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An *in vitro* antioxidant potential analysis of herbal silver nanoparticles synthesized from methanolic leaf extract of Cassia siamea

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ABSTRACT

Aim and Objectives: Synthesis and evaluation of in vitro antioxidant potential of silver nanoparticles (AgNPs) made from methanolic leaf extract of Cassia siamea, To develop a herbal, potential, cost-effective source of antioxidative agents.

Background : Herbal matter have been a good source of nutrition and antioxidant agents from the ages, to fulfil the requirements of one by its natural phytoconstituents. Present study compiles the, green synthesis of AgNPs using the methanolic leaf extract of C. siamea. Synthesized AgNPs were characterized and used for the detection of their in vitro antioxidant potential. The activity was analysed through DPPH and FRAP assays. While the antioxidant potential of AgNPs is known, the use of C. siamea extract for this purpose is relatively underexplored, making this study a significant contribution to the field of nanotechnology and natural antioxidants. The findings highlight the potential of plant-mediated AgNPs for future applications in biomedicine, particularly as natural, eco-friendly antioxidants.

Materials and Methods : The collection and identification of the plant leaf was done in University Campus. The AgNPs were characterized through several techniques as UV-Vis spectrophotometry, FTIR, XRD, particle size and Zeta potential analysis, SEM, confirming the formation of stable AgNPs. The in vitro antioxidant potential of the synthesized AgNPs was evaluated using DPPH and FRAP assays, with ascorbic acid serving as the reference standard.

Results : By current study this can be concluded that after successful synthesis and characterization of AgNPs, DPPH Assay demonstrated that AgNPs had a concentration-dependent % scavenging action, with considerable radical inhibition at all examined levels, but they are lower than ascorbic acid. Similarly, FRAP assay demonstrated the reducing power of AgNPs, which increased with concentration. The IC_{50} values obtained from both assays indicate that synthesized AgNPs possess substantial antioxidant activity.

Conclusion : The bioactive chemicals in the leaf extract play an important role in nanoparticle formation and their stability. These findings suggest that green-synthesized AgNPs from Cassia siamea exhibit considerable antioxidant potential, making them promising candidates for applications in biomedicine and nanotechnology. This study contributes to the growing body of research on plant-mediated nanoparticle synthesis and the potential of such nanoparticles as natural antioxidants.

1. Introduction

Medicinal plants and natural products have been a rich source of medicinally active compounds. Plants include polyphenolic alkaloid compounds known as flavonoids such as resveratrol, curcumin, ginsenosides, triptolide, and others that have antioxidant activity. Antioxidant substances have antibacterial, anti-atherosclerotic, anti-tumour, antimutagenic, anti-carcinogenic and antiviral

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properties.[1] A study done by Mali et al, 2013, suggests oral administration of a 50% ethanol extract of Withania sominifera resulted in reduced fertility in male rats.[2] Several mushroom species was found useful as a source of nutrients for health benefits and having high nutritional value. It shows antioxidant, antibacterial, DNA protecting, anti-inflammatory, cytotoxic and hepatoprotective activities. Researches show that antioxidant molecules can help manage ageing and other conditions. The WHO also supports phytotherapy and promotes fundamental pharmacological validation methods, which are part of plant origin.[3]

Cassia siamea, (Fabaceae), A small to medium size tree, that is widely planted in Southeast Asia. It is often used to treat fever, skin diseases, hypertension, sleeplessness, diabetes, and asthma. This plant contains several phytochemicals, including β-sitosterol and flavonoids. The compounds responsible for pharmacological effects are glycosides, anthraquinones, bianthraquinones, alkaloids, and kaempferol.[4] A study indicates that the alcoholic extract of C. siamea flowers has substantial antioxidant activity against free radicals, prevents oxidative damages and provides significant protection in the liver.[5] Another previous study suggested by Musa et al, 2021, AgNPs of Senna siamea (C. siamea) flower extract was synthesized, characterized and their results shows great antibacterial properties.[6] Following these important activities and phytochemical potential, In vitro antioxidant activity of AgNPs of C. siamea is comprehended further in this research.

Nanotechnology has emerged as a new interdisciplinary field of study, that blends the science of materials, biotechnology and bionanosciences. Due to their unique potential in the realms of electrical, magnetic, information storage and drug delivery, metal nanoparticle production is a major area of research in current biomaterial science.[7] Nanoparticles are small particles ranging from 1-100 nm in size. As they are small in size and their high surface area to volume ratio increase and so their physical and chemical applications.[8] There has been great development in the field of nanoscience from past decades, with an array of technologies developed to synthesize nanoparticles that have precise morphological (i.e., shape and size) and distribution features. Even though there are a number of techniques for creating specific and pure nanoparticles, they are highly costly and involve the use of dangerous chemicals that are harmful to humans, the environment and biological systems. This issue was resolved by the ecologically friendly synthesis of nanoparticles employing components that are safe for the environment, such as plants, fungi, seaweed, bacteria and enzymes. It has many benefits, including being non-toxic, time and money-efficient.[9] Among many types of nanoparticles, the green synthesis of plant mediated nanoparticles has made an entirely different space, because of their wide variety of applications which includes, in biomedicine, wound healing, waste processing and food packaging. It has various advantages over other synthesis methods, since it is a simple and Straight forward strategy that uses extracts from plants, avoiding the requirement for sterile surroundings and the tedious processes required by other methods for nanoparticles synthesis.[10] The use of AgNPs for biological applications has grown due to their stabilization by plant-derived bioactive substances. Nanoparticles derived from medicinal plants have considerable antioxidant activity.[11,12] Thus, this study's objectives were to create and evaluate antioxidant capabilities of herbal AgNPs using methanolic extract of C. siamea leaf.

