAIR /RHMD/CONSULT/2020/3766-67-3). All the reagents used were purchased from CDH (Central drug House) Fine Chemical, Delhi. Mueller-Hilton Agar (MHA) was obtained from HiMedia laboratories Pvt. Ltd, Mumbai. All chemicals were used as received without any further purifications.

### Preparation of extract

Stems were washed with distilled water and then by ethanol (99.8%) to avoid microbial contamination. The rinsed stems were dried in shade at room temperature for 1-2 hrs. 5 g of the stem was cut into fine pieces and put into 100 ml of distilled water in conical flask and heated at 60° C for 1 h on temperature controlled magnetic stirrer. Thereafter the cooled extract was filtered with the help of Whatman no 1 (11µm, Sigma Aldrich) filter paper. The extract was stored at 4°C and was used later for the synthesis of gold NPs.

### Biosynthesis of Gold NPs

For the synthesis of gold NPs aqueous solution of Chloroauric acid (1 mM, 50 mL) was added slowly to 25 mL of 50 mg/mL *C. reflexa* stem extract with continuous stirring on magnetic stirrer at 500 rpm at 70° C for 30 min until the color of the solution changed from light yellow to purple color which confirmed the synthesis of gold NPs. The solution was spun at 12,000 rpm for 30 min to collect gold NPs, washed with distilled water three times and collected in powder form by lyophilization.

**Characterization of nanoparticles**

Biosynthesised NPs were characterized by various analytical techniques. The UV-Vis spectrophotometer Shimadzu UV-1800 series was used to study the bioreduction of gold ion and formation of gold NPs. To determine the biological moeities used in the formation and stabilization of the nanoparticles Fourier transform infrared spectrometer Perkin Elmer Frontier FTIR-FIR Spectrophotometer (ATR) was used. Morphology, shape and size of the biosynthesise nanoparticles was determined by using Dynamic light scattering (DLS) and Zeta potential by Zetasizer nano series –Nano ZS90 (Malvern Panalytical), Scanning electron microscope (SEM) -JEOL-SM-610LA, X-ray diffractometer (Bruker, D2-Phaser), Transmission electron microscope (TEM) JEOL 2010 LaB6.

**Antibacterial activity of gold nanoparticles by disc diffusion method**

The antibacterial activity of biosynthesised gold NPs using stem of *C.reflexa* was determined by disc diffusion method (Kirby-Bauer method) using *Bacillus subtilis* (MTCC 1133) Gram positive bacteria, and *Escherichia coli* (MTCC 40) Gram negative bacteria. Mueller-Hilton Agar (MHA) was used as nutrient media. Bacterial culture strain with cell density 1 x $10^6$ cells/mL adjusted using 0.5 McFarland standards was homogeneously swabbed on the petri plates containing Mueller Hilton Agar medium. Thereafter 5mm of filter disc was placed in the petri disc containing 20 µL of AuNPs of concentration (1000 µg/mL and 2000 µg/mL) and plant extract 2000 µg/mL. Distilled water served as negative control and Gentamycin 250 µg/mL was taken as positive control. The plates were then incubated at 37° C for 24 h. The inhibition zones was measured and recorded. Antibacterial investigation was carried in triplicate analysis and the ZOI was stated as as mean ± standard deviation (SD). [28]

**MTT assay for cytotoxic potential**

The cell lines (Cervical cells HeLa) were procured from National Centre for Cell Science, Pune, India. The cytotoxic activity of prepared NPs was assayed by MTT method. Cells were seeded in triplicate at a concentration of $5x10^4$ cells/mL on 96 well plates for 24 h. Gold NPs with different concentration (10 µg/mL - 1000 µg/mL) were aded to each well. Untreated cells were considered as negative control and Doxorubicin as positive control. The medium was removed after 48 h and cells were incubated with 20 µL of MTT for 3 h at 37° C. DMSO was added to solubilize formazan crystals. The optical density was measured using a microplate spectrophotometer at a wavelength of 570 nm. [29]

Percentage cell viability was calculated using the equation shown below:

