



# Green Synthesis and Characterisation of Silver Nanoparticles Using *Cassia tora* Seed Extract and Investigation of Antibacterial Potential

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## Abstract

Nanoparticle research is fascinating and getting hold of consequences due to the wide variety of applications in the biomedical field. Green synthesis of nanoparticles is a cost-effective and eco-friendly approach. It can be synthesised using fungi, algae, plant, yeast, bacteria, microbial enzymes etc. Our current research study focuses on the green synthesis of silver nanoparticles using seed extract of *Cassia tora*. The colour change from yellow to red colour confirms the formation of silver nanoparticles. The synthesised silver nanoparticles were characterised by Ultraviolet–Visible spectroscopy, Fourier-transform infrared (FTIR), X-ray diffraction analysis (XRD), Scanning Electron Microscopy (SEM) and antibacterial efficacy against three different strains were analysed. The surface plasmon resonance of synthesised AgNPs using *Cassia tora* seed extract shows maximum absorption peak at 423 nm in UV–visible spectroscopy. X-ray diffraction displays the crystalline nature of synthesised AgNPs and they exhibited four distinct peaks at 36.69°, 42.92°, 63.27° and 76.46°. The particle size of synthesised AgNPs observed through SEM was found to be 55.80 nm, 58.97 nm, 61.06 nm, 63.26 nm and 64.80 nm. *S.aureus* exhibited maximum zone of inhibition of 12 mm and 13 mm when treated with 25 and 50 µl of the synthesised nanoparticles. Thus, the green synthesised silver nanoparticle using *Cassia tora* seed extract proved to possess strong anti-bacterial activity.

**Keywords** Silver nanoparticle · *Cassia tora* · Seed extract · Antibacterial · Green synthesis

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## Introduction

Infectious diseases have a great incidence and high outbreak rate. It may cause due to bacteria, fungi, parasite or viruses. They can be communicable or non-communicable diseases. Common signs for infectious diseases are diarrhoea, fever, coughing, severe throat, muscle pain, weakness of muscle and bones. Person who gets infection are recommended to consult a physician and to follow up treatment or else it can become a life-threatening problem [1]. According to world health organisation, infectious diseases can be divided into three categories such as high-level mortality, high population and diseases caused due to their rapidness and unexpected nature of outcome. They can multiply hastily from one host to another [2]. A survey described that about 3.0 million deaths occurred due to pneumoniae and diarrhoea illness and 1.3 million deaths due to tuberculosis. Worldwide, single infectious diseases like HIV/AIDS, tuberculosis and malaria are the major causes of death, top ten ranks in global list for causes of death [3]. Among, the common diseases like heart disease, cardiac arrest, stroke, Alzheimer, lung cancer and diabetes, bacterial infections are more common in humans. Urinary tract infection is one of the bacterial infections caused by *E.coli* and Enterobacter like *Klebsiella* and around 30 to 50% of men are suffered from these contagious diseases. Bacterial vaginosis is mostly common in women [4]. Skin, the largest and important organ of the body, provides protection against infection. However, due to the stability of pathogenic organisms, they expose different type of illness like cellulitis, impetigo, leprosy [5]. Remedy for these contagious sicknesses are drugs, vaccines and clean surface, hygiene. Amoxicillin, doxycycline, ciprofloxacin, clindamycin, metronidazole, azithromycin, cephalixin, sulfamethaxozole are regular antibiotics for ailment [6]. Traditional medicine or herbal plants were used for treatment of the above-mentioned diseases due to unavailability of drugs and vaccines. However, most of the drugs mimic or else get inspired from traditional herbal medicine chemical components [7]. The main advantages of using herbal medicines are safety, durability, inexpensive and do not cause any aftermath. They demonstrate promising effect in disease like cancer, diabetes, cardiovascular infection, atherosclerosis, cholesterol, cognitive complications and toxicity [8]. One of the edible herbal therapeutic plants is *Cassia tora* also named as *Senna tora*. Foetid *cassia*, the sickle *senna* and wild *senna* are the English name for this plant. It belongs to the family *Leguminosae*. It is usually grown in the wasteland. The roots, leaves and seeds of this herbal plant possess remedial value [9]. The important chemical constituents in *Cassia tora* roots are 1, 3, 5-trihydroxy-6, 7-dimethoxy-2-methylanthraquinone and  $\beta$ -sitosterol. *Cassia tora* seeds have Naphtho- $\alpha$ -pyrone-toralactone, chrysophanol, physcion, emodin, rubrofusarin. Kaempferol, palmitic and d-tartaric acids uridine, quercitin and isoquercitrin are found in the leaves of *C. tora* [10]. The seeds are 1 cm long, 3–4 mm thick, greenish to brown in colour, smooth and shiny. Similar to emodin compound, glucosidal substances may be seen in seeds of *Cassia tora*. Seeds from this plant protect the liver from the damage and used to treat herpes. They can be used in ayurvedic medicine as anthelmintic, ophthalmic, liver tonic, laxative, cough relief and treatment for constipation, ringworm, bronchitis, cardiac disorders. It is also used as protein source for birds and livestock. Commercially, roasted *C. tora* seeds are used instead of coffee and *C. tora* gums are used as binding and emulsifying agent [11]. Seed paste with buttermilk can be functional for ringworm and scabby infections [12]. Dosage of powder seed is 1 to 3 g for therapeutic treatment. High dosage intake may cause adverse effects like loose motions, reduced sperm

