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Bioactive Nanoparticles from *Cassia fistula* L. Seed Aqueous Extract: Assessment of Multiple Biological Activities

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ABSTRACT

Background: *Cassia fistula* L., a flowering plant of the leguminous family (Fabaceae), has been explored for its potential in synthesizing silver nanoparticles. This plant is known for its medicinal properties, making it a promising candidate for applications in nanobiotechnology.

Methods: The researchers employed a combination of hot and cold extraction procedures to synthesize silver nanoparticles using seed extracts of *C. fistula*. The identity and characteristics of these nanoparticles were verified through various techniques, including chromatography, imaging microscopy, X-ray diffraction and scanning electron microscopy. Biological activities such as antioxidant and antibacterial properties were tested. For antioxidant activity, a reaction mixture consisting of 50 µl of seed extract and 5 ml of a 0.04% (w/v) DPPH solution was used. Antibacterial activity was evaluated against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus pyogenes* at concentrations of 0.125, 0.25, 0.5 and 1 mg/ml of AgNPs. Additionally, the antibiofilm effect and hemolysis impact were studied.

Result: The synthesized silver nanoparticles demonstrated significant biological activities, including antioxidant and antibacterial effects. The diameters of the zones of inhibition for *P. aeruginosa* at a concentration of 1 mg/ml were 20 mm for cold extract and 18 mm for *E. coli* using hot extract. These findings highlight the potential of *C. fistula* as a sustainable source for environmentally friendly nanomaterials, contributing to advancements in medical and environmental sciences. Finally, the use of silver nanoparticles from *Cassia fistula* encourages sustainable and productive agricultural practices, by improving plant health and yield, potentially revolutionizing agricultural methods.

Key words: Biological activity, *Cassia fistula*, Nanoparticles, Seeds.

INTRODUCTION

The environmentally friendly methods have been the focus of global scientific studies since the emergence of metallic nanoparticles, which became possible through green synthesis. They have gained great attention and are known as the green revolution (Ihum *et al*., 2024). As per the literature, extensive research has been undertaken on the synthesis of Ag-NPs *via* utilizing plant extracts derived from their various (Chen *et al*., 2020). Herbalists have noted many pharmacological effects of *Cassia fistula* (Meena *et al*., 2022). *Cassia fistula* is a flowering plant of the leguminous family (Fabaceae), subfamily Caesalpiniaceae, known as Amaltas. *Cassia fistula* is a synonym for *Cassia exceisa* kunth.

It is a deciduous tree with opposing, pinnate leaves (Kirtikar and Basu, 2006). Flowers are arranged in scattered racemes that are 30-50 cm in length (Khan and Alam, 1996). Brown, pendulous-septate, cylindrical fruit has a diameter of 1-3 cm and a length of 25-45 cm. Reddish-brown seeds that are lenticular (Bhatnagar *et al*., 2010). The plant appreciates deep, well-drained, fairly rich, sandy, loamy soil (Pawar and Killedar, 2017). *Cassia fistula* is historically used to cure various disorders (Gupta *et al*., 2000; Jehangir *et al*., 2010). It treats asthma, leprosy, heart disease and fever. The root treats flu and colds, while the leaves relieve pain and reduce skin irritation from swelling. In addition, stem bark and fruit extracts remove blood toxins (Jung *et al*., 2016). In other study, Sharma *et al*. (2022) test *Cassia fistula* ethanolic extracts for antipyretic, analgesic and anti-inflammatory effects in rats. Bark extract had the greatest benefits in all experiments, whereas leaf extract was antipyretic and anti-inflammatory. Research supports its historic therapeutic usage.

An increasingly common strategy for attaining sustainability and protecting the environment is the “green” synthesis of NPs (Kanwal *et al*., 2025). A study by Pawar and Killedar (2017) mentioned that *C. fistula* has several therapeutic uses, especially in traditional medicine and its flower extracts are used in the widespread manufacture of silver nanoparticles (Ag-NPs). From a medical standpoint, notable outcomes have communicated the potential for biocompatibility, non-toxicity, economic viability and environmental friendliness (AL-Musawi and AL-Abedy, 2020;

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Omran, 2024). When contrasted with more traditional approaches, the new area of green synthesis within nanobiotechnology offers substantial benefits to both the environment and the economy (Ganesan and Rengarajan, 2024). This study, therefore, seeks to assess the inhibitory efficacy of AgNP nanoparticles synthesized using *C. fistula* seed.

MATERIALS AND METHODS

Methodology for the production of cold and hot water extracts from a plant

Cold and hot water extracts were generated from *C. fistula* seeds using Harborne's Maceration Technique (1998), as shown in Fig 1.

Synthesis of silver nanoparticles with *C. fistula* seed extract

To make silver nanoparticles, 5 mL of *Cassia* seed aqueous extract was mixed with 0.017 g of silver nitrate in 100 mL of deionized water and shaken in an incubator for 24 h. As the brownish hue deepened, it became clear that silver nanoparticles were taking shape. Alteration in colour indicates the production of Ag-NPs. After letting the solution sit at room temperature for one day, we could be sure the nanoparticles would be stable. Using a centrifuge at 15,000 rpm for 15 min, the nanoparticles were separated, washed with deionized water, frozen and subjected to further examination (Choudhary *et al*., 2015).

Synthesized Ag-NPs description

The morphology and size of the synthesized silver nanoparticles were determined using atomic force microscopy, which allows visualization of the surface structure by plotting the microscopic data (Bailey and Clark, 2006). The structure and size of the synthesized particles were also examined using Scanning Electron Microscopy (SEM) and the three-dimensional structure was studied by X-ray diffraction and the heterogeneous structure of the particles was evaluated by Fourier transformation spectroscopy.

The method described by Jothy *et al*. (2011) was used to quantify the radical scavenging tests. The reaction mixture included 50 ìl of seed extract (0.125-1 mg/ml) and 5 ml of a 0.04% (w/v) DPPH solution in 80% methanol. Butylated hydroxytoluene (BHT), a commercial antioxidant, was employed for comparison or as a positive control. Control and blank were DPPH without seed extract and 80% methanol, respectively. Incubation in the darkroom for 30 min produced 517 nm discolorations. The following equation determined the DPPH free radical percentage:
Where,

$$\text{Scavenging effect (\%)} = \frac{A0 - A1}{A0} \times 100$$

A0 = The absorbance of the control.
A1 = The absorbance in the presence of the seeds extract of *C. fistula*.