Cell viability (%) = Absorbance of test/ Absorbance of control *100

**Result and Discussion**

**UV analysis of gold NPs**

The stem extract of *Cuscuta reflexa* **(Figure. 1A)** was used for the synthesis of gold NPs **(Figure. 1B [E]).** The color of the colloidal solution changed from light yellow to purple color which showed the formation of gold NPs **(Figure 1B [G]).** UV-Vis spectroscopy further confirms the formation of NPs. The interaction of incident light with electrons of metal NPs leads to the formation of intense SPR (surface plasmon resonance) band in UV-Vis spectrum which is characteristic for every metal. SPR of AuNPs was seen at λ 562 nm in UV-Vis spectra when stem extract of *C.reflexa* was added to gold salt solution which confirmed the biosynthesis of gold NPs. [30] **(Figure 2)**

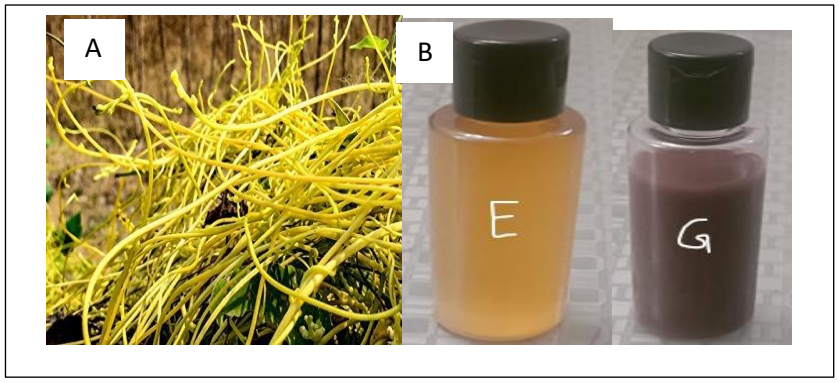



**Figure 1.** A) *Cuscuta Reflexa* stem B) E. *Cuscuta Reflexa* stem extract, G. AuNPs in solution.

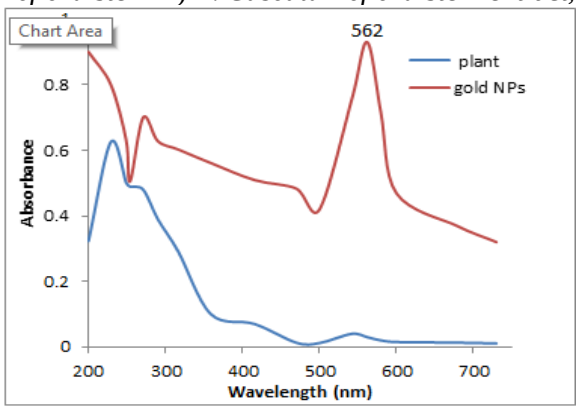
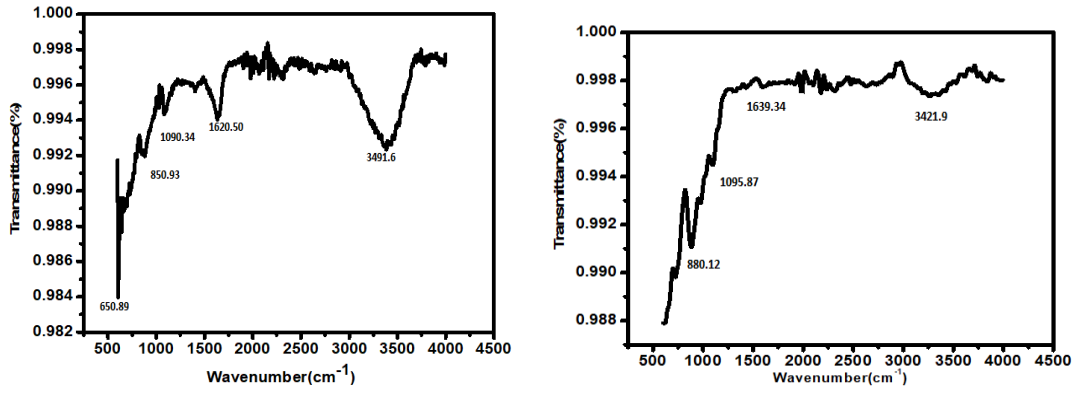



**Figure 2.** The UV-Visible spectrum of plant extract and AuNPs solution

**FTIR analysis of gold NPs**

FTIR spectral studies was carried out to determine the biomolecules that were responsible for the capping and stabilizing of gold NPs. FTIR spectra of both the stem extract and the synthesized gold NPs showed similar peaks with minute shift in the spectra.