count, red blood cell level will get lower and weight loss can be noted [13]. Commercially, it is also used as an antiseptic cream as it possesses broad spectrum of antibacterial activity towards pathogenic microorganisms [14].

Nanotechnology is the future approach in research study which is spreading all over the world. Basic research and development in nanotech products are carbon nanotubes, quantum dots, etc. [15, 16]. They are used in medical imaging, nano-glue, nanorepel, and in computers as 10 nm chip. It is commonly used in tennis ball to keep bouncing for longer duration. It can be practical to industrial and medicinal field as nanomedicine like developing vaccine, follow up of patients, diagnosis and to deliver drug [17, 18]. Peculiar properties of nanoparticles are small in size, large surface area and mechanical property helpful in antibiotic, antioxidant and anticancer activity. Important branch in nanomedicine is preparation of metal nanoparticle like silver, gold, platinum, copper, zinc, magnesium and titanium. Top-down and bottom-up approaches are two methods for preparing nanoparticles [19]. Synthesis of nanomaterial is an innovative idea but also, they had some challenges like lack of mechanism understanding, expensive, problem in device and toxicity. To overcome this, a new technique termed as green synthesis method was introduced. Green synthesis of metallic nanoparticles is produced from microorganism like bacteria, fungi, algae and plant source like leaves, roots, stem, flower, fruit and seed [20]. It gains their attention due to low toxicity, cheap, eco-friendly and less time consumption, minimization of waste and reduction of pollution. Plant encompasses good bioactive metabolites such as alkaloid, tannins, carbohydrate, proteins, polyphenols, flavonoids, amines and aldehydes which can condense, stabilise and act as capping agent for metal nanoparticles during the conversion from metal ions [21]. Among the various metallic nanoparticles, AgNP emerge as a most prominent non-toxic nanoparticle to human health. Antimicrobial, anticancer studies were reported in silver nanoparticles and other functions like anti-inflammation, antioxidant were seen [22]. They will break through into the cell wall of bacteria and transform the cell membrane structures which escort cell death. Following this mechanism, the development of natural antibacterial agent is much needed to limit the multidrug resistant pathogens [23]. The list of natural product mediated synthesis of silver nanoparticles and the inhibitory effect on certain bacterial species were represented in Table 1. The main objective of our study is the green synthesis of silver nanoparticle from the aqueous seed extract of *Cassia tora* and characterisation of the synthesised AgNP by UV–visible spectroscopy, FT-IR, XRD and SEM. The antimicrobial efficiency of the synthesised silver nanoparticle against pathogens such as *Staphylococcus aureus*, *Escherichia coli* (MTCC 433) and *Klebsiella pneumoniae* (MTCC 432) is shown in Fig. 1.

