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Green synthesis characterization and antimicrobial activities of Silver Nanoparticles using leaves of *Chrozophora Tinctoria*

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The study of particles at the nanoscale is known as nanotechnology, which has found various applications in biomedicine, industry, and agriculture. The biosynthesis of nanoparticles is gaining great attention of scientists due to their unique characteristics as well as the green method is cost effective and safe method. The aim of this investigation was to produce silver nanoparticles by utilizing the ethanolic extract of *Chrozophora tinctoria* leaves, followed by an assessment of their effectiveness in inhibiting microbial growth via the disc diffusion technique. The biogenic nanoparticles were characterized through FTIR, XRD, UV-Vis and SEM. The peak absorbance of FTIR at 3221cm^{-1} indicated the stretching vibration of hydroxyl groups which are specific to alcohols. The 2912cm^{-1} peak of absorbance showed the presence of amine salts while the absorption bands at 1466, and 1026cm^{-1} were assigned to C–H and C–O functional groups. The cubic face-centered form of nanoparticles was confirmed through XRD at 2θ. Confirmation of silver nanoparticles formation was achieved through the measurement of the surface plasmons resonance at λ-max = 400 nm. The nanoparticles were observed using scanning electron microscopy and appeared to be cubical in shape, with an average size of 73.792±35.243 nm. Their antimicrobial properties were tested against three types of bacteria and three types of fungi. Results revealed that the nanoparticles had shown strongest antibacterial activity against *S. aureus* at the maximum concentration of biogenic nanoparticle (300 mg/ml) with highest zone of inhibition (25.55±1.714 mm) followed by *E. coli* and *P. aeruginosa* (24.77±1.708 mm), (22.0±1.802 mm) and the lowest zone of inhibition (5.77±0.386 mm) shown by *E. coli* at minimum (100 mg/ml) nanoparticle concentration followed by *P. aeruginosa* (6.766±0.929) and *S. aureus* (7.863±0.703). The silver nanoparticles produced through biosynthesis displayed notable antifungal properties against *Aspergillus flavus* at the highest concentration of 300 mg/ml, with a zone of inhibition of 26.88±0.835 mm. The nanoparticles also exhibited antifungal activity against *A. terreus* (25.00±1.00 mm) and *C. albicans* (26.55±1.345 mm) at this concentration. However, the lowest antifungal activity was observed against *C. albicans* (5.40±1.058 mm) at the lowest nanoparticle concentration of 100 mg/ml. Hence, it's determined that the *C. tinctoria* mediated silver nanoparticles could be used as potent therapeutic agent in treatment of various microbial infections.

Keywords: Silver nanoparticles, *in vitro* antimicrobial activity, *Chrozophora tinctoria*

INTRODUCTION

Researchers have increasingly focused on traditional medicine, specifically herbal remedies, to address various health concerns. The World Health Organization recognizes the valuable contribution of herbal medicine to healthcare systems, particularly in less developed nations, for prevention, promotion, and treatment purposes. Traditional medicine, including herbal medicines, has been an integral part of healthcare systems worldwide for centuries (Karbab et al. 2020).

Nanotechnology has a tremendous impact on medicine and healthcare in addition to agriculture. treatment delivery to particular bodily sites using nanoparticles can increase treatment efficacy and minimize negative effects. For the early diagnosis of diseases, nanoscale sensors can identify biomarkers, and nanomaterials can be utilized in regenerative medicine and tissue engineering applications (Li et al.

2022). Along with other applications, nanotechnology has made significant contributions to the field of pharmaceuticals, revolutionizing drug delivery, diagnostics, and therapies. Nanoparticles can also be engineered to carry drugs, enhancing their stability, solubility and targeting capabilities. Nanocarriers such as liposomes, polymeric nanoparticles and dendrimers can deliver drugs to specific cells or tissues, improving therapeutic efficacy and reducing side effects (Mostafavi et al. 2019).

Nanoparticles are small particles that typically have sizes between 1 and 100 nanometers (nm). They can be manufactured from a variety of substances, including metals, metal oxides, polymers, and organic molecules. Due to their small size, nanoparticles exhibit physical, chemical, and biological differences from their larger counterparts. They are generally defined by their nanometer-scale size and can take on various shapes such as spheres, rods, tubes, or cubes depending on their surroundings. The methods and materials used in their production also contribute to their unique properties. The characteristics and behavior of nanoparticles are substantially influenced by their size and form (Batlle et al. 2022).