2. Materials and Methods

2.1. Collection of raw material

The fresh and healthy leaves of C. siamea was collectedFrom University of Rajasthan Campus and In and around Jaipur district of Rajasthan, in a fresh polythene packet. Specimen was submitted to the herbarium for verification, in, Department of Botany, at University of Rajasthan and got the voucher specimen RUBL21231 (Figure 2).

2.2. Preparation of leaf extract

The collected leaves samples were washed with double distilled water, shade dried and then ground into fine powder. 250 gm of the powder is weighed and then used for 50% v/v methanolic extract preparation using methanol and milli-Q water in Soxhlet apparatus at 40-45°C for 48 hours (according to CG-04 protocol, WHO,1983). Extract prepared was then filtered, and kept for drying at room temperature (25°C - 30°C) in oven, for further use.[4]

2.3. Synthesis of silver nanoparticles

5 ml of fresh aqueous leaf extract was prepared using dried extract powder. In a conical flask 55 ml of 1 mM (0.017 gm) silver nitrate (Aq.) solution was prepared using $AgNO_3$ dissolved in Milli-Q water. Both solutions were mixed together and heated on a hot plate to a temperature of 45 ± 2.5°C, following continuous stirring by magnetic stirrer for 15 minutes.[11]

3. Characterization of AgNPs

3.1. UV- Vis Spectrophotometry

UV-vis spectrophotometry is a sensitive, rapid, and simple equipment, that is frequently used to examine the initial quality of biologically produced metal nanoparticles. Secondary metabolites in metal-reducing organic

compounds of plants can absorb light in the UV and UV-Vis spectrums. Phytometabolites can mediate the reduction of silver ions, act as electron donors, and generate AgNPs.[13]

The absorption behavior of synthesized AgNPs were identified using Thermoscientifics multiskan Go spectrophotometer, present in the Central Instrumentation Facility (CIF), in the Department of Zoology, University of Rajasthan, Jaipur. According to the protocol suggested by Chaudhari et al, 2016 in the range of 300-700 nm with sample in quartz cuvette.[7]

3.2. FTIR spectroscopy

The FTIR (Fourier transformation infrared) study gives important details about AgNPs functional groups and chemical bonds. It depicts the vibrational bands of the existing chemical bonds and functional groups that cap and stabilize the nanoparticles, and shows various absorbance in infra-red range. Bands can be formed in between wavenumber (cm^{-1}) of 2850-3300,1680-1750,1000-1300,3230-3550,2500-3300 showing functional group C-H (alkanes), C=O (Carbonyl group), C-O*, O-H (alcohols), O-H (acids), respectively. The FTIR spectrum was formed using Perkin Elmer Infra-red spectrum 10.4.00, facility was sourced by MRC-MNIT, Jaipur. FTIR was performed on KBr pellet mode in the wavenumber 4000-450 cm.

3.3. X-ray Diffraction

To detect the distribution of molecules within any crystalline structure, XRD technique is used. Dried sample was prepared for detection of structure of AgNPs according to the process mentioned by Ravichandaran et al, 2016.[14]The crystal nature of AgNPs were analyzed using the PANalytical X'pert PRO X-ray diffractometer system, for the detection of crystalline lattice of the material. Using Cu-Kα radiation system, at a setting of 40ma/40kv, from a range of 2 theta 20̊- 80̊.[15]

3.4. Field Emission-Scanning Electron Microscopy

The surface topography of nanoparticles was detected facilitated by FE-SEM (Nova nano SEM 450) at magnification of 25000x and HV (High Vacuum) at voltage of 15.0 Kv, facility was sourced by MRC-MNIT, Jaipur. The sample was provided as a thin film on a small glass slide.[15]

3.5. Zeta-potential

In order to determine the stability of the composition of nanoparticles and surface charge, zeta potential studies are typically performed. This analysis measures the velocity of the nanoparticles, allowing one to assess the colloidal stability of the green synthesised AgNPs.[16]The surface electric charge of AgNPs was identified by zeta potential analyzer, using water as dispersant, with 12 zeta runs in a zetasizer ver. 7.11 Malvern instruments Ltd. (MRC-MNIT, Jaipur), when electric repulsion occurs between the particles.[17]

4. In- vitro Antioxidant Activity

To understand the total In-vitro antioxidant potential of leaf extract and synthesized AgNPs of C. siamea, it was identified by the, two assays, DPPH and FRAP assay. Various concentrations of the samples were prepared by dissolving extracts/AgNPs into Dimethyl sulphide (DMSO). Nanoparticles were sonicated before use.

4.1. DPPH radical % scavenging assay

The antioxidant activities that are related to the ability of the extract and AgNPs, to transfer electrons or hydrogen atoms by bleaching the purple color of the DPPH (1,1, diphenyl 1-2 picrylhydrazyl) into yellow solution.[18]

The scavenging potential of the leaf extracts and AgNPs from methanolic leaf extract was determined using the method of Akintola et al, 2020.[19]Concentration was taken as 200, 400, 600, 800 and 1000 μg/ml. The samples were subjected for DPPH free radical scavenging activity by adding 100 μl of leaf extract/AgNPs of each concentration and 3.9 ml of DPPH solution (1mM solution: prepared by dissolving in methanol). For each sample, 5 test tubes were kept in a set. Besides samples, one blank/negative control (methanol only) and one positive control (DPPH only) were taken. As standard drug, similar set for ascorbic acid was also prepared. All tubes were kept in dark for 30 minutes. After that absorbance were measured at 517 nm.