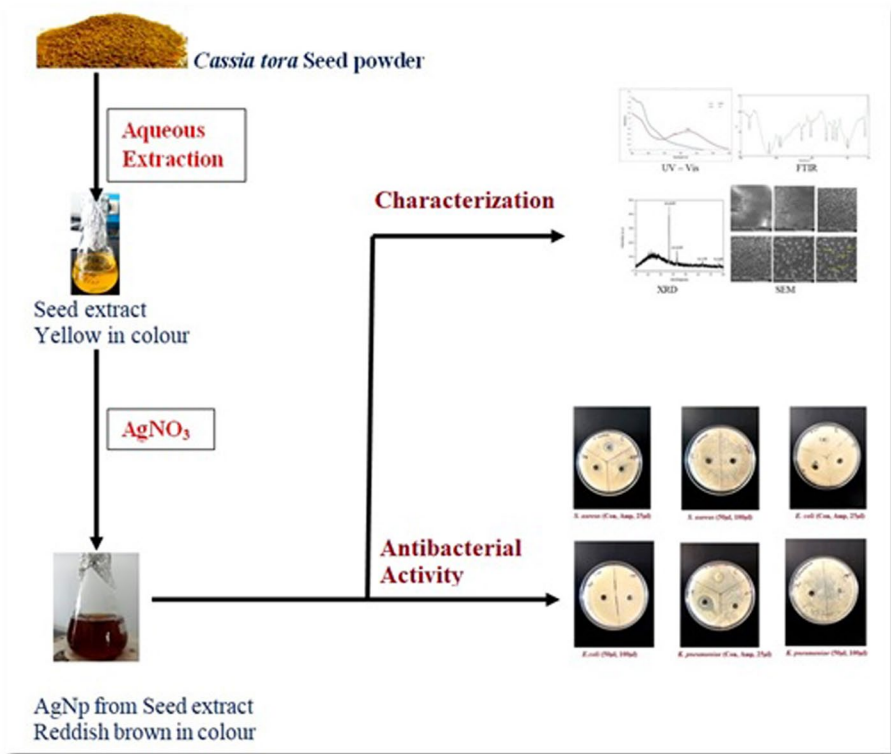
## Materials and Methods

### Chemicals

Silver nitrate ( $\text{AgNO}_3$ ), Luria Bertani agar, Luria Bertani broth, concentrated hydrochloric acid, concentrated sulphuric acid, Mayer's reagent, 5% ferric chloride and 10% ferric chloride, Fehling's solution A and Fehling's solution B, 1% copper sulphate, 5% sodium hydroxide, chloroform and glacial acetic acid of analytic grade were used in the study.

**Table 1** The list of natural products used to synthesis of silver nanoparticles

S. no	Name of natural agent	Antibacterial activity upon	References
1	Piper nigrum seed extract	Bacillus subtilis ( <i>B. subtilis</i> ) and Escherichia coli ( <i>E. coli</i> )	[24]
2	Pomegranate peel	<i>L. monocytogenes</i>	[25]
3	Phoenix dactylifera Seed extract	Bacillus subtilis, Escherichia coli, Staphylococcus aureus, methicillin-resistant Staphylococcus aureus and Streptococcus pneumoniae,	[26]
4	Pteris vitata extract	<i>P. aeruginosa</i>	[27]
5	Punica granatum peel extracts	Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumoniae and Escherichia coli	[28]
6	Cannabis sativa Leaf Extracts	Escherichia coli, Klebsiella pneumoniae, Pseudomonas fluorescens and Staphylococcus aureus	[29]
7	Grapefruit peel extracts	Staphylococcus aureus, clinical Enterococcus faecalis and <i>S. aureus</i>	[30]



**Fig. 1** Graphical representation of synthesis and characterisation of AgNP. From *C. tora* seed extract and their antibacterial activity

## Bacterial Strain

*Staphylococcus aureus*, *Escherichia coli* (MTCC 433), *Klebsiella pneumoniae* (MTCC 432) were procured from Microbial Type Culture Collection and Gene Bank (MTCC), India.

## Collection of Seed

*Cassia tora* seed were purchased from LIFERR Ayurveda marketed by Sinhal Herbs, industrial estate, Neemuch, Madhya Pradesh, India. The seeds were authenticated by a botanist. About 200gm of *C.tora* seed were sun dried for 24 h and finely powdered. Then, it was stored it in a clean air tight container.

## Seed Extraction

The aqueous seed extraction method was carried out in the ratio of 1:30. Fifteen grams of powdered seed was dissolved in 300 ml of distilled water and kept in hot plate for 2 h at

50 °C. Then, it was filtered through Whatman No 1 filter paper and stored at 4 °C for further use [31].