There are several methods for synthesizing nanoparticles, such as physical chemical and biological method. Chemical method involves the reduction of metal salts using reducing agents in a solution**.** By altering reaction parameters like temperature, pH, and reactant concentrations, one may modify the size and structure of the nanoparticles. (Yaqoob et al. 2020). Synthesizing nanoparticles through physical and chemical methods can be costly, time-consuming, and pose risks to living organisms. In contrast, the biological or green approach is inexpensive, environment friendly, and non-toxic. As a result, scientists have increasingly turned to using natural resources such as plant extracts or microorganisms to synthesize nanoparticles. This green method relies on the reducing and stabilizing properties of biological components to regulate the formation of nanoparticles. Green synthesis of nanoparticles refers to the use of natural sources, such as plant extracts, microorganisms, or biocompatible materials, in the synthesis process. It offers several advantages, including environmental sustainability, biocompatibility, and cost-effectiveness (Zhang et al. 2020).

Characterization of nanoparticles is a crucial step to understand their physical, chemical, and structural properties. It helps the researchers to determine the size, shape, composition, surface characteristics, and other relevant parameters of nanoparticles. Nanoparticles can be characterized through various parameters such as Transmission Electron Microscopy (TEM) provides high-resolution imaging of nanoparticles. Researchers can use TEM to visualize the shape, size and distribution of non-particles at nanoscale, and obtained information about their crystal structure and lattice fringes. SEM, on the other hand, allows for examination of nanoparticles on a larger scale and provides detailed information about their surface morphology, size, and shape. When coupled with EDS, SEM can also be used for elemental analysis and mapping of nanoparticles. The final technique is XRD, which is used to analyze the phase composition and crystal structure of nanoparticles. It provides information about the lattice spacing and crystallographic orientation of nanoparticles (Modena et al. 2019).

Silver nanoparticles have great interest in the medical and healthcare fields. They can be incorporated into wound dressings, coatings, and implant materials to prevent infections and promote wound healing. Silver nanoparticles also have potential applications in drug delivery, diagnostics, and imaging. They can be used in water and air purification systems to remove contaminants, including heavy metals and organic pollutants (Arif and Uddin, 2021).

The emergence of pathogens to resist the action of drugs is known as microbial resistance. The microbial resistance causes great economic burden and increase the rate or morbidity and mortality round the world. To treat microbial infections, numerous synthetic drugs are available in local market but these drugs can also cause a lot of adverse effects on human health. It is therefore, necessary to investigate novel antimicrobial agents as alternative to chemical drugs and antibiotics. Antibiotics are a class of antimicrobial drugs used to treat bacterial infections. Antibiotics can be classified into several groups based on their mechanism of action and the type of bacteria they target such as penicillin G and amoxicillin are one of the earliest discovered and widely used antibiotics. They inhibit bacterial cell wall synthesis, leading to cell death (Acharjee et al. 2023).

Dyer's croton or giradol, scientifically known as *Chrozophora tinctoria*, is a member of the Euphorbiaceae family and is indigenous to the Mediterranean region, Asia, and Africa. This herbaceous annual plant is compact and typically attains a height of 30-60 cm. The plant's greenish-yellow, unremarkable flowers are small in size, according to Marzouk et al. (2016). In traditional medicine, *Chrozophora tinctoria* has been used for its medicinal properties. Different parts of the plant, including leaves, seeds and resin are used to treat various ailments such as rheumatism, joint pain, respiratory disorders, gastrointestinal issues and urinary problems. It is believed to have anti-inflammatory, analgesic, antiseptic and wound-healing properties (Sher et al. 2022).

MATERIALS AND METHODS

Plant collection

Fresh and healthy leaves of *Chrozophora tinctoria* L. were collected from Charsadda and washed with distilled water to remove dust and dirt particles. The leaves were shade dried at room temperature. The plant identification was performed by a plant taxonomist.

Extraction

The shade dried leaves of selected plant were dissolved in 1L of 70% ethanol. The mixture was shaken at orbital shaker for 30-60 minutes. After shaking, the mixture was kept in dark for 72 hours and then filtered with the help of filter paper. The filtrate was stored in refrigerator for further investigations while plant residue was discarded (Sohail et al. 2020).