Free radical scavenging activity or the percent antioxidant activity was measured by following formula- % activity = (Ac-At)/Ac*100 Ac, is the absorbance of control (DPPH radicle +methanol); At- Absorbance of test (Cs-E/Cs-AgNPs) + DPPH radicle.

4.2. FRAP % reducing ability assay

The Ferric Reducing Ability of Plasma (FRAP) assay, developed by Benzie and Strain in 1996, is a simple and automated method used to assess the "antioxidant power" of a sample by measuring its ability to reduce ferric ions. At a low pH, ferric ions (Fe^{3+}) are reduced to ferrous ions (Fe^{2+}), forming a blue-coloured ferrous-tripyridyl triazine complex from an initially colourless solution. The FRAP value is determined by comparing the change in absorbance at 593 nm between the test samples and standard solutions of ferrous ions at known concentrations.[20]

The FRAP assay was slightly modified to assess the overall antioxidant potential of leaf extracts and AgNPs. Stock solutions included 300 mM acetate buffer (0.3 M acetic acid and sodium acetate, pH 3.6), 10 mM

TPTZ (2,4,6-tripyridyl-s-triazine in 40 mM HCl), and 20 mM $FeCl_3 \cdot 6H_2O$. A fresh working solution was prepared by combining 25 ml of acetate buffer with 2.5 ml of $FeCl_3 \cdot 6H_2O$ and TPTZ solutions, with the temperature increased to 37°C just before use. Leaf extracts and nanoparticles, prepared in 100 μl of DMSO, were reacted with 290 μl of FRAP solution for 30 minutes in dark conditions. The absorbance of the resulting ferrous-tripyridyl triazine complex was measured at 593 nm. In the negative control (blank), only FRAP solution was used, while ascorbic acid served as the positive control (standard).

The catalase enzyme concentration was found out using following equation- % catalase activity = 100- ((Control-test/control) *100).

5. Results

5.1. UV- Vis spectral and color change analysis

After keeping the reaction mixture for some time of incubation, color change of the solution was observed (Figure 3 A) from white to light brown and then turns darker with respect to time. Change of the color is also the primary indication of Ag^+ion has been reduced into $Ag^0$and synthesis of AgNPs.

After the color change was observed, the absorbance of AgNPs was measured with UV- spectrophotometer, between the range of 350nm-700nm in a quartz cuvette, and they show a range between 410nm- 450 nm, best peaks were observed at 434 nm, as shown in Figure 3 B. This absorbance shows conformation that AgNPs has been synthesize. Figure 3 C is showing comparison and difference in absorbance of UV-vis light between crude leaf extract and synthesized AgNPs of plant leaf extract.

Table 1: Average crystal size of C. siamea- AgNPs was calculated using parameters mention in table below

Sample	2 θ(°)	hkl	FWHM (°)	Crystal size D (nm)
C. siamea-AgNPs	27.97	(100)	0.1378	59.38
	32.39	(122)	0.1574	21.00
	46.38	(231)	0.1378	62.69
	54.99	(142)	0.2755	32.49
	57.64	(241)	0.2755	53.85
	64.68	(220)	0.3936	23.88
	76.84	(311)	0.1574	64.39
D average Size				45.38 nm

5.2. FTIR analysis

The FTIR analysis aims to identify and study interaction of functional group and chemical bonds present between plant secondary metabolite and $Ag^+$to form AgNPs. The biomolecules present in leaf extract were responsible for

Table 2: DPPH free radicalscavenging activity of extract and AgNPs of C. siamea

Concentration (μg/ml)	DPPH Scavenging activity (%)		
	Extract	Cs/AgNP	Ascorbic acid
200	32.73±0.27	47.96±0.30	71.30±3.02
400	35.90±0.27	59.84±0.23	81.65±0.30
600	36.98±0.23	68.40±0.27	85.83±0.31
800	37.97±0.27	76.49±0.27	88.47±0.34
1000	38.17±0.34	79.76±0.23	94.93±3.72
IC_{50}	2711.60±56.6	188.88±7.01	-

(Data are represented in Triplicates (n=3) as Mean±S.D. at p<0.05)

Table 3: FRAP % scavengingactivity of extract and AgNPs of C. siamea.

Concentration (μg/ml)	FRAP Scavenging activity (%)		
	Extract	Cs/AgNP	Ascorbic acid
20	8.46±0.20	29.13±0.26	46.05±0.20
40	9.25±0.33	31.26±0.36	56.50±0.36
60	10.40±0.33	33.60±0.28	60.15±0.24
80	11.13±0.24	36.62±0.41	74.80±0.28
100	11.87±0.24	39.59±0.28	80.96±0.24
IC_{50}	975.98±19.99	181.40±2.51	28.90±0.66

(Dataare represented in Triplicates (n=3) as Mean±S.D. at p<0.05)

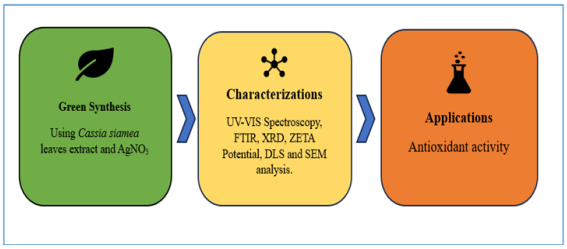

Figure 1: Graphical representation of synthesis, characterization and in vitro antioxidant activity evaluation of C. siamea mediated AgNPs