## Synthesis of Silver Nanoparticle from *Cassia tora* Seed Extract

1 M aqueous solution of silver nitrate ( $\text{AgNO}_3$ ) was prepared and used for synthesis of silver nanoparticles. To 100 ml of 1 M  $\text{AgNO}_3$ , 100 ml of *Cassia tora* seed extract (25 ml made upto 100 ml) was added and stirred overnight. The colour change from yellow to red colour indicates the formation of silver nanoparticles and it was characterised by UV–visible spectroscopy. The synthesised silver nanoparticles were subjected to high-speed centrifugation at 10,000 rpm for 10 min. Then, it was dried in thermostat for 1 h at 100 °C. Finally, it was powdered and used for further characterisation [32].

## Characterisation of Silver Nanoparticle from *Cassia tora* Seed Extract

### UV–Visible Spectroscopy

The formation of silver nanoparticle by the reduction of silver ions was determined by ultraviolet–visible spectrophotometer instrument in the range of 200–800 nm. Distilled water was used for the baseline correction [33].

### X-Ray Diffraction Study

The synthesised silver nanoparticle powder was subjected to X-ray diffractometer to analyse its nature. The powder was placed on the slide and the diffraction pattern was operated at a voltage of 30 kV and current 12 mA. The data were obtained over the range of 20 to 80 °C ( $2\theta$ ) with a scanning speed of 8.0000 deg/min and step width of 0.200 deg using a nickel monochromator [34].

### Scanning Electron Microscopy

The fine powder of the obtained silver nanoparticle was used for the visualization of morphological features and particle size using scanning electron microscopy (SEM) analysis using (TESCAN VEGA3). The powder sample was dispersed in a very less amount of ethanol and sonicated for equal distribution of the nanoparticle. Then, a small droplet was placed on the SEM grid using a micropipette, allowed to dry followed by visualization [35].

### Fourier-Transform Infrared Spectroscopy

To identify the functional groups, present in the synthesised silver nanoparticles, Fourier-transform infrared spectroscopy recordings were performed in the range of 400–4000  $\text{cm}^{-1}$  using (JASCO P2000, USA) [36].

## Preparation of Bacterial Suspensions

Bacterial strains were cultured in Luria Bertani broth and kept in a shaker incubator for 24 h at 37 °C [37].

## Antibacterial Activity

### Agar Well Diffusion Method

Luria Bertani agar was prepared. Fifty microlitres of *S.aureus*, *E.coli* and *K.pneumoniae* was added into the petri plate using micropipette. It was evenly spread all over the plate using sterile L rod. The wells of 5 mm diameter were made and the synthesised AgNO<sub>3</sub> of 25 µl, 50 µl and 100 µl was added. *Cassia tora* seed extract of 100 µl and 100 µl of antibiotic ampicillin served as positive control [38].

## Results and Discussion

In the present study, *Cassia tora* seeds were dried in sun light for 24 h and finely powdered by a mixer. Later, the seed powder was used for aqueous extraction method. Sterile-distilled water and seed extract were taken in the ratio 1:30 and it was kept in a hot plate and filtered. Thus, extracted aqueous seed appeared in yellow colour which is shown in Fig. 2.

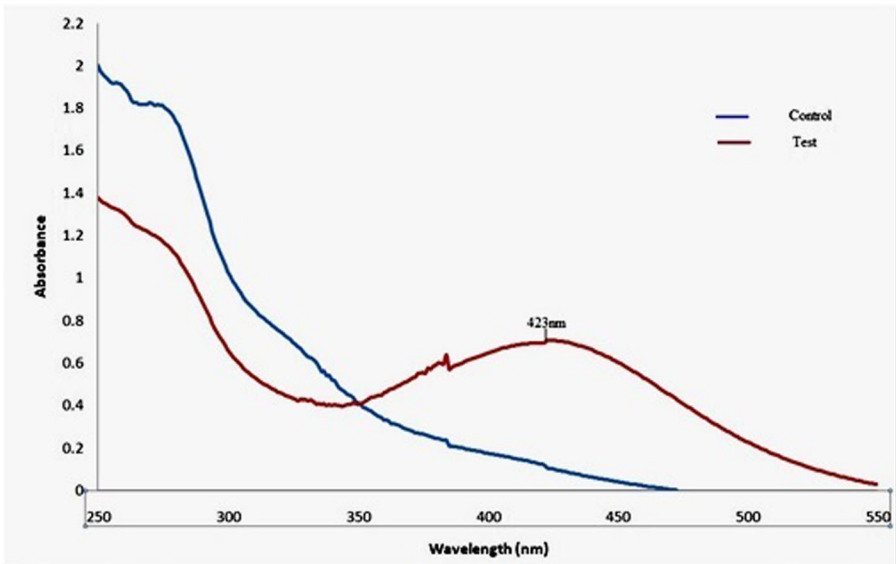
Visual assessment of colour change confirms the presence of silver nanoparticle. Reduction of silver ions to silver nanoparticle while adding seed extract of *Cassia tora* is the reason for colour change. AgNO<sub>3</sub> was a colourless solution and it was mixed with seed extract in 1:1 ratio (100 ml of 1 M AgNO<sub>3</sub> + 100 ml of *C. tora* seed extract) and it is yellow in colour. It was kept in magnetic stirrer for overnight. The observation of colour change from yellow to reddish brown indicates the formation of silver nanoparticles which was compared in Fig. 3.