IR spectra of *C.Reflexa* stem extract and AuNPs (**Figure 3 [A] & [B])** show a strong peak at 3491 $cm^{-1}$ and 3421 $cm^{-1}$ which corresponds to the stretching vibration of (O-H) of the phenolic group or amine functional group (N-H). The other band at 1620 $cm^{-1}$ and 1639 $cm^{-1}$ is assigned to the amide I band and -C=C- stretching vibration band. This amide I band could be due to the presence of proteins in the stem extract. Stretching vibration of ether group (C-O-C) was observed at 1090 $cm^{-1}$ and 1095 $cm^{-1}$ respectively. Likewise the band at 850 $cm^{-1}$ and 880 $cm^{-1}$ corresponds to C-H wags of alkenes in plant extract and AuNPs respectively. [31,32] From FTIR spectral study it can be estimated that the biomolecules that could be involved in the stabilization and capping of gold NPs may be phenolics, proteins or flavonoids present in the *C.reflexa* stem extract.




A B

**Figure 3.** (A) FTIR spectrum of C. Reflexa stem extract, (B) FTIR spectrum of AuNPs

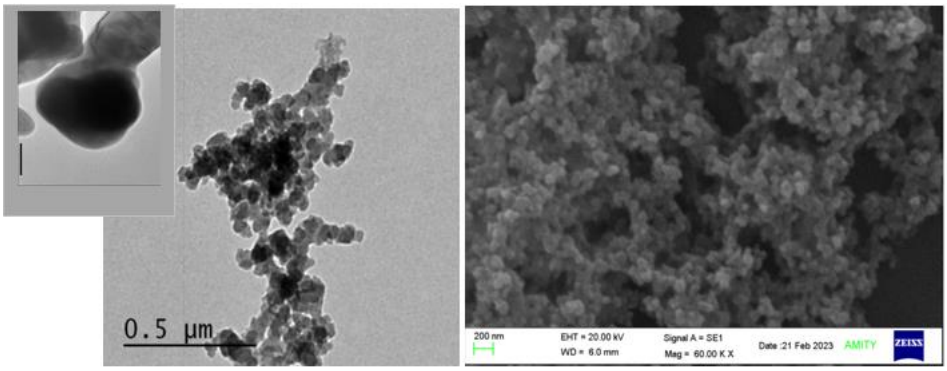



**Figure 4.** (A) TEM image of AuNPs, (B) SEM image of AuNPs

**Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) analysis**

SEM and TEM analysis are done to determine the morphology, shape and size of the synthesized NPs. Results of SEM and TEM images analysis of *C.Reflexa* extract mediated gold nanoparticles showed that the NPs were spherical in shape, polydispersed and aggregated which may be during the sample preparation **(Figure 4. [A] & [B]).** The particle size of the NPs ranges from 30-80 nm with the average particle size of 48 nm. By comparing the SEM and TEM results, it is observed that the gold NPs forms aggregates by absorbing moisture and ultrasonication can disaggregate them to form low size NPs.

**XRD diffraction study**

The XRD characterization is done to determine the crystalline nature and purity of gold NPs. The XRD diffraction pattern for gold NPs is shown in **Figure 5.** The diffraction peaks were observed at $2\theta$ values 38.2, 44.6, 64.8, 77.2 relating to (111), (200), (220), (311) crystal planes of face centered gold crystals respectively, (JCPDS 04-0784) which is in agreement with the work reported previously. [33]