Figure 2: RUBL21231 C. siamea

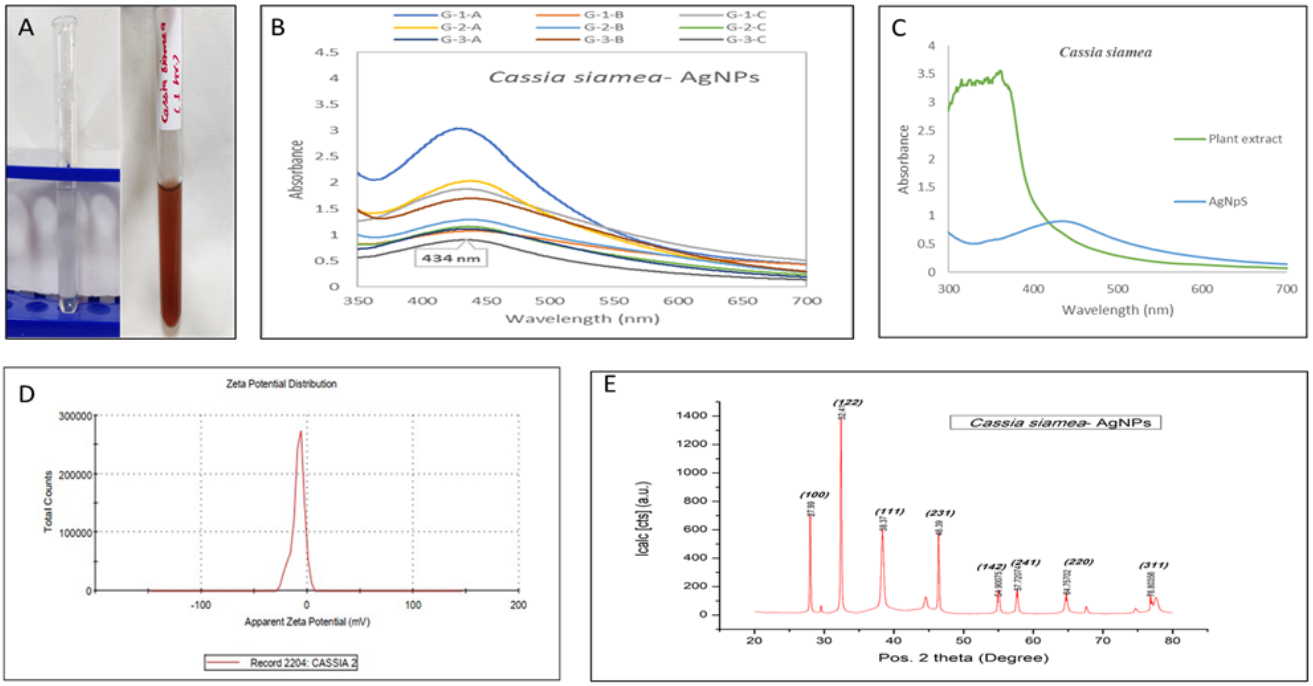

Figure 3: A- Colour change of the solution observed, during the synthesis of AgNPs. **B-** UV-visible spectra of AgNPs at different concentrations. **C-**Comparative UV-Visible spectrums of plant extract and AgNPs. **D-** Zeta Potential analysis. **E-** X-ray Diffractogram of synthesized AgNPs