After AgNPs were synthesised, it undergoes different characterisation methods. As simple and reliable method is UV–visible spectroscopy to monitor the stability of synthesised AgNP. The combined vibrations of free electrons in the metal nanoparticle give rise to surface plasmon resonance (SPR) absorption band. Previous studies reported that spherical-shaped nanoparticles were observed in the SPR region about 410 to 450 nm [39]. As shown in Fig. 4, the synthesis of AgNPs was confirmed in the highest absorption peak at



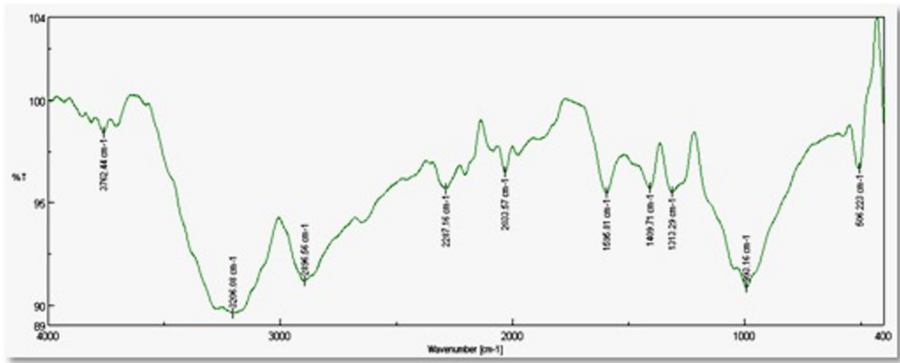
**Fig. 2** The work flow of *Cassia tora* aqueous seed extraction was

**Fig. 3** Synthesis of AgNPs from *Cassia tora* seed extract **A** before synthesis of AgNPs, **B** After synthesis of AgNPs with *Cassia tora* seed extract. **A** *Cassia tora* seed extract + AgNO<sub>3</sub>, **B** AgNP from *Cassia tora* seed extract



**Fig. 4** UV–visible spectra for synthesised AgNP and *Cassia tora* seed extract





**Fig. 5** FTIR analysis of synthesised AgNP. The representative peaks were tabulated

**Table 2** FTIR Analysis of AgNP from *C.tora* seed extract

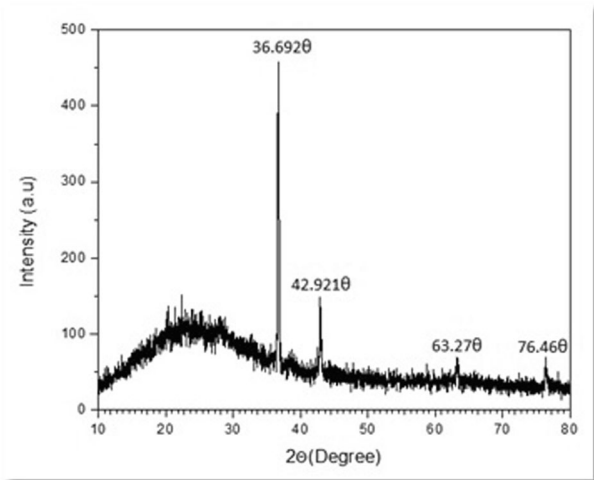
No	Peak appearance	Absorption region of AgNP seed extract	Functional group
1	Strong, broad	3206.08 cm <sup>-1</sup>	O–H stretching, alcohol group, intermolecular bonding
2	Medium	2896.56 cm <sup>-1</sup>	C–H stretching, alkane group
3	Medium	1595.81 cm <sup>-1</sup>	C=C stretching, cyclic alkene. N–H bending, amine group
4	Medium	1409.71 cm <sup>-1</sup>	O–H bending, alcohol and carboxylic acid group
5	Medium	1313.29 cm <sup>-1</sup>	O–H bending, phenol group
6	Strong, broad	993.16 cm <sup>-1</sup>	C=C bending, alkene group