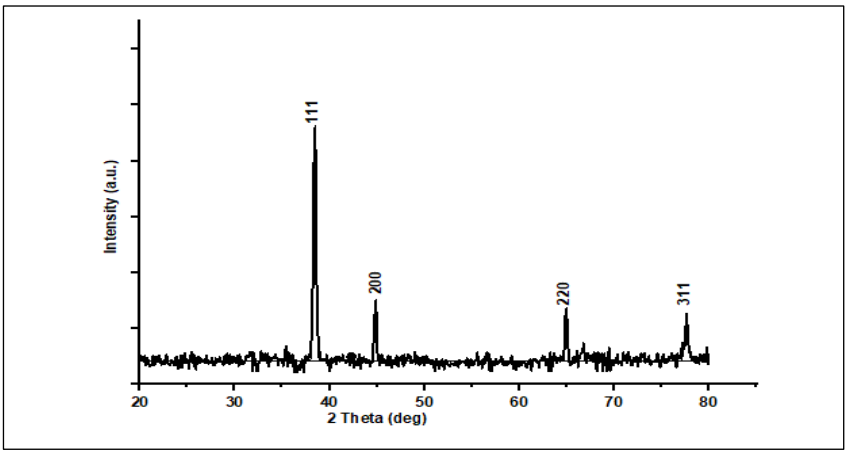
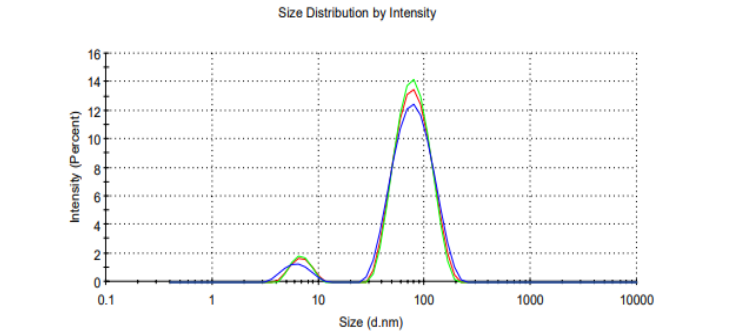



**Figure 5.** XRD spectrum of AuNPs

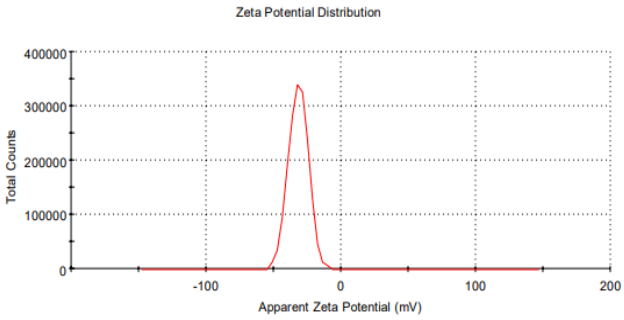
**Dynamic light scattering and Zeta Potential Analysis**

DLS is used to determine both the particle size and Zeta potential of the biosynthesised AuNPs. The results are shown in (**Figure. 6 [A] & [B]).** As seen in **Figure 6[A]** two peaks are seen at 81 nm, 7 nm with the mean particle size distribution of gold NPs is 76.46 nm. The polydispersity index is 0.311.The zeta potential of AuNPs was -31.6 mV shown in **Figure 6[B].** Zeta potential is an important parameter for stability of the colloid, higher is the value of zeta potential higher is the electrostatic repulsion between colloidal particles and greater will be the stability. The value -31.6 mV indicates that the biosynthesised NPs shows good stability. [34]




**A**

| | | Mean (mV) | Area (%) | St Dev (mV) |
|---|---|---|---|---|
| Zeta Potential (mV): -31.6 | Peak 1: | -31.6 | 100.0 | 7.26 |
| Zeta Deviation (mV): 7.26 | Peak 2: | 0.00 | 0.0 | 0.00 |
| Conductivity (mS/cm): 0.0115 | Peak 3: | 0.00 | 0.0 | 0.00 |
| Result quality : Good | | | | |




**B**

Figure 6. (A) DLS of AuNPs, (B) Zeta potential of AuNPs

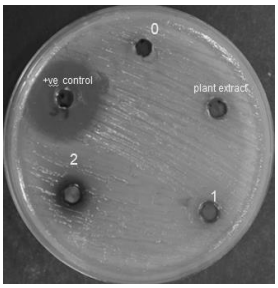
**Antibacterial Activity**

**Antibacterial Activity**

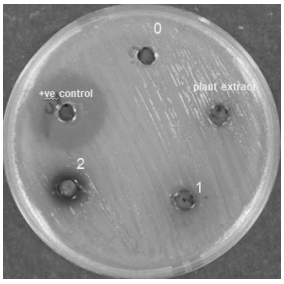
The antibacterial activity of plant extract and green AuNPs by disc diffusion method against Gram-positive bacteria *Bacillus subtilis* and gram-negative bacteria *Escherichia coli* are shown in **Table 1** and **Figure 7**. The zone of inhibition (mm) was measured and calculated after adding drugs and then incubating at room temperature for 24 h. Zone of inhibition was 15.5 mm and 15 mm for *E. coli* and *S. aureus*. Plant extract also have very less antibacterial activity against the bacterial strains. The mechanism behind antibacterial activity of AuNPs is that when these AuNPs comes in contact with the bacteria it gets attached to the surface of bacteria through electrostatic attraction. The small size enables their permeation into the bacterial cell through membrane bound proteins such as sulphur groups which are present in the protein to form thiols thus leading to DNA damage. It has also been seen that the interaction of AuNPs with cysteine residue leads to the formation of reactive oxygen species (ROS) thus leading to bacterial death. [35]