Biosynthesis of AgNPs

The plant extract was combined in a 1:1 ratio with a 1mM AgNo3 solution to create silver nanoparticles. At the magnetic stirrer, the mixture was swirled for an hour. Centrifuging the mixture for 15-20 minutes at 10,000 rpm was done after stirring. After centrifugation, the nanoparticles were collected and the supernatant was discarded. The obtained nanoparticles were dried in oven, water bath or autoclave and then stored for further investigations (Bavanilatha et al. 2019).

Characterization of AgNPs

The bio-fabricated nanoparticles were characterized through various parameters including X-ray diffraction technique, Fourier transform infrared spectroscopy, UV-Visible spectroscopy and scanning electron microscope (El-Ghmari et al*.* 2021).

X-ray diffraction technique

The X-ray diffraction technique was used to study the crystalline powder nature of nanoparticles. The X-rays penetrate deeply in the particles and scan the crystal structure of nanoparticles. By the hel TEM TEM TEM TEM TEM p of XRD technique, different planes of Bragg's reflection were determined which are used to study the crystal symmetry of nanoparticles.

Fourier transforms infrared spectroscopy

The functional groups that were connected to the surface of the nanoparticles made from the leaves of *Chrozophora* tinctoria and in charge of turning unstable silver ions into nanosized particles were investigated using FTIR spectroscopy. The 4000-400 cm-1 range was used for the FTIR spectroscopy.

UV-Visible spectroscopy

UV–Visible Spectroscopy was used to establish the characteristics of nanoparticles. The nanoparticles were analyzed via spectrophotometer, UV-Vis between the wavelength range of 200 to 800 nm. The sample showing maximum absorption between 350-450 nm showed the formation of nanoparticles.

Scanning electron microscope:

The nanoparticles were characterized using scanning electron microscopy based on their surface appearance and size. The nanoparticles were examined using several scales of scanning electron microscopy (JEOL, CRL, Peshawar).

Antimicrobial Analysis

The disc diffusion method was used to measure the microbiological activity of silver nanoparticles in accordance with the methodology of Martinez-Gutierrez et al. (2010).

Antibacterial Analysis

For antibacterial activity, 28 grams of agar was mixed in 1000 ml of distilled water. The agar media was placed in petri dishes and 4 wells of 3mm diameter were made on agar plate. About 0.1 ml of nanoparticles was poured in 3 peripheral wells while central well was filled with standard drug ciprofloxacin. Three bacterial strains i.e. *E. coli, P. aeruginosa* and *S. aureus* were employed for antibacterial activity. The petri dishes were then incubated for 72 hours at 37 ^{o}C Inhibitory zones around each well were measured for the assessment of antibacterial potential of nanoparticles.

Antifungal Activity

For antifungal activity, 4 wells of 3mm diameter were made on agar plate. About 0.1 ml of nanoparticles was poured in 3 peripheral wells while central well was employed as standard filled with ciprofloxacin. Three fungal strains i.e. *Aspergillus flavus, A. terreus* and *Candida albicans* was tested for antifungal activity. Inhibitory zones around each well were measured for the assessment of antifungal potential of nanoparticles.

Statistical analysis

The data obtained from results was analyzed through *t*-test and one-way ANOVA using SPSS software. The obtained results were considered as significant having P value less than 0.05 (Metwally et al. 2022).

RESULTS

Synthesis of Ct-AgNPs

The efficacy of bioactive compounds with the emergence of metal nanoparticles as reducing, capping and stabilizing substance is well known since the development of nanotechnology. The bioactive compounds of plants are responsible for silver nitrate is converted into silver by a process. In this study, AgNPs were synthesized using 1 mM solution of silver nitrate and leaves extract of *C*. *tinctoria*. The ethanolic extract of *C*. *tinctoria* and silver nitrate solution was mixed in the ratio 1:1. During the fabrication of silver nanoparticles, AgNo3 was acting as a precursor while the extract was acting as reducing and capping agent. The reaction mixture was stirred at room temperature for 60 minutes until the solution turned a dark brown hue, indicating the formation of nanoparticles. A change in the fluid's hue was the first indication that nanoparticles had formed.