Antibacterial effect study

According to Pratiyanto *et al*. (2022), the researchers studied the inhibition zone of *C. fistula* seed Ag-NPs suspensions against gram-negative bacteria "*Escherichia coli* and *Pseudomonas aeruginosa*" and gram-positive bacteria "*Staphylococcus aureus* and *Streptococcus pyogenes*" using the well diffusion.

Antibiofilm effect study

To know the effect of silver nanoparticles prepared using hot and cold extracts of *C. fistula* seeds on the ability of bacteria to form a thin biofilm, a test of the nanomaterial was performed by making five concentrations for each sample for two types of bacterial genera, namely gram-positive bacteria such as *S. aureus* and gram-negative bacteria such as *E. coli*. According to the method of Barapatre *et al*. (2016), a 96-well microtiter plate technique was used to test the effectiveness of silver nanoparticles in biofilm development. The following equation was used to calculate the percentage of biofilm inhibition.

Study of the hemolysis impact

One healthy donor's blood was subjected to hemolysis tests using the methodology of Laloy *et al*. (2014), Blood



Fig 1: *Cassia fistula* fruits and seeds.

samples were mixed with 15 µL of nanoparticles at concentrations of (1, 0.5, 0.25 and 0.125 µg/ml ,negative control was tyrode.285 microliters of whole blood are mixed with the positive control (Triton X-100).For four hours, the suspension is incubated in an incubator shaker. Following the incubation period, the suspension is centrifuged for five minutes at 10,000 g. The Elisa Reader is used to read the supernatant in a 96-well plate at 550 nm.

The percentage haemolysis was calculated as:

$$H(\%) = \frac{OD550\ nm\ sample - OD550\ nm\ tyrode}{OD550\ nm\ Triton\ X\text{-}100\ 1\% - OD550\ nm\ tyrode} * 100$$

RESULTS AND DISCUSSION

Characteristic of the synthesized Ag-NPs

As particles were deposited throughout the production process of silver nanoparticles, the colour of both the hot and cold extracts of *C. fistula* seeds changed from brown to a darker shade of brown and the consistency also altered. After being exposed to 1 mM of silver nitrate ($AgNO_3$), the *Cassia* plant demonstrated its capacity to produce silver nanoparticles *via* biosynthesis. The colour change of the reaction mixture after 24 h of incubation in a shaking incubator at 37°C is clear evidence of the formation of silver nanoparticles by a group of environmentally friendly reducing agents present in *cassia* seeds, which reduced silver ions (Ag+) to elemental silver (Ag0) as confirmed by Mohammed *et al*. (2018). According to Korbekandi *et al*. (2013) and Benakashani *et al*. (2016), the colour change of the reaction mixture from light brown to dark brown indicates the successful production of silver nanoparticles.

Fig 2 and Fig 3 show the 2D and 3D atomic number microscopy images of silver nanoparticles synthesized using hot and cold-water extract of *Cassia* seeds, respectively. The average size of the silver nanoparticles is 37 and 40 nm, respectively. The atomic number microscopy technique deals with images that allow quantitative measurements of the surface of the material, such as average roughness (Ra) (Kent and Vikesland, 2012).

The results showed that the nanoparticle size of the hot seed extract was smaller than that of the cold seed extract. The difference in the size of the nanoparticles measured by SEM for the hot and cold extracts of silver nanoparticles from *C. fistula* seeds was clearly observed. This indicates that different extraction methods have different properties of bioactive silver nanoparticles. According to the nanoparticle characterization results, there was a difference in the ability of each extract to synthesize bioactive silver nanoparticles. It is clear that the hot extract was more effective than the cold extract. The smaller the size of the

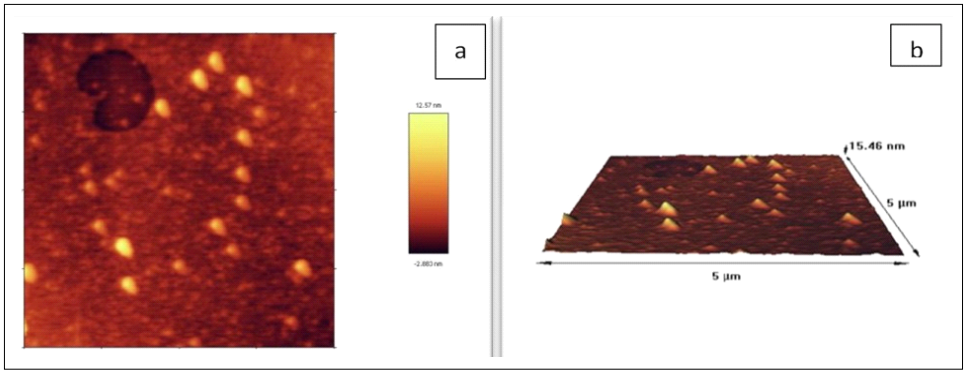


Fig 2: The 2D (a) and 3D (b) AFM images of silver nanoparticles synthesized by hot water extract of *Cassia fistula* seed.