| **Compounds (µg/mL)** | **Zone of inhibition (mm) Average ±SD** | |
|---|---|---|
| | *E.coli* | *S.aureus* |
| Distilled water (0) | *0* | *0* |
| Plant extract 2000 | 6 ± 0.23 | 5 ± 0.12 |
| AuNPs 1000 | 10± 0.10 | 9 ± 0.09 |
| AuNPs 2000 | 15.5 ± 0.07 | 15 ± .06 |
| +ve control 250 | 20 ±0.08 | 22± 0.04 |

**Table 1**. Zone of inhibition (mm) for blank discs, plant extract, AuNPs and positive control against tested bacteria. Data represent mean ± SD (n=3, p< 0.05).




**A**




**B**

**Figure 7.** Zone of inhibition of AuNPs against bacterial strains A. E.coli and B. S.aureus, 0: Distilled water, Plant extract, 1: 1000 µg/mL, 2: 2000 µg/mL, Positive control (Gentamycin)

**In-Vitro cytotoxicity**

The AuNPs treated HeLa cells were analyzed through MTT assay after 24 h of adding various concentrations of 10-1000 µg/mL for AuNPs. $IC_{50}$ value for AuNPs was calculated as 54.34 µg/mL. **Table 2** The anticancer activity of the metal NPs is because of the production of reactive oxygen species which decreases the cellular ATP uptake and decreases the dehydrogenase activity which destroys the mitochondrial respiratory chain and other component of the cell and finally leads to the cell death which is clearly represented in the study that the toxicity of the AuNPs shows dose-dependent response in the tested cancer cells. (**Figure 8. [A]&[B])** shows the anticancer activity of AuNPs biosynthesised from fresh stem of *C. reflexa* against HeLa cells[.36]

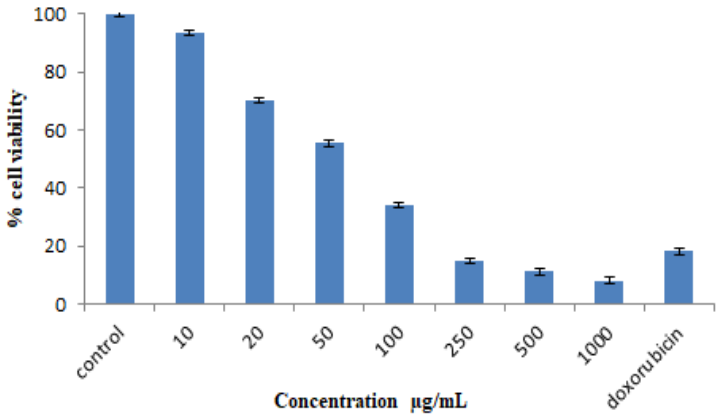
| **Concentration of AuNPs (µg/mL)** | **Absorbance @ 570nm ± SD** | **Percentage viability (Mean ± SD)** | **$IC_{50}$** |
|---|---|---|---|
| **0** | 0.1165±0.14 | 100±0.02 | 54.34 µg/mL |
| **10** | 0.109±0.08 | 93.5622318±0.01 | |
| **20** | 0.08175±0.012 | 70.1716738±0.03 | |
| **50** | 0.0645±0.15 | 55.3648069±1.23 | |
| **100** | 0.03975±0.23 | 34.1201717±0.83 | |
| **250** | 0.0175±0.02 | 15.0214592±0.25 | |
| **500** | 0.013±0.05 | 11.1587983±0.09 | |
| **1000** | 0.0095±0.008 | 8.15450644±0.12 | |