Characterization of silver nanoparticles

UV-Visible spectroscopy

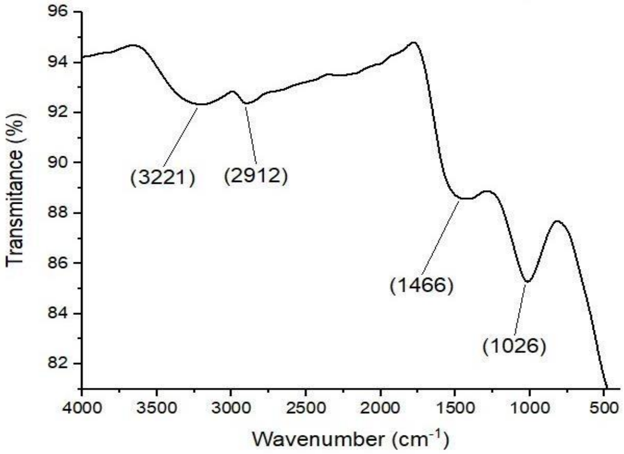

Figure 1: UV- Visible spectroscopy

Ultraviolet light of different wavelengths was passed through the *C. tinctoria* mediated silver nanoparticles to determine the colloidal solution. The maximum absorption for biogenic AgNPs in the given spectrum is at 400 nm. The presence of maximum peak roughly between 300 to 500 nm indicated the formation of silver nanoparticles (Fig 2).

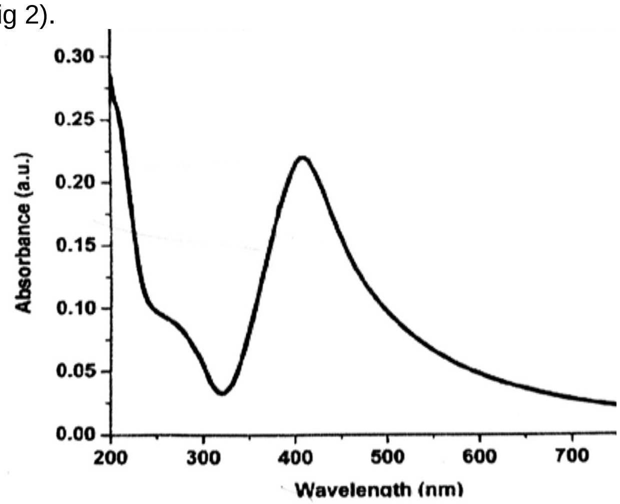

Figure 2: FTIR spectroscopy of *C. tinctoria* mediated silver nanoparticles

FTIR

For the determination of functional groups, FTIR was carried out to assess the bond linkage and identify the associated functional groups of *C. tinctoria* (Fig.1) treated with silver nitrate. These functional groups are considered as responsible for silver nitrate's conversion into silver ions which were then stabilized in to silver. The peak absorbance at 3221 cm^{-1} indicated the stretching vibration of hydroxyl groups which are specific to alcohols and phenols. The 2912 cm^{-1} peak of absorbance was corresponded to amine salts while the absorption bands at 1466, and 1026 cm^{-1} were assigned to C–H and C–O functional groups (Table 1) (Fig 2). Thus, the findings revealed that the reduction of silver nitrate and confirmation that it functions as a capping agent for the creation of silver nanoparticles were both accomplished using the leaf extract of *C. tinctoria.*

X-Ray diffraction technique

The X-ray diffraction pattern of *Chrozophora tinctoria* mediated silver nanoparticles is presented in (Fig 3). The XRD was originated four peaks at 2 theta values of 38^o, 44^o, 64^o and 77^o representing (111), (200), (220) and (311) Bragg's reflections of the face-centered cubic structure of silver, respectively. The XRD peak at 38^o range which falls within 111 plane showed highest intensity, broadening at their end determined that they were nano-size and the peak broadening of other peaks indicated their reduced crystal size (Fig 4).