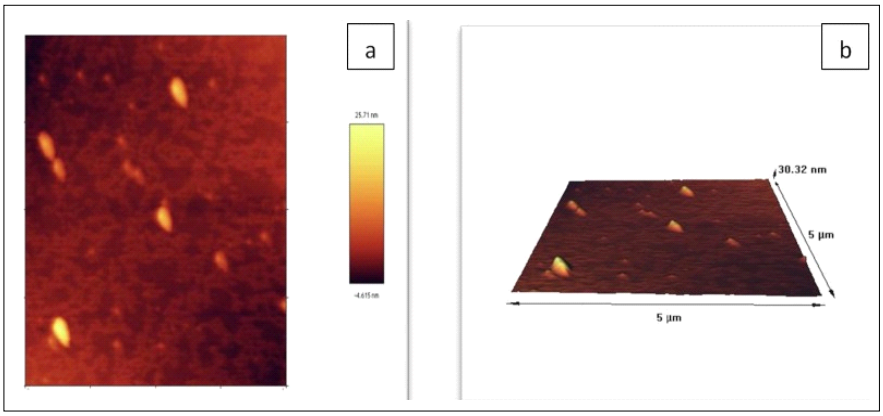


Fig 3: The 2D (a) and 3D (b) AFM images of silver nanoparticles synthesized by cold water extract of *Cassia fistula* seeds.

nanoparticles, the greater is their effectiveness (Agnihotri *et al*., 2014). Fig 4 shows the silver nanoparticles resulting from hot and cold extraction of *C. fistula* seeds, respectively.

X-ray diffraction (XRD)

The materials made from silver nitrate with hot and cold seed extract were found to have a polycrystalline structure based on the results of the X-ray diffraction examination. Fig 5 shows the presence of multiple peaks that correspond to the composition of the material being worked on with a hexagonal structure. The X-axis (2θ) represents the diffraction angle, which is related to the spacing between atomic planes in the crystal lattice and the Y-axis (counts) represents the intensity of the diffracted X-rays at each angle.

Fourier transform infrared spectroscopy (FT-IR)

Table 1, 2 and Fig 6 and 7 indicate the peak values and functional groups for silver nanoparticles produced by hot and cold extraction of *C. fistula* seeds, respectively. The X-axis (wave number, cm^{-1}) represents the frequency of the infrared light and the Y-axis (transmittance or absorbance) indicates how much light is transmitted through the sample (transmittance) or absorbed by the sample (absorbance). The IR spectrum indicates the presence of a molecule containing both O-H and C=O functional groups, suggesting a hydroxy ketone, hydroxy aldehyde, carboxylic acid or amide. Such as a strong, broad peak around 3431.17 cm^{-1} is characteristic of O-H stretching vibrations, typically from alcohols or carboxylic acids and the broadness suggests hydrogen bonding.

Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging

Researchers have looked at the antioxidant properties of *C. fistula* seed extract to find a new natural antioxidant source. It is well-known that DPPH tests accurately measure the antioxidant capacity of the material under study. The idea behind the DPPH test is that the colour of the DPPH solution would shift from purple to yellow when the antioxidant gets rid of radicals. Fig 8 displays the screening findings showing that the *C. fistula* seed extract effectively scavenged the DPPH radical. Also, it shows that the scavenging percentage decreases as the concentration of the extract decreases, indicating that the extract is more effective at higher concentrations. This suggests that *C. fistula* seed extract possesses antioxidant properties and can effectively neutralize DPPH radicals. Therefore, the *C. fistula* seed extract acts as a free radical scavenger, transforming them into stable molecules.

Antibacterial activity

An inhibitory effect on various bacteria "*Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli* and *Pseudomonas aeruginosa*" was detected using two different extracts made from *C. fistula* seeds. The concen-trations of the extracts were 0.125, 0.25, 0.5 and 1 mg/ml.

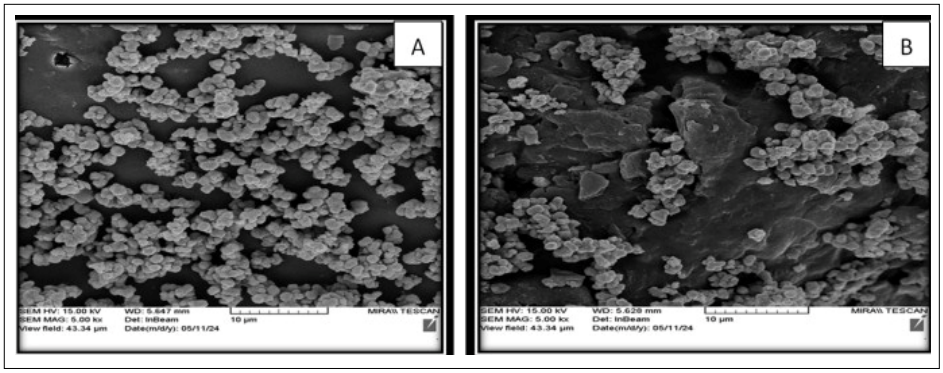


Fig 4: Silver nanoparticles produced by (A) Hot and (B) Cold extraction of *Cassia fistula* seeds.

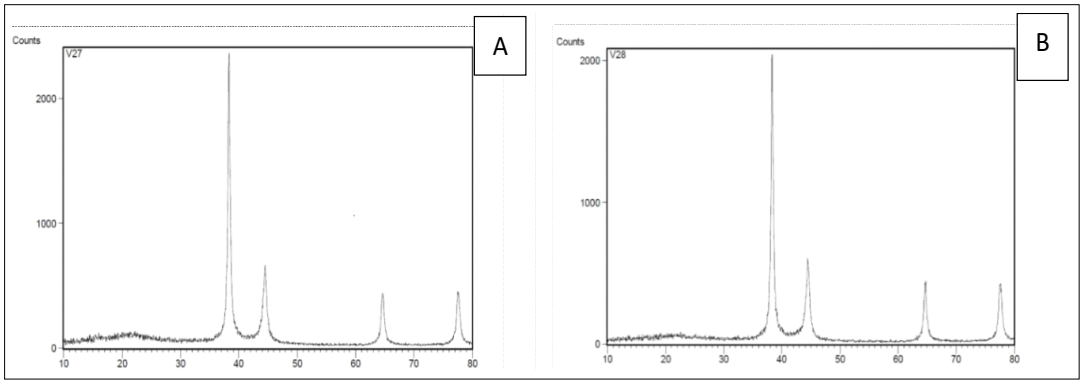


Fig 5: XRD analysis of Biosynthesized nanoparticles by (A) hot and (B) cold extraction of *Cassia fistula* seeds.