**A**

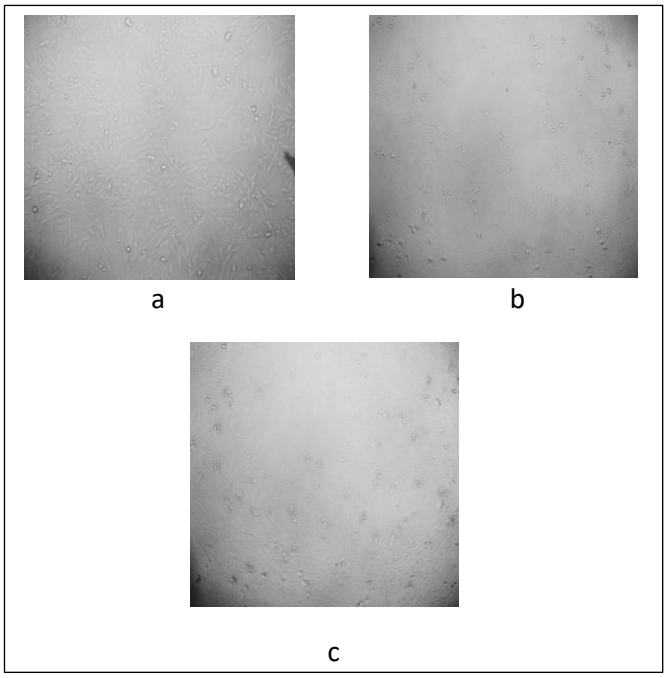
| **Concentration of Doxorubicin (µg/mL)** | **Absorbance @ 570nm(mean ± SD** | **Percentage inhibition(Mean ± SD)** | **$IC_{50}$** |
|---|---|---|---|
| **0** | 0.636±0.02 | 0.00 | 18.11 µg/mL |
| **1.560** | 0.617±0.06 | 3.03±0.02 | |
| **3.125** | 0.516±0.12 | 18.84±0.06 | |
| **6.25** | 0.481±1.2 | 24.35±0.05 | |
| **12.5** | 0.362±0.34 | 43.15±0.06 | |
| **25** | 0.231±0.51 | 63.65±0.12 | |
| **50** | 0.130±0.07 | 79.54±0.23 | |
| **100** | 0.038±0.02 | 93.99±0.54 | |

**B**

**Table 2:** Cytotoxic activity of (A) biogenic AuNPs, (B) Doxorubicin against HeLa cells




**A**




**B**

**Figure 8.** A. Anticancer activity of AuNPs against HeLa cell line. B. Images of HeLa cell line treated with control (a) Doxorubicin (b) phytogenic AuNPs (c)

## Conclusion

In this study we used stem of *C.reflexa* extract to synthesise AuNPs. The NPs were further characterised using modern analytical techniques like UV-Visible, FTIR, TEM, SEM, DLS, XRD etc. The NPs synthesised were spherical and within 100 nm range. The biosynthesised AuNPs showed bactericidal effect on gram positive and gram negative bacteria. The NPs further show promising anticancer activity against HeLa cell line. C.reflexa was found to have a potential and this plant can be exploited in the production of metal NPs on large scale.

## References

1. Azharuddin, M., Zhu, G. H., Das, D., Ozgur, E., Uzun, L., Turner, A. P., & Patra, H. K. (2019). A repertoire of biomedical applications of noble metal nanoparticles. Chemical Communica-tions, 55(49), 6964-6996.
2. Liu, F., Ma, D., Chen, W., Chen, X., Qian, Y., Zhao, Y., & Zhao, W. (2019). Gold nanoparticles suppressed proliferation, migration, and invasion in papillary thyroid carcinoma cells via downregulation of CCT3. Journal of Nanomaterials, 2019, 1-12.
3. Ge, L., Li, Q., Wang, M., Ouyang, J., Li, X., & Xing, M. M. (2014). Nanosilver particles in medical applications: synthesis, performance, and toxicity. International journal of nanomedicine, 9, 2399-2407.
4. Wiley, B., Sun, Y., Mayers, B., & Xia, Y. (2005). Shape-controlled synthesis of metal nanostructures: the case of silver. Chemistry–A European Journal, 11(2), 454-463.