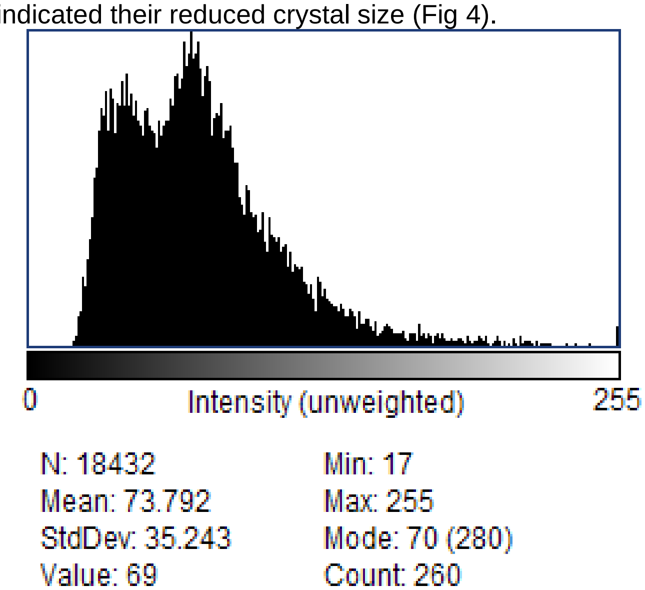

Figure 3: X-ray diffraction analysis of biosynthesized silver nanoparticles.

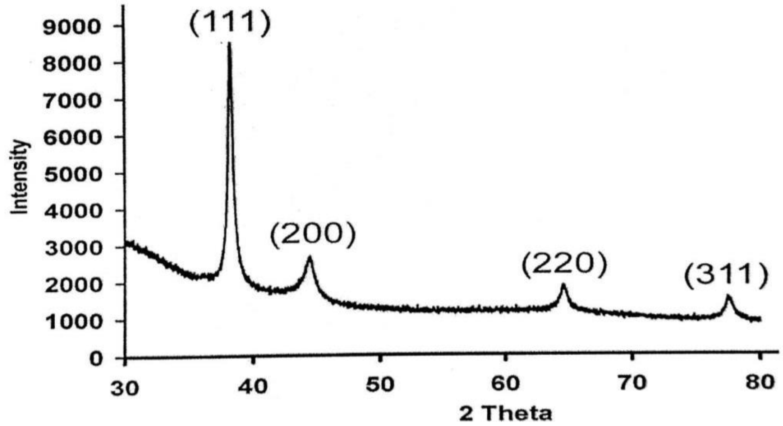

Figure 4: Histogram showing average size of nanoparticles

Table 1: List of FTIR analysis of *C. tinctoria* mediated silver nanoparticles

S. No.	Wavelength	Intensity	Functional group	Compound
1.	3221 cm^{-1}	Weak stretching	OH	Alcohols, Phenols
2.	2912 cm^{-1}	Weak	NH	Amine salts
3.	1466 cm^{-1}	Medium	C–H	Aromatic compounds
4.	1026 cm^{-1}	Strong	C–O	Carboxylic esters

Scanning electron microscope

SEM showed the detail 3D image on the surface of silver nanoparticles with high magnification of 65 with 20 kilovolts. Surface image determined average size (73.792±35.243 nm) of nanoparticles which was calculated using image J software. The SEM photogram showed that the nanoparticles were cubical in shape. (Fig 5)

Antimicrobial activity

Antibacterial activity

The antibacterial activity of silver nanoparticles of *Chrozophora tinctoria* (Fig 6) showed that the highest zone of inhibition (25.55±1.714mm) was measured against *S. aureus* at the maximum concentration of biogenic nanoparticle (300 mg/ml) followed by *E. coli* and *P. aeruginosa* (24.77±1.708 mm), (22.0±1.802 mm) and the lowest zone of inhibition (5.77±0.386 mm) shown by *E. coli* at minimum (100 mg/ml) nanoparticle concentration followed by *P. aeruginosa* (6.766±0.929) and *S. aureus* (7.863±0.703) (Table 2).

Antifungal activity

Antifungal activity shown that the silver nanoparticles of *Chrozophora tinctoria* (Fig 7) had a significant antifungal effect (26.88±0.835 mm) against *Aspergillus flavus* at the maximum (300 mg/ml) nanoparticle concentration followed by *C. albicans, A. terreus* (26.55±1.345 mm) (25.00±1.00 mm) and lowest antifungal activity against *C. albicans* (5.40±1.058 mm) at minimum (100 mg/ml) nanoparticles concentration (Table 3).