According to Table 3, the study shows that the concentration of each plant extract went up, so did the size of the inhibition zones. The diameters of the inhibition zones of the hot aqueous extract towards the growth of bacteria varied from 20 mm in Pseudomonas aeruginosa at a concentration of 1 mg/ml (Fig 9A) to 11 mm in *Streptococcus pyogenes* and *E. coli* at a concentration of 0.25 mg/ml (Fig 9B and C). However, no inhibition was observed for any type of bacteria at a concentration of 0.125 mg/ml and similarly, no inhibition was observed for Staphylococcus aureus at a concentration of 0.25 mg/ml.

The AgNP suspension in a cold-water extract of *C. fistula* seed had the largest inhibition zone, about 20 mm, against gram-positive bacteria, specifically *Streptococcus pyogenes,* at a concentration of 1 mg/ml (Fig 10A). On the other hand, the *Pseudomonas aeruginosa* bacteria had the smallest inhibition zone, about 10 mm, at a concentration of 0.125 mg/ml (Fig 10B). Also, there was no inhibition zone in *E. coli* or *Staphylococcus aureus* bacteria at a concentration of 0.125 mg/ml (Fig 10C and D) and there was no inhibition zone in *Staphylococcus aureus* bacteria at a concentration of 0.25 mg/ml (Fig 10D). There are a number of hypotheses regarding the potential effects of Ag-NPs on cell membrane permeability. Another theory is that nanoparticles' large surface area, which allows them to come into contact with organisms more easily than larger particles, is responsible for their strong antibacterial activity. In addition to interacting with the surface of the membrane, Ag-NPs may also interact with the bacteria inside (Sahayaraj and Rajesh, 2011; Al-Shugeairy *et al.,* 2021).

Three lectins isolated from *C. fistula* seeds were shown to have antibacterial properties against several types of harmful bacteria, according to Kuo *et al.* (2002). Aqueous and alcoholic extracts of the stem bark have also shown promising antibacterial activity. According to Verma (2016), *C. fistula* contains a lot of tannins, flavonoids and glycosides, which give it a wide range of pharmacological effects, including those against diabetes, inflammation, cancer, bacteria, fungal infections and others.

C. fistula is the subject of phytochemical research and is widely used by traditional Indian medicinal systems (Ali, 2014). It is an important medicinal plant due to its many beneficial uses and medicinal properties; moreover, it has hepatoprotective, anti-inflammatory, antitussive, antifungal, anti-wound and antibacterial properties. Its high concentration of glycosides, flavonoids and tannins has made it famous, as noted by Danish *et al.* (2011).

Table 1: Peak values and functional group for silver nanoparticles produced by hot extraction of *Cassia fistula* seeds.

Peak values	Functional group	Group
3431.17	Alcohol and hydroxy compound	O-H stretching
1627.8	Carbonyl compound /Quinone or conjugated ketone	C=C stretching
1265.69	Ether and oxy compound / Aromatic ethers, aryl -O stretch	C-N stretching
992.6	Ether and oxy compound / Peroxides, C-O-O- stretch	C-O-O- stretch

Table 2: Peak values and functional group for silver nanoparticles produced by cold extraction of *Cassia fistula* seeds.

Peak values	Functional group	Group
3430.47	Alcohol and hydroxy compound	O-H stretching
2887.38	Carbonyl compound /Transition metal carbonyls	N=C=S stretching
1618.82	Carbonyl compound /Quinone or conjugated ketone	C=C stretching
1270.11	Ether and oxy compound /Aromatic ethers, aryl -O stretch	C-N stretching
1049.71	Ether and oxy compound / Primary amino / Primary amine, CN stretch	CN stretch
991.42	Ether and oxy compound / Peroxides, C-O-O- stretch	C-O-O- stretch

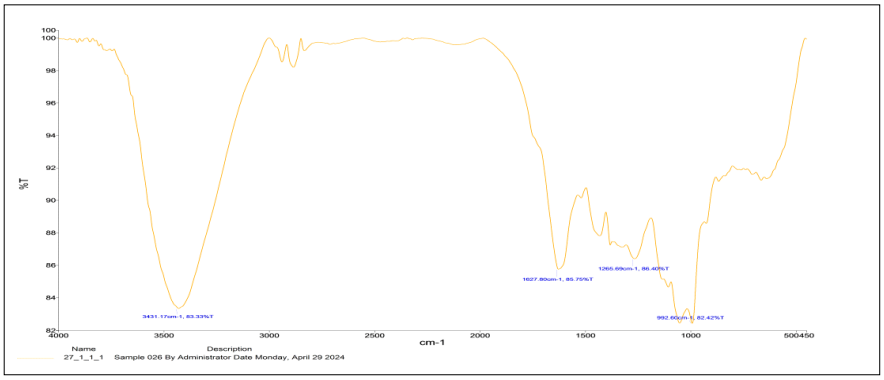


Fig 6: FT-IR of Biosynthesized nanoparticles by hot extraction of *Cassia fistula* seeds.

Previous research has demonstrated that solvent extracts of *C. fistula* have antibacterial and antifungal properties. These extracts were tested against various bacteria and yeasts, including *E. coli, S. aureus, P.aeruginosa* and *Candida albicans, Aspergillus clavatus* and *Aspergillus niger* (Bhalodia *et al*., 2012). In another study by Almuhayawi *et al*. (2024), under *in-vitro*, the antibacterial activity of ZnO nanomaterials shows the maximum zone of inhibition for nanoparticles prepared from the stem against *Proteus mirabilis, Staphylococcus albus* and *Lactobacillus*.

Antibiofilm effect

The most significant characteristic of bacteria that improves their ability to adhere to instrument surfaces is biofilm. Biosynthesized Ag-NPs, their capacity to suppress biofilm formation against *S. aureus* and *E. coli* was examined using the 96-well microtiter plate technique (Barapatre *et al*., 2016).