5. Behera, A.; Mohapatra, S.S.; Verma, D.K. Nanomaterials: Fundamental Principle and Applications. In Nanotechnology and Nanomaterial Applications in Food, Health, and Biomedical Sciences; Apple Academic Press: Cambridge, MA, USA, 2019.
6. Boroumand Moghaddam, A.; Namvar, F.; Moniri, M.; Azizi, S.; Mohamad, R. Nanoparticles biosynthesized by fungi and yeast: A review of their preparation, properties, and medical applications.
7. Fouda, A., Eid, A. M., Abdel kareem, A., Said, H. A., El-Belely, E. F., Alkhalifah, D. H. M.,& Hassan, S. E. D. (2022). Phyco-synthesized zinc oxide nanoparticles using marine macroalgae, Ulva fasciata Delile, characterization, antibacterial activity, photocatalysis, and tanning wastewater treatment. Catalysts, 12(7), 756.
8. Rónavári, A., Igaz, N., Gopisetty, M. K., Szerencsés, B., Kovács, D., Papp, C., & Pfeiffer, I. (2018). Biosynthesized silver and gold nanoparticles are potent antimycotics against opportunistic pathogenic yeasts and dermatophytes. International journal of nanomedicine, 13, 695.
9. Al-Ansari, M. M., Al-Dahmash, N. D., & Ranjitsingh, A. J. A. (2021). Synthesis of silver nanoparticles using gum Arabic: Evaluation of its inhibitory action on Streptococcus mutans causing dental caries and endocarditis. Journal of Infection and Public Health, 14(3), 324-330.
10. Annamalai, A., Christina, V. L. P., Sudha, D., Kalpana, M., & Lakshmi, P. T. V. (2013). Green synthesis, characterization and antimicrobial activity of Au NPs using Euphorbia hirta L. leaf extract. Colloids and Surfaces B: Biointerfaces, 108, 60-65.
11. Gopinath, V., Priyadarshini, S., Loke, M. F., Arun kumar, J., Marsili, E., Mubarak Ali, D., & Vadivelu, J. (2017). Biogenic synthesis, characterization of antibacterial silver nanoparticles and its cell cytotoxicity. Arabian Journal of Chemistry, 10(8), 1107-1117.
12. Chandrasekaran, R., Yadav, S. A., & Sivaperumal, S. (2020). Phytosynthesis and characterization of copper oxide nanoparticles using the aqueous extract of Beta vulgaris L and evaluation of their antibacterial and anticancer activities. Journal of Cluster Science, 31(1), 221-230.
13. Majdalawieh, A., Kanan, M. C., El-Kadri, O., & Kanan, S. M. (2014). Recent advances in gold and silver nanoparticles: synthesis and applications. Journal of nanoscience and nanotechnology, 14(7), 4757-4780.
14. Singh, P., Kim, Y. J., Wang, C., Mathiyalagan, R., & Yang, D. C. (2016). The development of a green approach for the biosynthesis of silver and gold nanoparticles by using Panax ginseng root extract, and their biological applications. Artificial cells, nanomedicine, and biotechnology, 44(4), 1150-1157.
15. Fu, L. H., Yang, J., Zhu, J. F., & Ma, M. G. (2017). Synthesis of gold nanoparticles and their applications in drug delivery. Metal Nanoparticles in Pharma, 155-191.
16. Rónavári, A., Igaz, N., Adamecz, D. I., Szerencsés, B., Molnar, C., Kónya, Z., & Kiricsi, M. (2021). Green silver and gold nanoparticles: Biological synthesis approaches and potentials for biomedical applications. Molecules, 26(4), 844.
17. Priya Velammal, S., Devi, T. A., & Amaladhas, T. P. (2016). Antioxidant, antimicrobial and cytotoxic activities of silver and gold nanoparticles synthesized using Plumbago zeylanica bark. Journal of Nanostructure in Chemistry, 6, 247-260..
18. Baruah, D., Goswami, M., Yadav, R. N. S., Yadav, A., & Das, A. M. (2018). Biogenic synthesis of gold nanoparticles and their application in photocatalytic degradation of toxic dyes. Journal of Photochemistry and Photobiology B: Biology, 186, 51-58.
19. Gopinath, V., Priyadarshini, S., MubarakAli, D., Loke, M. F., Thajuddin, N., Alharbi, N. S., ... & Vadivelu, J. (2019). Anti-Helicobacter pylori, cytotoxicity and catalytic activity of biosynthesized gold nanoparticles: multifaceted application. Arabian journal of chemistry, 12 (1), 33-40.
20. Barai, A. C., Paul, K., Dey, A., Manna, S., Roy, S., Bag, B. G., & Mukhopadhyay, C. (2018). Green synthesis of Nerium oleander-conjugated gold nanoparticles and study of its in vitro anticancer activity on MCF-7 cell lines and catalytic activity. Nano convergence, 5(1), 1-9.
21. Bhau, B. S., Ghosh, S., Puri, S., Borah, B., Sarmah, D. K., & Khan, R. (2015). Green synthesis of gold nanoparticles from the leaf extract of Nepenthes khasiana and antimicrobial assay. Advanced Materials Letters, 6(1), 55-58.
22. Kumar, K. P., Paul, W., & Sharma, C. P. (2011). Green synthesis of gold nanoparticles with Zingiber officinale extract: characterization and blood compatibility. Process Biochemistry, 46(10), 2007-2013.