Figure 5: Scanning electron microscopy

Table 2: Antibacterial activity of Ct-AgNPs

Bacteria	Zone of inhibition (mm)			
	100 (µg/ml)	200 (µg/ml)	300 (µg/ml)	Ciprofloxacin
E. coli	5.77±0.386	18.440±7.311	24.77±1.708	30
P.aeruginosa	6.766±0.929	11.533±1.327	22.0±1.802	29
S. aureus	7.863±0.703	15.330±0.882	25.55±1.714	29

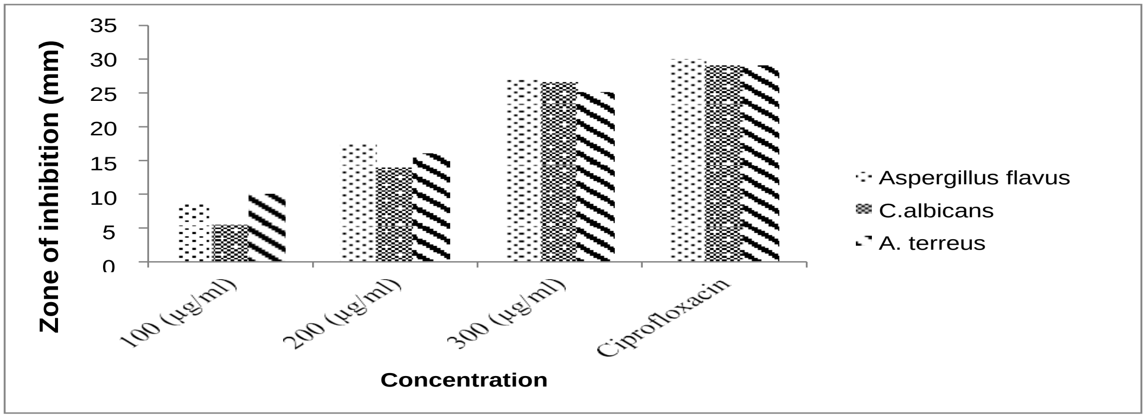

Figure 6: Antibacterial activity of Ct-AgNPs

Table 3: Antifungal activity of Ct-AgNPs

Fungi	Zone of inhibition (mm)			
	100 (mg/ml)	200 (mg/ml)	300 (mg/ml)	+ve Control
Aspergillus Flavus	8.333±0.5773	17.550±0.840	26.88±0.835	30
C.albicans	5.40±1.058	13.88±1.174	26.55±1.345	29
A. terreus	10.0±1.00	16.0±1.00	25.00±1.00	30