Fig 11 demonstrates that silver nanoparticles synthesized from *C. fistula* seeds by hot extraction have significant antibiofilm activity against *S. aureus* and *E. coli*. These nanoparticles' effectiveness depends on their concentration,

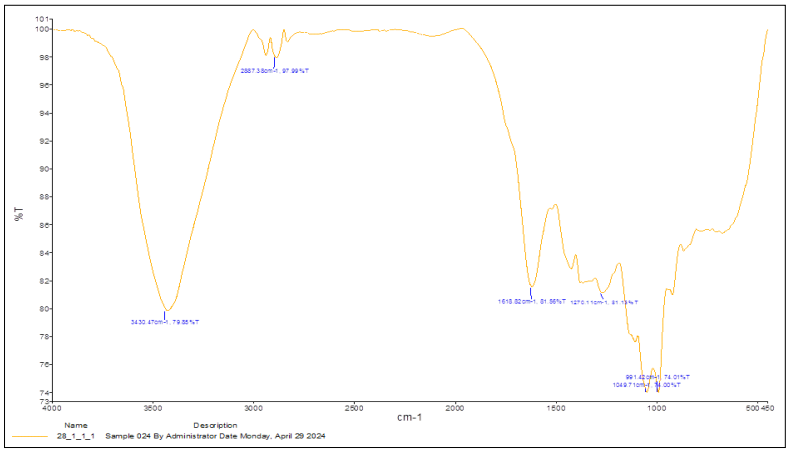


Fig 7: FT-IR of Biosynthesized nanoparticles by cold extraction of *Cassia fistula* seeds.

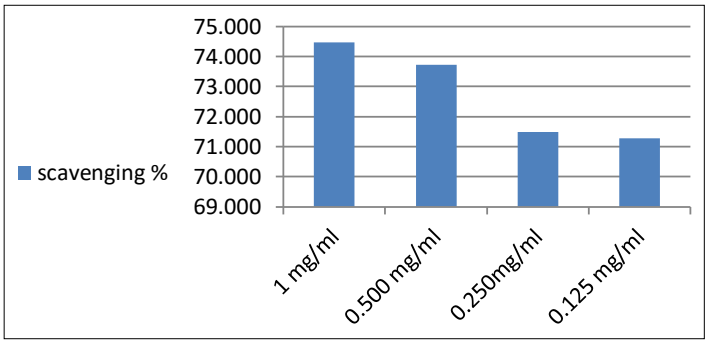


Fig 8: Effect of the *C. fistula* seed extract on scavenging the DPPH radical.

Table 3: The inhibition zone diameters of four bacterial species against *C. fistula* seeds treated with Ag-NPs.

Material	Concentration mg/ml	Gram positive bacteria		Gram negative bacteria	
		Streptococcus pyogenes	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*E. coli*
Control	Control	Zero	Zero	Zero	Zero
Hot water	1	19	17	20	18
	0.5	14	12	16	14
	0.25	11	Zero	12	11
	0.125	Zero	Zero	Zero	Zero
Cold water	1	21	16	19	20
	0.5	18	13	14	14
	0.25	13	Zero	11	11
	0.125	11	Zero	10	Zero

with higher concentrations leading to greater inhibition. *Staphylococcus aureus* appears to be more susceptible to the anti-biofilm effects of these Ag-NPs compared to *E. coli*. Thus, these silver nanoparticles can be used as a very effective agent against bacterial infections, including those involving biofilm formation. The results showed a significant decrease in biofilm production and increasing the concentration of silver nanoparticles reduced the amount of biofilm development in both hot and cold seed extracts. Silver nanoparticles for hot extracts (1 mg/ml) reduced biofilm production in both *S. aureus* and *E. coli* by up to 95.53% and 78.03%, respectively (Fig 11). While the silver nanoparticles of cold extracts (1 mg/ml) reduced the biofilm production in both *S. aureus* and *E. coli* by up to 90.42 and 83.40 per cent, respectively, In Fig 12 it confirmed that the silver nanoparticles synthesized through cold extraction from *C. fistula* seeds possess remarkable anti-biofilm properties against both *S. aureus* and *E.coli*.

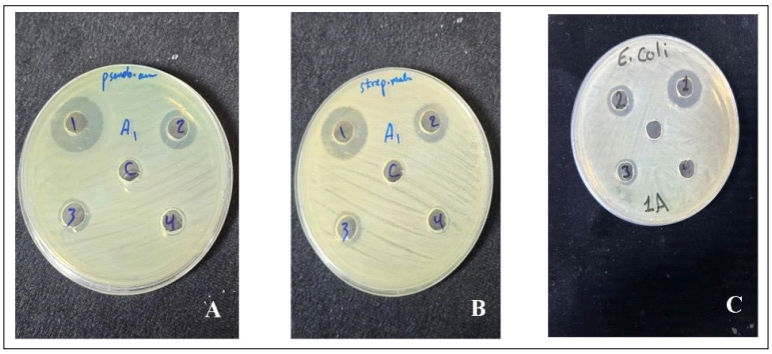


Fig 9: Antibacterial effect of silver nanoparticles prepared from hot extract of *C. fistula* seeds against (A) *P. aeruginosa*, (B) *Str. pyogenes* and (C) *E. coli*.

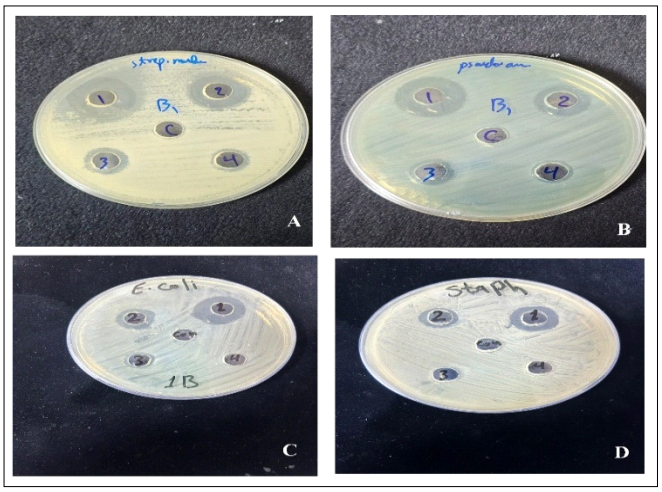


Fig 10: Antibacterial effect of silver nanoparticles prepared from cold extract of *C. fistula* seeds against (A) *P. aeruginosa*, (B) *Str. pyogenes* (C) *E. coli* and (D) *Staph. aureus*.