23. Patel, S., Sharma, V., Chauhan, N. S., & Dixit, V. K. (2012). An updated review on the parasitic herb of Cuscuta reflexa Roxb. Zhong xi yi jie he xue bao= Journal of Chinese integrative medicine, 10(3), 249-255.
24. Pal, D. K., Mandal, M., Senthilkumar, G. P., & Padhiari, A. (2006). Antibacterial activity of Cuscuta reflexa stem and Corchorus olitorius seed. Fitoterapia, 77(7-8), 589-591.
25. Ali, I., Suhail, M., Fazil, M., Ahmad, B., Sayeed, A., Naqshbandi, M. F., & Azam, A. (2019). Anti-cancer and Anti-oxidant Potencies of Cuscuta reflexa Roxb. Plant Extracts. Am. J. Adv. Drug Deliv, 14, 92-113.
26. Ranjan, R., Dandapat, S., Kumar, M., & Sinha, M. P. (2019). Synthesis and characterization of Cuscuta reflexa (Roxb.) aqueous extract mediated silver nanoparticles. J Anal Pharm Res, 8(2), 80-83.
27. Soni, N., & Dhiman, R. C. (2020). Larvicidal activity of Zinc oxide and titanium dioxide nanoparticles Synthesis using Cuscuta reflexa extract against malaria vector (Anopheles stephensi). Egyptian Journal of Basic and Applied Sciences, 7(1), 342-352.
28. Bharadwaj, K. K., Rabha, B., Pati, S., Choudhury, B. K., Sarkar, T., Gogoi, S. K., ... & Edinur, H. A. (2021). Green synthesis of silver nanoparticles using Diospyros malabarica fruit extract and assessments of their antimicrobial, anticancer and catalytic reduction of 4-nitrophenol (4-NP). Nano-materials, 11(8), 1999.
29. Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. Journal of pharmaceutical analysis, 6(2), 71-79.
30. Sekar, V., Al-Ansari, M. M., Narenkumar, J., Al-Humaid, L., Arunkumar, P., & Santhanam, A. (2022). Synthesis of gold nanoparticles (AuNPs) with improved anti-diabetic, antioxidant and anti-microbial activity from Physalis minima. Journal of King Saud University-Science, 34(6), 102197.
31. Osibe, D. A., & Aoyagi, H. (2019). A novel strategy for the synthesis of gold nanoparticles with Catharanthus roseus cell suspension culture. Materials Letters, 238, 317-320.
32. Nakkala, J. R., Mata, R., Gupta, A. K., & Sadras, S. R. (2014). Biological activities of green silver nanoparticles synthesized with Acorous calamus rhizome extract. European journal of medicinal chemistry, 85, 784-794.
33. Alghuthaymi, M. A., Rajkuberan, C., Santhiya, T., Krejcar, O., Kuča, K., Periakaruppan, R., & Prabukumar, S. (2021). Green Synthesis of Gold Nanoparticles Using Polianthes tuberosa L. Floral Extract. Plants, 10(11), 2370.
34. Thirumagal, N., & Jeyakumari, A. P. (2020). Structural, optical and antibacterial properties of green synthesized silver nanoparticles (AgNPs) using Justicia adhatoda L. leaf extract. Journal of Cluster Science, 31(2), 487-497.
35. Nagaraj, B., Musthafa, S. A., Muhammad, S., Munuswamy-Ramanujam, G., Chung, W. J., Alodaini, H. A.,& Ranganathan, V. (2022). Anti-microbial and anti-cancer activity of gold nanoparticles phytofabricated using clerodin enriched clerodendrum ethanolic leaf extract. Journal of King Saud University-Science, 34(4), 101989.
36. Andleeb, A., Andleeb, A., Asghar, S., Zaman, G., Tariq, M., Mehmood, A.,& Abbasi, B. H. (2021). A systematic review of biosynthesized metallic nanoparticles as a promising anti-cancer-strategy. Cancers, 13(11), 2818.