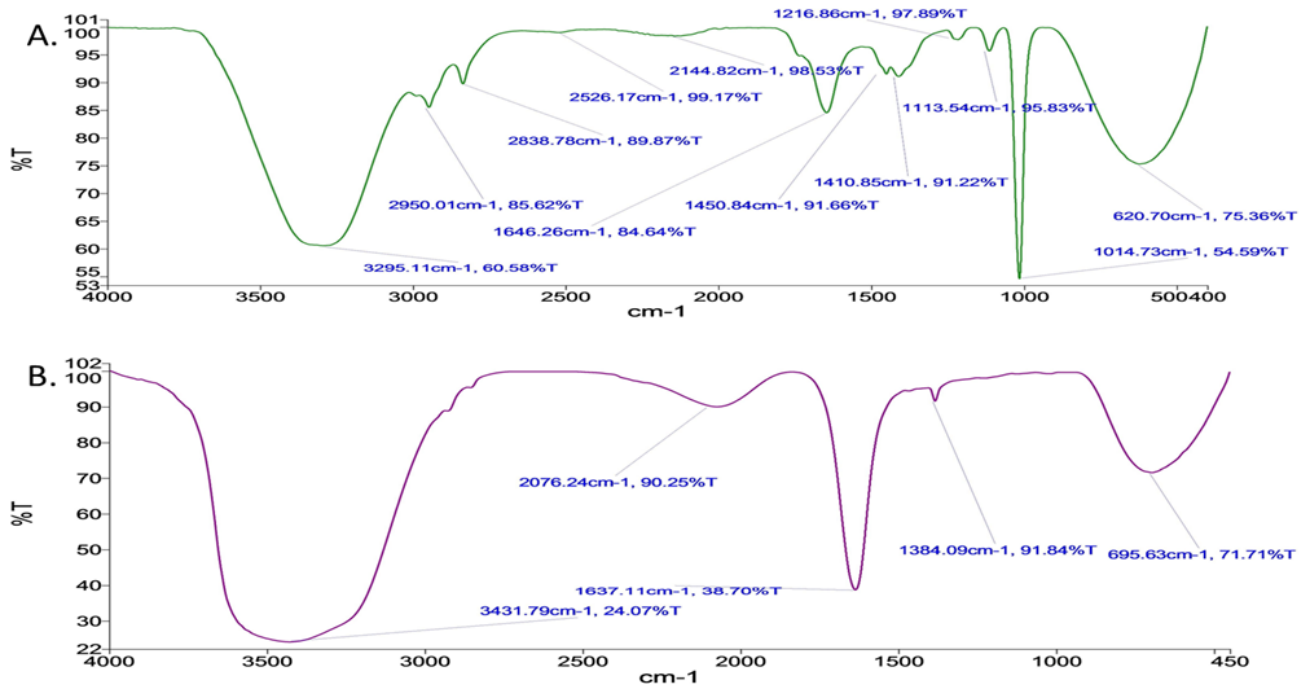

Figure 4: (A): and **(B):** Comparative FTIR spectrum of C. siamea leaf extract and C.siamea-AgNPs.

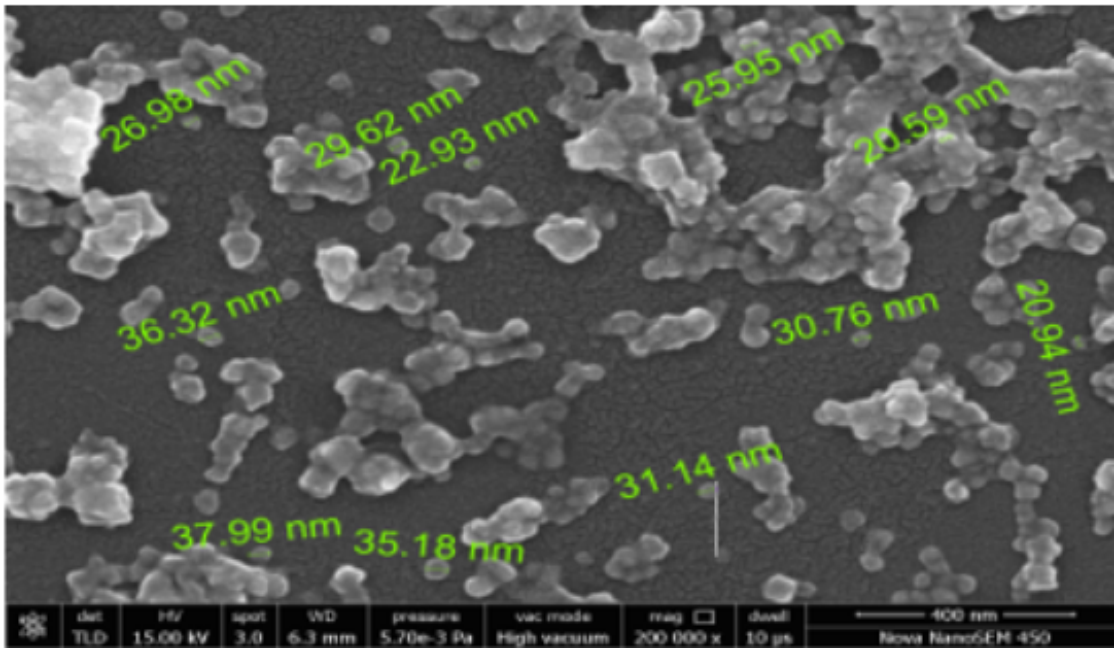

Figure 5: SEM images of C. siamea silver nanoparticles.

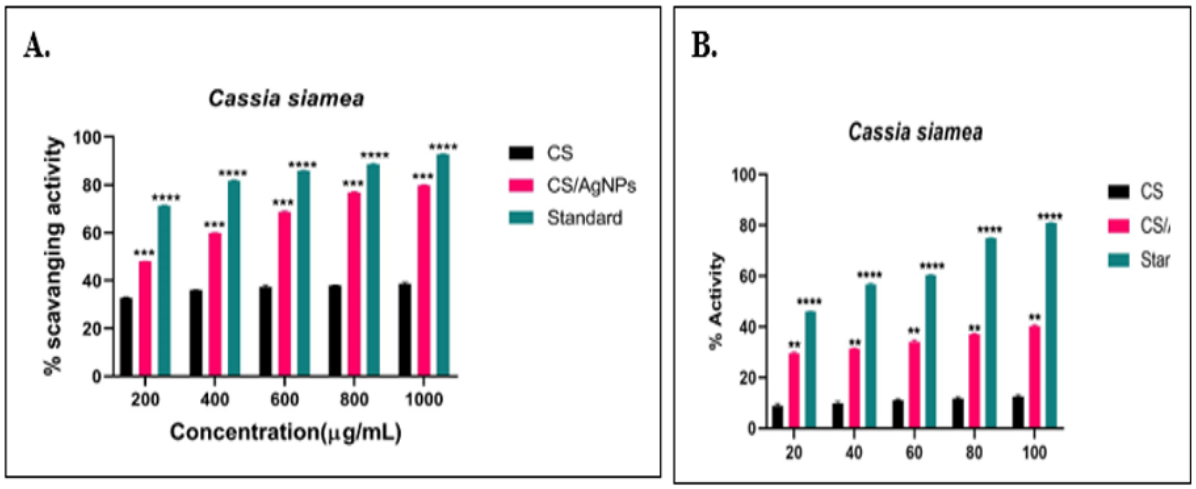

Figure 6: (A): DPPH % radical Scavenging activity and **(B):** FRAP reducing activity of AgNPs synthesized by C. siamea.