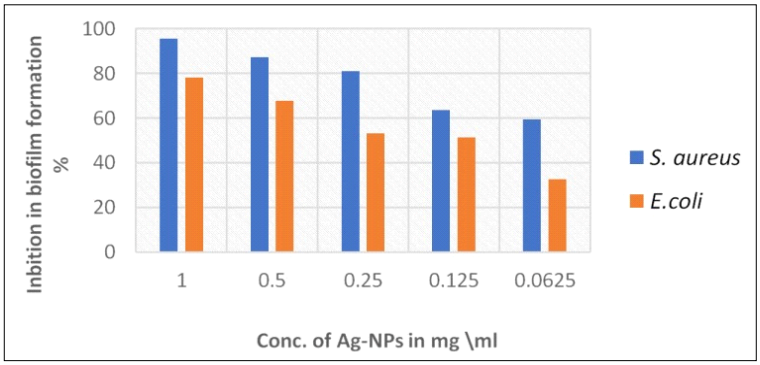


Fig 11: The antibiofilm inhibition rate of silver nanoparticles synthesized by hot extraction of *C. fistula* seeds.

Table 4: Per cent of hemolysis of the for hot and cold extracts of *C. fistula* seeds.

Concentration (mg/ml)	Hot water extracts		Cold water extracts	
	Test result	hemolysis %	Test result	hemolysis %
1	0.5366	0	0.7092	0
0.5	0.5086	0	0.5244	0
0.25	0.4624	0	0.3321	0
0.125	0.2978	0	0.2553	0
Positive	2.6327	100	2.6327	100
Negative	0.2355	0	0.2355	0

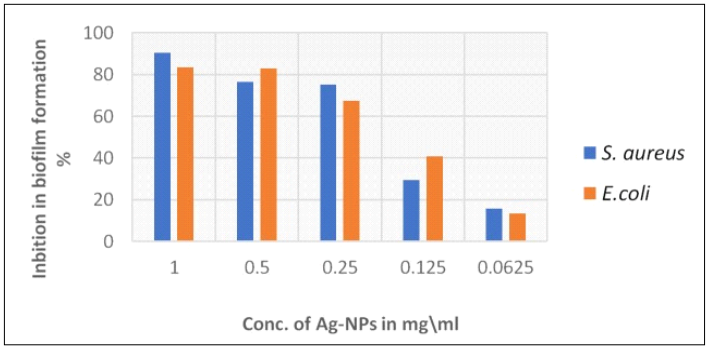


Fig 12: The antibiofilm inhibition rate of silver nanoparticles synthesized by cold extraction of *C. fistula* seeds.

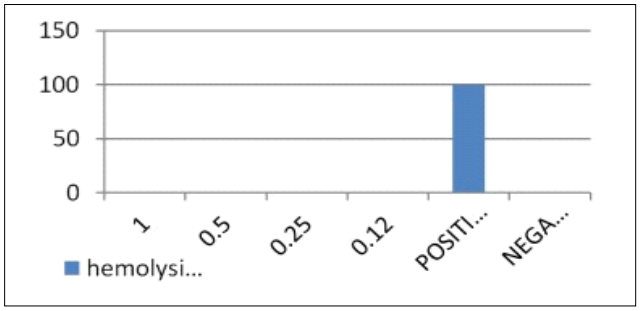


Fig 13: Per cent of hemolysis of the for both hot and cold extracts of *C. fistula* seeds.

The effectiveness of these nanoparticles is concentration-dependent. While both bacteria are inhibited, *S. aureus* generally shows a higher sensitivity to the Ag-NPs than *E. coli*.

The results also showed great anti-biofilm activity for both hot and cold extracts of *C.fistula* seeds against *S.aureus* as a model for gram-positive bacteria and also good activity, but to a lesser extent, against biofilm formation in gram-negative bacteria. The results showed that the effectiveness of nanoparticles made from hot *fistula* seed extracts was lower than that of the cold extract against Gram-negative *Escherichia coli* and the effectiveness of the cold extract was lower than that of the hot extract against biofilm formation of gram-positive *Staphylococcus aureus*.

The difference in inhibitory activity between silver nanoparticles of hot and cold *C. fistula* seed extracts can be attributed to a number of factors including antimicrobial activity, physical properties such as nanoparticle size, which affects restricted penetration and some other chemical properties such as affinity of the material to biofilms (Park *et al*., 2013).

Greeshma *et al.* (2024) emphasized the need to explore these antibiofilm medications in vivo following successful in vitro research. Using current antimicrobials, these antibiofilm drugs may treat biofilm-forming infections. How much sensitivity increases depends on the antibiotics and biofilm inhibitors utilized.

Effect of hemolysis

Since all substances entering the bloodstream come into contact with red blood cells, the solubility of biological materials may need to be assessed. The American Society for Testing and Materials (ASTM) states that hemolysis of less than 5% is normal (Luna-Vázquez-Gómez *et al*., 2021). The results of our study showed that no hemolysis occurred even at the highest concentration used for both hot and cold extracts of *C. fistula* seeds, which was 1 mg/ml as shown in Table 4 and Fig 13, given that hemoglobin release can result in negative health outcomes such anemia, pulmonary hypertension and renal toxicity, these findings may have therapeutic implications (Rother *et al*., 2005). The non-hemolytic concentration range found here is superior to the effective concentration to develop its microbicide, anticancer and immune-stimulating effects.

The results indicate that the use of Ag-NPs made from *Cassia fistula* can result in creative farming methods that are both efficient and sustainable for the environment. This strategy encourages the shift to more environmentally friendly farming methods in addition to addressing the

demand for efficient agricultural inputs. Environmentally friendly practices are promoted by using green synthesis techniques to produce silver nanoparticles from *Cassia fistula*. By lowering dependency on dangerous chemicals and improving agricultural resource efficiency, this supports the objective of guaranteeing sustainable patterns of use and production. Thus, achieving the twelfth goal of sustainable development: (responsible consumption and production).