423 nm and the seed extract of *C. tora* shows a band at 280 nm. The blue line describes control seed extract and the dark brown red line represents AgNPs which were synthesised.

To identify the functional group of biomolecules, FTIR was measured at 4000–400 cm<sup>-1</sup>. Prior examine detailed O–H hydroxyl group stretching vibration in region of 3100–3500 cm<sup>-1</sup> and C–H stretching in 2800–3000 cm<sup>-1</sup> IR region [40]. Another research study explains that the functional group of C–O, C–H, C=C, C=O and C–N was observed in region between 1800 and 900 cm<sup>-1</sup>. IR region from 1750 to 1600 cm<sup>-1</sup> represent (C=O) carbonyl functional group of flavonoids [41]. FTIR spectra showed prominent peaks at 3762.44, 3206.08, 2896.56, 2287.16, 2033.57, 1595.81, 1409.71, 1313.29 and 993.16 cm<sup>-1</sup> for AgNP and they exhibit the functional groups such as –OH stretching, –NH stretching, –CH bending, C–H stretching, C=C bending respectively in Fig. 5. C–H bending vibrations were resulted to absorptions which are below 1000 cm<sup>-1</sup>. The peak at 3762.44 and 3206.08 cm<sup>-1</sup> broad spectrum represents –OH vibration stretching of hydroxyl group from water, alcohol, phenols, carbohydrate, etc., and also amine N–H stretching. Another broad-spectrum peak at 993.16 cm<sup>-1</sup> found to be C=C bending alkene compound. Absorption in 2896.56 cm<sup>-1</sup> found to be C–H stretching which is alkane which was shown in Table 2. Therefore, FTIR results represent the presence of functional groups like alkane, alkene, phenol, carboxylic acid, aromatic compound, benzene and amine is responsible for capping and stabilisation of synthesised AgNPs.

AgNPs exhibit crystalline formation which is confirmed by XRD study. The X-ray diffraction configuration of synthesised AgNPs from *Cassia tora* seeds was shown in Fig. 6.

**Fig. 6** XRD pattern for synthesised AgNP



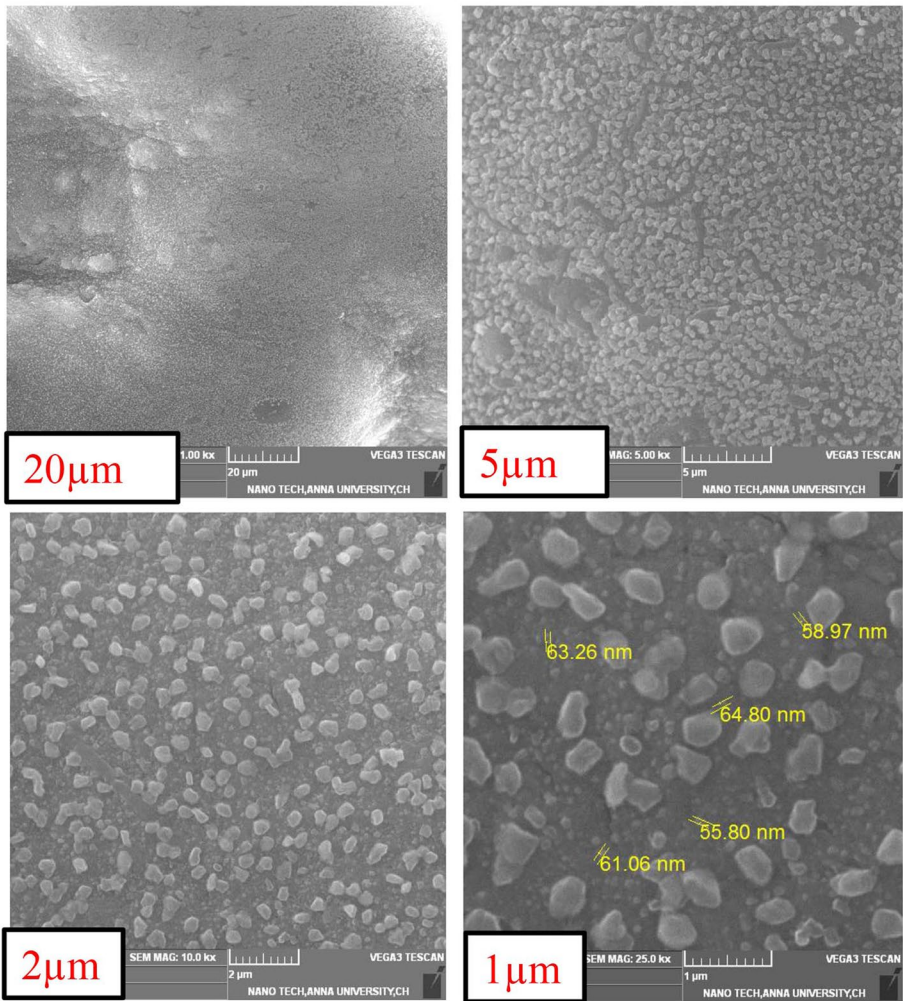
Four distinct peaks were observed at  $2\theta$  values of  $36.69^\circ$ ,  $42.92^\circ$  and  $63.27^\circ$  and  $76.46^\circ$  can be accredited to (111), (200), (220) and (311) respectively. XRD was operated at a voltage of 30 kV and 12 mA. The data were obtained in the range of 10 to 80  $^\circ\text{C}$ .