reducing silver nitrate. Figure 4 A and B , depicting various bands at different % transmittance, for both the plants extract and AgNPs, respectively. Presence of different peaks suggest the various bond types and functional groups (alkane, alkene, hydroxyl, carbonyl, ketone) present in AgNPs, which absorbs at different wavelength.[21]

Various bands are prominently visible in FTIR spectra of C. siamea leaf extract (Figure 4 A) at which corresponds to different vibrations of alcoholic O-H, Alkanes C-H, Phenolic O-H and C-O stretches. Analysis of C. siamea-AgNPs (Figure 4 4B) shows a strong and broad round band, O-H stretching at 3431.79, strong N=C=S Isothiocyanate band at 2076.24, a medium C=C (Conjugated alkene) band at 1637.11, a medium C-H alkane band at 1384.09 and a strong C=C alkene band at 695.63, variation in these band from leaf extract bands shows vibrational pattern of the molecules present and they form chemical bonds.

5.3. Zeta- potential analysis

AgNPs synthesized using methanolic leaf extract of C. siamea, shows an average negative charge with potential of -8.28±5.81 mV (Mean±S.D.), at conductivity 0.191 mS/cm, in zeta potential report. Generally higher the negative charge, shows higher stability of formed nanoparticles (Figure 3 D). Concentration of $AgNO_3$ and other factors

may affect the stability of synthesized AgNPs. Here AgNPs are showing good negative charge which confirmed that synthesized nanoparticles are stable, for a longer period of time and can be used for further analysis.[16]

5.4. XRD analysis

X-ray diffraction was used to analyse the crystalline nature of the nanoparticles, as illustrated in the Figure 3 E, The measured spectrum displays eight primary peaks at varied 2θ, in the range of $2\mathring{0}$ - $8\mathring{0}$, corresponding to various planes as (100), (122), (111), (220), and (311).

Sharpening of the peaks indicated their presence in the nano area, and this XRD pattern demonstrates the presence of synthesised AgNPs in the FCC (Face cubic centre) crystalline structure, according to Helfish et al, 2021.[22]These results shows that AgNPs has been synthesized. The crystalline size of AgNPs was calculated using full width half maximum, Bragg reflection by Debye-Scherrer equation-

$$D = 0.9\lambda/\beta Cos\theta$$

where D is the crystallite size, λ = 1.5406 Å is the wavelength of X-ray, β is the full width half maximums (FWHM) of the peak in radians, and θ is the Bragg angle. The crystallite size of the AgNPs, calculated by the above-mentioned equation using hkl value and FWHM value are 45.35 nm.[23]

5.5. FE-SEM analysis

SEM analysis (Figure 5) helps in direct visualization of nanoparticles by analyzing their surface morphology. From surface morphology it has been observed that nanoparticles are spherical in shape and shows uniform aggregation. The aggregation of AgNPs shows that there is free static charge at their surface. They produce crystalline structure. The particle diameter of AgNPs were ranging between 15.0 nm-51.91nm, thus nanoparticles are below 100 nm in size.

5.6. DPPH assay analysis

The IC_{50} value represents that concentration of a compound, which is required to achieve 50% of its maximal effect, which, in this context, reflects the scavenging potential of the sample. A lower IC_{50} value indicates a higher antioxidant capacity, as it signifies that a lower concentration is sufficient to achieve 50% DPPH radical inhibition. Thus, IC_{50} values are inversely related to antioxidant potential.[23,24]

In Table 2, the results of the DPPH analysis show no IC_{50} value for ascorbic acid (standard), as the 50% inhibition threshold was reached at a concentration lower than the minimum concentration tested. The AgNPs exhibited a concentration-dependent increase in DPPH scavenging activity, as demonstrated by their corresponding IC_{50} values. The gradual increase in scavenging activity with increasing concentrations of AgNPs shows their antioxidant potential.

5.7. FRAP assay analysis

At a concentration of 20 μg/ml, the leaf extract displayed the lowest reducing power, with a ferrous reducing activity of 8.46±0.20% (Mean±S.D.), this lower activity is likely due to the lower concentration of bioactive compounds in the crude extract. In contrast, the AgNPs showed a significantly higher reducing activity at the same concentration, indicating that the incorporation of bioactive compounds onto the nanoparticle surface enhances their antioxidant capacity.[25,26] The enhanced activity of AgNPs is likely attributed to the surface-bound phytochemicals acting synergistically, amplifying the antioxidant effects. The results also highlight the benchmark antioxidant activity of ascorbic acid, which showed the highest reducing power across all concentrations. However, while the AgNPs did not match the reducing potential of ascorbic acid, they demonstrated a considerable antioxidant capacity, particularly at higher concentrations.

As the concentration increased, both the AgNPs and the leaf extract demonstrated improved FRAP activity; however, the AgNPs consistently outperformed the leaf extract. At the highest concentration tested (100 μg/ml), the AgNPs exhibited a maximum ferrous reducing activity of 39.59±0.28%, which was significantly higher than the leaf extract's activity of 11.87±0.24%, but significantly lower than the standard ascorbic acid 80.96±0.24.

5.8. Statistical analysis

GraphPad PRISM 8.3 software (GraphPad, La Jolla, CA, USA) and Microsoft Xl was used for statistical analysis. Data is calculated in Triplicates (n=3) as Mean±SD. Tukey's one-way ANOVA test was used to determine significant differences between control and different concentrations of samples for their antioxidant activity ($p<0.05$).