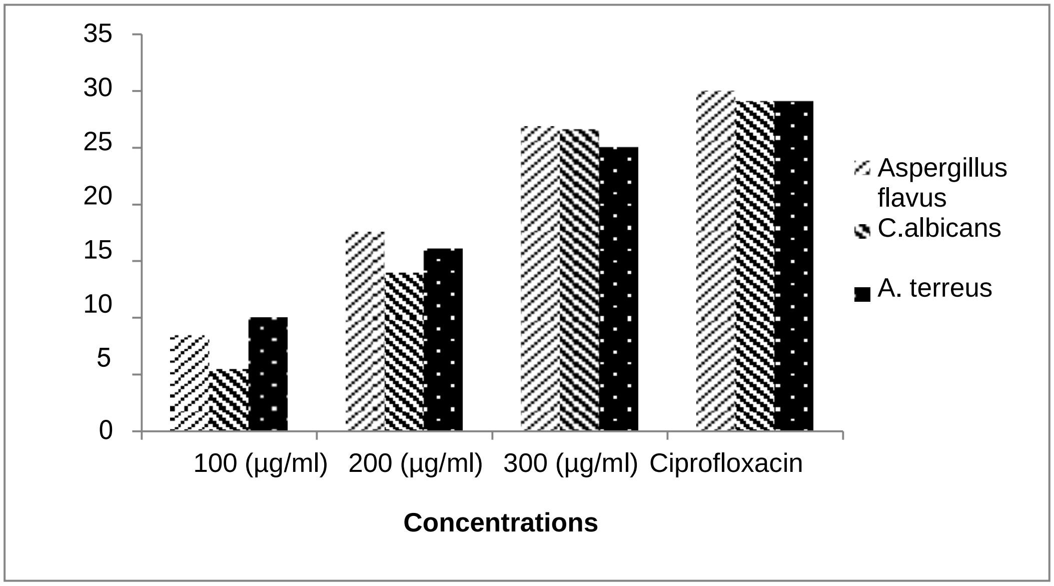

Figure 7: Antifungal activity of Ct-AgNP

DISCUSSION

In the last few decades, the field of nanotechnology is the most emerging fields of science in which different metals have been used to formulate nanosized particles. These nanoparticles have been used in almost every field such as textile industry, paper industry, medicines and food industry. These nanosized particles are also used as the most promising antimicrobial agents which are mostly applied to target the pathogens as alternative to conventional medicines Due to their unique physicochemical properties such as large surface area to volume ratio, the can easily penetrate into cell and target the biomolecules and microbes within the body of individual (Singh et al. 2021). Physical, chemical, and biological processes can all be used to create nanoparticles. While the green approach is environmentally benign, less expensive, and not toxic to the environment, the physical and chemical procedures are seen as being costly, time-consuming, and dangerous. In this study, the ethanolic extract of *C. tinctoria* was used for the formation for silver nanoparticles. Ethanolic extract of *C. tinctoria* was mixed with 1mM solution of silver nitrate following 1:1. The reaction was allowed to stir for 60 minutes and after 60 minutes physical color change was observed from less colour to dark brown color. The change in color of solution from colorless to dark brown was confirmed with the study of Shah et al. 2020. Thus we can conclude that the formation of AgNPs using leaves extract of *C. tinctoria* is a cheap and less time consuming method and the methodology which we followed was very easy and simplest method without any toxic effect. The results of our study are similar with the findings of Palithya et al. 2021 who described the use of leaves extract in the green synthesis of AgNps *Decaschistia crotonifolia*. After the precise synthesis of nanoparticles, it is important to analyze and characterize nanoparticles using various parameters on the basis of their shape, and size, stability and ability to absorb UV radiations. For this purpose, numerous techniques had been used in the current study including X-ray diffraction technique, scanning electron microscope, UV-Visible spectroscopy and Fourier Transforms Infrared Spectroscopy. UV-Vis is considered as one of the most commonly used techniques for structural analysis and characterization of nanosized particles. The absorbance of UV radiation at 470nm peak clearly showed the formation of silver nanoparticles in solution due to the presence of SPR electron on nanoparticles surface (Bilal et al. 2017). The surface plasmon resonance pattern is mainly dependent on characteristic features of metal nanoparticles such as shape, size and other physicochemical and electrochemical properties as well the dielectric features of solution used for the fabrication of nanoparticles. With time, the intensity of surface plasmon resonance bands are increasing which show the production of nanoparticles. The AgNps has been confirmed by UV visible spectrum. The extract of plants acts as reluctant and the formation of nanoparticles take place due to silver nitrate's conversion to silver ions which were then stabilized into silver nanosized particles. These nanoparticles have possessed free electrons on their surface due to which they produce surface plasmon resonance bands. When the UV rays were passed through colloidal solution of silver nanoparticles synthesized from *C. tinctoria*, they showed the maximum absorbance peak at 400 nm which clearly indicated the formation of silver nanoparticles in solution due to the occurrence of surface plasmon resonance electrons on the surface of AgNPs. Travieso et al. (2018) also reported that nanoparticles had shown maximum absorption spectrum at 470 nm due to surface plasmon resonance electrons which confirmed the synthesis of nanoparticles. The findings of our results were in line with previously reported study of Sivalingam et al. 2012 where increase in SPR leads to increase in intensity of nanoparticles production. Plants are God gifted with numerous chemical compounds also known as phytochemicals or secondary metabolites. These metabolites are responsible for therapeutic potential of plants as well these compounds are also involved in the reduction of many salts in to metallic ions. Fourier transform infrared spectroscopy is a technique which is involved in the identification and determination of functional groups of these chemical compounds which act as reducing agents when nanoparticles are created (Akintelu and Folorunso, 2020). In FTR spectroscopy, each group of compounds leads to the development of different peaks. In the current study, FTIR analysis was carried out to study different absorbance peaks including 3221 cm^{-1}, 2912 cm^{-1}, 1466 and 1026 cm^{-1}, respectively. These peaks are representing the presence on numerous chemical compounds groups and C-O which are corresponding to alcohol, amine salt, alkanes and alkyl aryl ether. The existence of these compounds had shown that the bioactive compounds of *C. tinctoria* were a part of the decrease of silver nitrate into silver naosized particles and these phytochemicals may be coated on the surface of nanoparticles. The results of FTIR are in