Topographical and morphological studies of synthesised AgNPs were determined by scanning electron microscopy. The AgNPs samples were used for SEM analysis. The abovementioned Fig. 7 represents the surface morphology of AgNPs which is crystalline in nature, truncate and non-aggregated. Different sizes of AgNPs were experimental such as 55.80, 58.97, 61.06, 63.26 and 64.80 nm. Therefore, average size of AgNPs from *Cassia tora* seeds may be 60 nm.

The synthesised and characterised AgNP from *Cassia tora* seeds were evaluated for their antibacterial potential. Green synthesised AgNP from *Cassia tora* seed; control antibiotic ampicillin and aqueous seed extract were tested against the pathogenic bacteria such as *Staphylococcus aureus*, *Escherichia coli* MTCC 433, *Klebsiella pneumoniae* MTCC 432 in the concentration of 25, 50 and 100  $\mu\text{l}$  which were tabulated in Table 3.

Figure 8 shows the zone of inhibition of AgNP against the pathogens. The result revealed 12 mm and 13 mm size zone of inhibition in *S. aureus* in 25 and 50  $\mu\text{l}$  concentration. The control seed extract showed 13 mm size zone of inhibition. *E. coli* and *K. pneumoniae* showed their resistance to AgNP from *Cassia tora* seed extract.