6. Discussion

The present study successfully synthesized and characterized AgNPs using UV-vis, FTIR, XRD, Zeta potential analysis and SEM Techniques, using methanolic leaf extract of C. siamea. The use of plant-based extracts for the biosynthesis of nanoparticles is an eco-friendly and cost-effective alternative to traditional chemical synthesis methods.[27]

One of the study's important finds was the antioxidant activity of the synthesized AgNPs, when compared to methanolic leaf extracts, AgNPs from C. siamea displayed greater free radical scavenging capacity. The DPPH assay showed that AgNPs could donate electrons or hydrogen atoms to neutralize DPPH free radicals, demonstrating

their potential antioxidant capacity and the FRAP assay confirmed AgNPs' ability to reduce ferric ions (Fe3+) to ferrous ions (Fe2+), providing additional evidence of their reducing power. The increased antioxidant activity of the AgNPs may be related to the synergistic effects of the silver and bioactive chemicals contained in C. siamea, though it was found lower than the standard.[28,29] This shows that the phytochemicals used in the production of AgNPs retained their antioxidant capabilities within the nanoparticles.[12,30,31] *s* The capacity of AgNPs to scavenge free radicals suggests that they could have therapeutic implications in reducing oxidative damage in biological systems. Future research should focus on characterizing the produced AgNPs, notably their size, shape, and surface charge, as these features can have a substantial impact on their biological activities. Furthermore, in vivo investigations are required to assess the safeguarding, biological compatibility, and therapeutic efficacy of the produced AgNPs in biological environments.

7. Conclusions

By the above investigation, we can conclude that synthesis of AgNPs from C. siamea methanolic leaf extract has been successfully done. AgNPs from C. siamea exhibited better antioxidant activity compared to the extract. This suggests that the phytochemicals acting as reducing agents during AgNPs synthesis retained their antioxidant properties in the nanoparticles. The nanoparticles effectively scavenge free radicals, as evidenced by their performance in DPPH and FRAP assays. In conclusion, AgNPs of C. siamea demonstrate promising antioxidant potential. Further studies are suggested to explore their biocompatibility and potential applications in antioxidant therapy and other biomedical fields, with beneficial biological activities, contributing to the development of eco-friendly and sustainable nanotechnologies.

8. Ethical Committee Approval

Not applicable.

9. Clinical Trial Registry

Not applicable for current study.

10. Author Contributions

Pratap Chand Mali- Supervision, investigation and Validation. Neha Bharti- Conceptualization, Data Curation, Funding acquisition, Methodology, Writing- Original Draft. Prity Yadav Formal Analysis, Resources, Writing and editing and Ashish Kumar Kansotiya- Visualization.

11. Data Availability Statements

The datasets utilized and analyzed in this study are accessible from the corresponding author upon reasonable request.

12. Source of Funding

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13. Conflict of Interest

None.

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References

1. Bharti N, Yadav P, Kumari S, Kansotiy AK, Mali PC. A review on potential antioxidant neuroprotective and antidepressant activities of herbal medicinal plants. *EJBPS*. 2023;5(10):122–33.
2. Mali PC. Control of fertility in male wistar rats treated with hydro alcoholic extract of Withania somnifera fruits. *Int J Pharmacol Bio Sci*. 2013;7:13–21.
3. Yadav P, Bharti N, Kansotiya AK, Kumari S, Yadav RK, Mali PC. Potential antifertility and antimicrobial activities of plants used in traditional medicines: A review. *J Pharmacogn Phytochem*. 2023;12(2):190–8.
4. Dewal S, Lakhne R, Gupta R. Antifertility activity of Cassia siamea (stem bark) in male albino rats and phytochemical analysis by GC-MS technique. *J Pharmacogn Phytochemi*. 2018;7(3):3050–3.
5. Kaur G, Alam S, Jabbar Z, Javed K, Athar M. Evaluation of antioxidant activity of Cassia siamea flowers. *J Ethnopharmacol*. 2006;108(3):340–8.
6. Musa A, Wada BH, Hadi MA, Danmusa MA. Green Synthesis of Silver Nanoparticles and Its Antibacterial Activity using the Flower Extract of Senna Siamea. *In Int J Nanosci Nanotechnol*. 2021;3(17):173–9.
7. Chaudhuri SK, Chandela S, Malodia L. Plant mediated green synthesis of silver nanoparticles using Tecomella undulata leaf extract and their characterization. *Nano Biomed Eng*. 2016;8(1):1–8.
8. Dhaka A, Mali C, Sharma S, Trivedi S. A review on biological synthesis of silver nanoparticles and their potential applications. *Res Chemi*. 2023;6:101108.
9. Arunkumar C, Nima P, Astalakshmi A, Ganesan V. Green synthesis and characerization of silver nanopartilces using leaves of Tecoma stans (L.) kunth. *Int J Nanotech App*. 2013;3(4):1–10.
10. Tariq H, Rafi M, Amirzada MI, Muhammad SA, Yameen MA, Mannan A. Photodynamic cytotoxic and antibacterial evaluation of Tecoma stans and Narcissus tazetta mediated silver nanoparticles. *Arab J Chem*. 2022;15(3):103652.
11. Seekonda S, Rani R. Eco-friendly synthesis, characterization, catalytic, antibacterial, antidiabetic, and antioxidant activities of Embelia robusta seeds extract stabilized AgNPs. *JSAMD*. 2022;7(4):1–12.
12. Burlacu E, Corneliu T, Năstaca-Alina C, Lavinia B. A Review of Bark-Extract-Mediated Green Synthesis of Metallic Nanoparticles and Their Applications. *Molecules*. 2019;24(23):4354.