CONCLUSION

The research found that Ag-NPs made from *Cassia fistula* seed extracts had a lot of biological activity. Although Ag-NPs were effectively generated by both the hot and cold extraction procedures, the nanoparticles were smaller and more effective when extracted using the hot approach. The structural characteristics and successful production of the nanoparticles were verified by characterization methods. The results indicate that *C. fistula* may be used as a sustainable and environmentally friendly source of nanomaterials, which might have medical and environmental benefits. In nanobiotechnology, this study promotes non-toxic and economically feasible alternatives to conventional nanoparticle manufacturing processes, highlighting the significance of green synthesis techniques.

The study's overall findings support the feasibility of employing *Cassia fistula* seed extracts for the environmentally friendly synthesis of Ag-NPs, which has the potential to transform farming methods by offering a sustainable and efficient way to improve plant production and health.

Conflict of interest

The authors say that there is no conflict of interest.

REFERENCES

Agnihotri, S., Mukherji, S. and Mukherji, S. (2014). Size-controlled silver nanoparticles synthesized over the range 5-100 nm using the same protocol and their antibacterial efficacy. *Rsc. Advances*. **4(8):** 3974-3983.

Ali, M.A. (2014). *Cassia fistula* Linn: A review of phytochemical and pharmacological studies. *Int. J. Pharm. Sci. Res*. **5(6):** 2125-2130. https://doi.org/10.13040/IJPSR.0975-8232.

Almuhayawi, M.S., Alruhaili, M.H., Solimcan, M.K., Tarabulsi, M.K., Ashy, R.A., Saddiq, A.A., Selim, S., Alruwaili, Y. and Salem, S.S (2024). Investigating the *in vitro* antibacterial, antibiofilm, antioxidant, anticancer and antiviral activities of zinc oxide nanoparticles biofabricated from *Cassia javanica*. *PLoS One*. **19(10):** e0310927.

AL-Musawi, B.H. and AL-Abedy, A. N. (2020). Genetic variability analysis of some selected rice (*Oryza sativa* L.) genotypes in Iraq. *International Journal of Agricultural and Statistical Sciences*. **16(1):** 191-197.

Al-Shugeairy, Z.K., Alogaidi, F.F. and Al-Abedy, A.N. (2021). Using a new method to induce targeted genetic mutations in Potato virus Y (PVY). *Journal of Agricultural and Statistical Sciences*. **17(1):** 2123-2131.

Bailey, N A., Booth, J. and Clark, B.C. (2006). Characterization of drug nanoparticles by atomic force microscopy. *NSTI-Nanotechnology*. **2(1):** 739-743.

Barapatre, A., Aadil, K.R. and Jha, H. (2016). Synergistic antibacterial and antibiofilm activity of silver nanoparticles biosynthesized by lignin-degrading fungus. *Bioresources and Bioprocessing*. **3:** 1-13.

Benakashani, F., Allafchian, A.R. and Jalali, S.A.H. (2016). Biosynthesis of silver nanoparticles using *Capparis spinosa* L. leaf extract and their antibacterial activity. *Karbala International Journal of Modern Science*. **2(4):** 251-258. https://doi.org/10.1016/j.kijoms.2016.08.004.

Bhalodia, N.R., Nariya, P.B., Acharya, R., Shukla, V. (2012). *In vitro* antibacterial and antifungal activities of *Cassia fistula* Linn. fruit pulp extracts. *Ayu*. **33:** 123.

Bhatnagar, M., Vimal, S., Vyas, Y., Sharma, D. and Sharma, K. (2010). Antioxidant activity of fruit pulp powder of *Cassia fistula*. *Pharmacognosy Journal*. **2(8):** 219-228.

Chen, K., Wang, F., Liu, S., Wu, X., Xu, L. and Zhang, D. (2020). *In situ* reduction of silver nanoparticles by sodium alginate to obtain silver-loaded composite wound dressing with enhanced mechanical and antimicrobial property. *International Journal of Biological Macromolecules*. doi.org/10.1016/j.ijbiomac.2020.01.156.

Choudhary, M.K., Kataria, J., Cameotra, S.S. and Singh, J. (2015). A facile biomimetic preparation of highly stabilized silver nanoparticles derived from seed extract of *Vigna radiate* and evaluation of their antibacterial activity. *Applied Nanosciences*. **6(1):** 105-111.

Danish, M., Singh, P., Mishra, G., Srivastava, S., Jha, K.K. and Khosa, R.L. (2011). *Cassia fistula* Linn. (Amulthus)-An important medicinal plant: A review of its traditional uses, phytochemistry and pharmacological properties. *J. Nat. Prod Plant Resour*. **1(1):** 101-118.

Ganesan, A. and Rengarajan, J. (2024). Green synthesis of chitosan nanoparticles using *Cassia fistula* leaf extract: Evaluation of antimicrobial, antioxidant, antibiofilm and cytotoxic activities. *3 Biotech*. **14(10):** 223.

Greeshma, A.J., Ramani Pushpa, R.N, Lakshmi Kavitha, K. and Srinivasa Rao, T. (2024). Efficacy of resveratrol and ursolic acid on biofilm inhibition and antimicrobial resistance of *Streptococcus uberis*. *Indian Journal of Animal Research*. **58(12):** 2177-2183. doi: 10.18805/IJAR.B-4697.

Gupta, M., Mazumder, U.K., Rath, N., Mukhopadhyay, D.K. (2000). Antitumor activity of methanolic extract of *Cassia fistula L.* seed against *Ehrlich ascites Carcinoma. Journal of Ethnopharmacology*. **72:** 151-156.

Harborne, J. (1998). Methods of extraction and isolation. *Phytochemical Methods*. **3:** 60-66.

Ihum, T.A., Oledibe, C.F., Kurrah, A.I., Akande, E.J., Ajayi, O.A., Olatunji, J.T. and Lawal, Z.O. (2024). Inhibitory activity of *Cassia fistula* synthesized selenium nanoparticles against moulds of groundnut. *Fudma Journal of Sciences*. **8(3):** 242-248. https://doi.org/10.33003/fjs-2024-0803-2548.