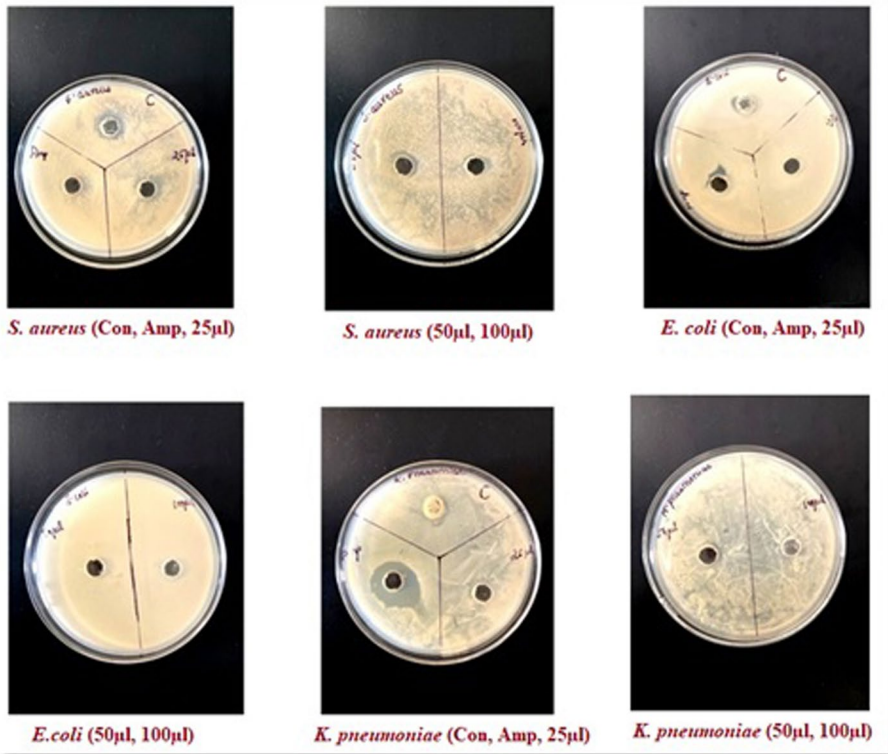
Green synthesised nanoparticles showed potential antibacterial activity against variety of bacterial pathogens [42–44]. In consistent with the previous studies, aqueous, ethanol and methanol extract of *Cassia tora* leaves, roots were used for the synthesis and characterisation of AgNP. Also, *Cassia tora* leaves, roots and seeds are having potent medicinal properties and they were well-known for their antimicrobial properties. *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Salmonella* showed their susceptibility to *Cassia tora* leaf, root and seeds also gum resin or paste from *C. tora*. Novel approach of our study is to synthesise AgNPs from *C. tora* seeds which is done and characterised by UV–visible spectroscopy, FTIR, XRD and SEM. Antibacterial studies revealed minor effectiveness and tested microorganisms showed their resistance. For future studies, synergistic effect of seeds or silver nanoparticle with antibiotic and increasing the concentration of synthesised AgNPs may yield good results.



**Fig. 7** SEM images of AgNP from seed extract of *Cassia tora*. Four different magnification of SEM images were represented (20, 5, 2 and 1 μm)

**Table 3** Inhibitory effect of AgNP from *C. tora* seed extract

Sample	Concentration (μl)	Zone of inhibition (mm)		
		<i>S. aureus</i>	<i>E. coli</i>	<i>K. pneumoniae</i>
Ag nanoparticle from <i>Cassia tora</i> seed extract	25	12	0	0
	50	13	0	0
	100	0	0	0
Control seed extract		13	0	0
Ampicillin antibiotic		10	0	20



**Fig. 8** Antibacterial activities of Synthesised AgNP from seed extract *C. tora* against pathogens *S. aureus*, *E. coli* and *K. pneumoniae*. The antibacterial assay was performed by well diffusion assay. The bacterial species were spread in the agar plate. Different concentrations of synthesised AgNPs were loaded in the well. After the incubation period, the zone of inhibition was measured and recorded

## Conclusion

Seeds of *Cassia tora* possessing gelling agent are applied in the food industries. Seeds along with leaves and roots of *Cassia tora* have many medicinal applications. They help out in digestion, pain relief, liver disorder, visual improvement, maintaining body temperature, bowel relaxation and protection from infections. In our current study, *Cassia tora* seeds were used in the synthesis of AgNPs, a novel, non-toxic, cost-effective and eco-friendly method. The presence of biomolecules in *C. tora* seeds may act as a reducing and capping agent for AgNPs. The characterised AgNPs showed UV–visible spectroscopy peak at 423 nm. Aldehydes, alkane, alkene, phenols, carboxylic acid and aromatic compounds were observed in FTIR analysis. XRD confirms the crystalline formation of AgNPs. SEM image results exhibit truncated shape silver nanoparticle with a size range between 55 and 65 nm. Antibacterial activity of synthesised AgNP was limited. Therefore, current research indicates a novel AgNPs from seed extract of *Cassia tora* were synthesised at a room temperature with a simple, rapid method without using any harmful agents. Thus, it can be used in large-scale industries, biomedical applications, nanomedicine, drug development and research.

**Author Contribution** SN and AKP designed the experiment; SN and PS performed the experiments. SN, AKP, SKA and AAB analysed the results. SN wrote the manuscript. AKP and SKA edited the final version of the manuscript.

**Data Availability** The authors confirm that the data supporting the findings of this study are available within the article and its supporting information.

## Declarations

**Ethics Approval** This article does not contain any studies with human participants or animals performed by any of the authors.

**Consent to Participate** The authors agreed to participate in this work.

**Consent for Publication** The authors agreed to publish this work.

**Conflict of Interest** The authors declare no competing interests.

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