13. Kemala P, Idroes R, Khairan K, Ramli M, Jalil Z, Idroes GM. Green Synthesis and Antimicrobial Activities of Silver Nanoparticles Using Calotropis gigantea from Ie Seu-Um Geothermal Area. *Molecules*. 2022;27(16):5310–5310.
14. Ravichandran V, Vasanthi S, Shalini S, Shah A, Harish R. Green synthesis of silver nanoparticles using Atrocarpus altilis leaf extract and the study of their antimicrobial and antioxidant activity. *Mater Lett*. 2016;180(1):264–7.
15. Gangadharan G, Abraham G, Krishnaswamy T. Biosynthesis and characterization of silver nanoparticles using Actinopteris radiata, Bedd and assessment of their antimicrobial activity. *JUSST*. 2022;6(24):253–64.
16. Jain N, Jain P, Rajput D, Patil UK. Green synthesized plant-based silver nanoparticles: therapeutic prospective for anticancer and antiviral activity. Micro and. *Nano Syst Lett*. 2021;9(1):1–5.
17. Lavudi K, Harika GV, Thirunavukarasou A, Govindarajan G, Patnaik S, Golla N, et al. Green Synthesis of Tecoma stans Flower and Leaf Extracts: Characterization and Anti-Proliferative Activity in Colorectal Cancer Cell Lines. . *Lett Appl NanoBioSci*. 2023;12(3):1–18.
18. Gebrehiwot H, Ensermu U, Dekebo A, Endale M, Hunsen M. Exploring the medicinal potential of Senna siamea roots: an integrated study of antibacterial and antioxidant activities, phytochemical analysis, ADMET profiling, and molecular docking insights. *Appl Biol Chem*. 2024;67(1):1–22.
19. Akintola AO, Kehinde BD, Ayoola PB, Adewoyin AG, Adedosu OT, Ajayi JF. Antioxidant properties of silver nanoparticles biosynthesized from methanolic leaf extract of Blighia sapida. *IOP Conference Series Mat Sci Eng*. 2020;805(1):22–4.
20. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal Biochem*. 1996;239(1):70–6.
21. Kalakonda P, Thodeti M, Ganneboina C, Ankathi K, Kathri S, Begari K. Eco-Friendly Fabrication of Silver Nanoparticles for Sustainable Water Purification and Antibacterial Synergy. *Plasmonics*. 2024;2:617.
22. Heflish AA, Hanfy AE, Ansari MJ, Dessoky ES, Attia AO, Elshaer MM. Green biosynthesized silver nanoparticles using Acalypha wilkesiana extract control root-knot nematode. *J King Saud Univ Sci*. 2021;33(6):101516.
23. Thangapushbam V, Rama P, Sivakami S, Jothika M, Muthu K, Almansour AI. Potential in-vitro antioxidant and anti-inflammatory activity of Martynia annua extract mediated Phytosynthesis of MnO2 nanoparticles. *Heliyon*. 2024;10(8):e29457.
24. Salari S, Bahabadi E, Samzadeh-Kermani S, Yosefzaei A. In-vitro Evaluation of Antioxidant and Antibacterial Potential of Green Synthesized Silver Nanoparticles Using Prosopis farcta Fruit Extract. *Iran J Pharm Res*. 2019;18(1):430–55.
25. Bedlovičová Z, Strapáč I, Baláž M, Salayová A. A brief overview on antioxidant activity determination of silver nanoparticles. *Molecules*. 2020;25(25):14.
26. Balciunaitiene A, Viskelis P, Viskelis J, Streimikyte P, Liaudanskas M, Bartkiene E. Green Synthesis of Silver Nanoparticles Using Extract of Artemisia absinthium L., Humulus lupulus L. and Thymus vulgaris L. Physico-Chemical Characterization. *Antimicrob Antioxidant Activity Proc*. 2021;9(8):1304.
27. Willian N, Syukri, Zulhadjri Z, Labanni A, Arief S. Bio-Friendly Synthesis of Silver Nanoparticles Using Mangrove Rhizophora stylosa Leaf Aqueous Extract and Its Antibacterial and Antioxidant Activity. *Rasayan J Chem*. 2020;13(3):14–85.
28. Mohammed A, Gokulapriya K, Rajeshkumar S. Antioxidant Activity of Ocimum gratissimum Stem Extract Mediated Silver Nanoparticles-An in Vitro Study. *IJMST*. 2024;10(5):662–6.
29. Erenler R, Geçer EN, Genç N, Yanar D. Antioxidant activity of silver nanoparticles synthesized from Tagetes erecta L. leaves. *Int J Chem Techno*. 2021;5(2):141–6.
30. Reddy NJ, Vali DN, and RMR. Evaluation of antioxidant, antibacterial and cytotoxic effects of green synthesized silver nanoparticles by Piper longum fruit. *Mat Sci Eng*. 2014;34:115–22.
31. Rizwana H, Aljowaie RM, Otibi A, Alwahibi F, and MSA. Antimicrobial and antioxidant potential of the silver nanoparticles synthesized using aqueous extracts of coconut meat (Cocos nucifera L). *Sci Rep*. 2023;13(1):16270.

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