Jehangir, A., Nagi, A.H., Shahzad, M., Zia, A. (2010). The hepatoprotective effect of *Cassia fistula* (AMALTAS) leaves in isoniazid and rifampicin induced hepatotoxicity in rodents. *Biomedica*. **26:** 25-29.

Jothy, S.L., Zuraini, Z. and Sasidharan, S. (2011). Phytochemicals screening, DPPH free radical scavenging and xanthine oxidase inhibitiory activities of *Cassia fistula* seeds extract. *Journal of Medicinal Plants Research*. **5(10):** 1941-1947. http://www.academicjournals.org/JMPR.

Jung, H.A., Ali, M.Y. and Choi, J.S. (2016). Promising inhibitory effects of anthraquinones, naphthopyrone and naphthalene glycosides, from *Cassia obtusifolia* on α-glucosidase and human protein tyrosine phosphatases 1B. *Molecules*. **22(1):** 28.

Kent, R.D. and Vikesland, P.J. (2012). Controlled evaluation of silver nanoparticle dissolution using atomic force microscopy. *Environ Sci Technol*. **46(13):** 6977-6984.

Kanwal, A., Singh, B.J., Kumar, S., Sehgal, R., Kumar, S., Upadhyay. and Singh, R. (2025). Green synthesis of nanoparticles using pea peel biomass and their assessment on seed germination of tomato, chilli and brinjal Crop. *Indian Journal of Agricultural Research*. 1-10. doi: 10.18805/IJARe.A-6301.

Khan, M.S. and Alam, K. (1996). Homestead flora of Bangladesh. 1st. edition Bangladesh Agricultural Research Council (BARC). 95-96.

Kirtikar, K.R. and Basu, B.D. (2006). Indian medicinal plants. *International Book Distributors*. **2:** 856-860.

Korbekandi, H., Ashari, Z., Iravani, S., Abbasi, S. (2013). Optimization of biological synthesis of silver nanoparticles using *Fusarium oxysporum*. *Iranian Journal of Pharmaceutical Research: IJPR*. **12(3):** 289.

Kuo, Y.H., Lee Ping Hung, K.Y., L.P. and Wein Yung Shun, W.Y. (2002). Four new compounds from the seeds of *Cassia fistula*. *Journal of Natural Products*. **65(8):** 1165-1167.

Laloy, J., Minet, V., Alpan, L., Mullier, F., Beken, S., Toussaint, O. and Dogné, J.M. (2014). Impact of silver nanoparticles on haemolysis, platelet function and coagulation. *Nanobiomedicine*. **1:**4.

Luna-Vázquez-Gómez, R., Arellano-García, M.E., García-Ramos, J.C., Radilla-Chávez, P., Salas-Vargas, D.S., Casillas-Figueroa, F., Balam Ruiz-Ruiz, Nina Bogdanchikova and Pestryakov, A. (2021). Hemolysis of human erythrocytes by Argovit™ Ag-NPs from healthy and diabetic donors: An *in vitro* study. *Materials*. **14(11):** 2792.

Meena, P.L., Surela, A.K., Poswal, K., Saini, J.K. and Chhachhia, L.K. (2022). Biogenic synthesis of Bi_2O_3 nanoparticles using *Cassia fistula* plant pod extract for the effective degradation of organic dyes in aqueous medium, Biomass Convers. *Biorefin*. https://doi.org/10.1007/s13399-022-02605-y.

Mohammed, A.E., Al-Qahtani, A., Al-Mutairi, A., Al-Shamri, B. and Aabed, K. (2018). Antibacterial and cytotoxic potential of biosynthesized silver nanoparticles by some plant extracts. *Nanomaterials*. **8(6):** 382.

Muteab, M.M. and AL-Abedy, A.N. (2025). Genetic diversity of *Trichoderma* spp. Isolated from soils in the Iraqi Environment. *Agricultural Science Digest*. 1-8. doi: 10.18805/ag.DF-734.

Omran, A.M. (2024). Green route synthesis of silver nanoparticles driven by *Cassia fistula* flower extract: Characterization, antioxidant, antibacterial, anticancer and photocatalytic assessment. *Biomass Conversion and Biorefinery*. **14(17):** 20405-20418.

Park, H., Park, S., Roh, J., Kim, S., Choi, K., Yi, J., Kim, Y., Yoon, J. (2013). Removal characteristicsof engineered nanoparticles by activated sludge. *J. Ind. Eng Chem*. **19:** 614-619.

Pawar, A.V. and Killedar, S.G. (2017). Uses of *Cassia fistula* Linn as a medicinal plant. *International Journal for Advance Research and Development*. **2(3).** doi:10.5281/zenodo.8079450.

Prastiyanto, M.E., Kartika, A.I., Sri, D. and Radja, O.K. (2022). Biopros-pecting of bacterial symbionts of sponge *Spongia officinalis* from Savu Sea, Indonesia for antibacterial potential against multidrug-resistant bacteria. *Biodiversitas*. **23(2):** 1118-1124. doi: 10.13057/biodiv/d230256.

Rother, R.P., Bell, L., Hillmen, P. and Gladwin, M.T. (2005). The clinical sequelae of intravascular hemolysis and extracellular plasma hemoglobin: A novel mechanism of human disease. *Jama*. **293(13):** 1653-1662.

Sahayaraj, K. and Rajesh, S. (2011). Bionanoparticles: Synthesis and Antimicrobial Applications. In: Science against Microbial Pathogens: Communicating Current Research and Technological Advances. [Méndez Vilas A, (editor)]. Badajoz: Formatex Research Center. p: 228-244.

Sharma, D.K., Sharma, S.K., Lonare, M.K., Kaur, R. and Dumka, V.K. (2022). Pharmacological evaluation of antipyretic, analgesic and anti-inflammatory activities of ethanolic extract of *Cassia fistula*. *Indian Journal of Animal Research*. **56(4):** 483-488. doi: 10.18805/IJAR.B-4531.

Verma, S. (2016). Pharmacological Review on *Cassia fistula* Linn (Amaltas). *International Journal of Pharmaceutical, Chemical and Biological Sciences*. **6(3):** 332-335.