line with the FTIR study of Othman et al. 2016 who synthesized silver nanoparticles using culture supernatant of *Penicillium politans*. XRD is an effective technique used for both quantitative and qualitative analysis of nanoscale particles. This technique is used to confirm the synthesis of nanoparticles and also help determination of their crystalline structure. Moreover, this technique has also been used to measure the size and degree of crystallinity of nanoparticles (Dey et al. 2009). The examination of particles using this technique is mainly dependent on the diffraction patterns because each and every material possesses a unique and distinctive diffraction beam. It has also been used to study purity of materials. XRD technique is basically based on Bragg reflection of nanoparticles. In the current study, the XRD analysis showed the remarkable. The X-ray diffraction analysis of *C. tinctoria* mediated nanoparticles had shown four peak at 2 theta values 38°, 44°, 64° and 77° which represented (111), (200), (220) and (311) Bragg's reflections of the face-centered cubic structure of silver, respectively. The XRD peak at 38° range which falls within 111 planes showed highest intensity, broadening at their end determined that they were nano-size and the peak broadening of other peaks indicated their reduced crystal size. The crystallographic planes for silver nanoparticles at 2 degree with distinct peaks as 111, 200, 300, 220, 311 recorded in our study are similar with the XRD pattern of Mashwani et al. 2015. The face centered cubic structure of AgNPs at 111, 200 and 220 diffraction peaks were in line with the XRD analysis of (Alaqad and Saleh, 2016). Scanning electron microscope is used to study the shape, size and distribution of metal nanoparticles due to their electrical conductivity (Sadeghi and Gholam, 2015). Scanning electron microscopy help in providing information about the aggregation and purity of nanoparticles but it cannot detect the internal morphology of nanoparticles. Silver nanoparticles are mostly triangular, oval, spherical, circular, pebble-like, and cubical in shape and may be found as single or in the form of aggregate (Ghabban et al. 2022). In the current study, the cubical shape nanoparticles with an average size of (73.792±35.243 nm) were determined through scanning electron microscope. (Abdellatif et al. 2022) also synthesized cubical shaped nanoparticles from bulb of *Allium cepa*. Since ancient times, people round the world are facing enormous health problems in which infectious diseases are taking tremendous part in affecting livestock and human population. The casual agents of infectious diseases are fungi, bacteria, virus, protozoa and some other parasites. These agents can affect different body parts of their host internally or externally and can lead to serious health issues leading to death (Dejen et al. 2023). For the treatment of microbial infections, many medications had been used but some pathogens showed resistant to these drugs. Along with microbial resistance, synthetic medications are also expensive and out of reach of most of people. For this purpose, many researches had been conducted on medicinal plants as alternative. Medicinal plants are considered as natural antimicrobial agents due to availability of numerous chemical compounds in different plant parts such as terpenoids, phenols, flavonoids and tannins etc. (Alkahtani et al. 2022). The current research was conducted to evaluate the antimicrobial activity of *C. tinctoria* mediated AgNps which the highest inhibition zone against bacteria *S. aureus* followed by *E. coli* and *P. aeruginosa* fungi including *Aspergillus flavus* followed by *C. albicans* and *A. terreus*. The findings of our study are in line with Renganathan et al. (2021) who studied the antimicrobial potential of *Bauhinia tomentosa* mediated silver nanoparticles.

CONCLUSIONS

The purpose of this research was to create silver nanoparticles by utilizing *Chrozophora tinctoria* leaf extract as a reducing agent. The nanoparticles were then analyzed using various methods such as XRD, FTIR, SEM, and UV-Visible spectroscopy. To evaluate their effectiveness, the silver nanoparticles produced by *C. tinctoria* were tested against three bacterial and three fungal strains using the disc diffusion method. The results showed that these nanoparticles had strong antibacterial effects against S. aureus, E. coli, and P. aeruginosa, and potent antifungal activity against *Aspergillus flavus, C. albicans, and A. terreus*. This study suggests that *C. tinctoria*-mediated silver nanoparticles could potentially be used to combat various pathogenic infections and could be used to create new antimicrobial agents.

Supplementary materials

The supplementary material / supporting for this article can be found online and downloaded at: https://www.isisn.org/article/

Author contributions

M, N, K: Project Administration, Methodology, M. A Original draft writing, M.N: Editing, supervision, visualization: M.A, M.N. K: Software, Data collection and analysis.

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Data Availability Statement

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Conflict of interest

The authors declare that they have no conflict of interest.

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