Green Mediator for Selenium Nanoparticles Synthesis with Antimicrobial Activity and Plant Biostimulant Properties under Heavy Metal Stress

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Nanotechnology is a valuable strategy for managing a number of medicinal, agricultural, and environmental concerns. *Cocculus pendulus* was used for selenium nanoparticles (SeNPs) synthesis to evaluate their usage for microbial inhibition and to enhance plant resistance to heavy metals. Mono-dispersed, spherical shape, and mean diameter (36.19 nm) of SeNPs were documented. More inhibitory potential was associated with SeNPs with inhibition zones of 38±0.3, 18±0.2, 18±0.1, 31±0.2, and 27±0.1 mm than extract of *C. pendulus* against *B. subtilis, S. aureus, E. coli, K. pneumoniae,* and *C. albicans*, respectively. SeNPs had successfully scavenged the free radicals of 2,2-diphenyl-1-picrylhydrazyl (DPPH) with lower IC_{50} (inhibitory concentration that inhibit 50% of DPPH) value (10.31 µg/mL) than that of *C. pendulus* extract (55.54 µg/mL). The effect of *C. pendulus* extract (200 mg/kg soil) alone or in combination with SeNPs (15 mg/kg soil of SeNPs) as a soil drench on shoot, root lengths, plant pigments, lead and cadmium contents of *Corchorus olitorius* under lead and cadmium stress (5 mg/L) was investigated. The pigments quantity and plant growth were decreased by cadmium and lead poisoning. Application of *C. pendulus* extracts or SeNPs decreased the Pb and Cd concentrations and improved the growth and metabolites of *Corchorus olitorius* plants.

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INTRODUCTION

Nanotechnology operating at a scale less than 100 nm, improves the surface area of small particles, unlocking their unique properties and enabling diverse applications across a wide array of disciplines, such as biological sciences, chemical sciences, material sciences, and electronics (Abdelghany *et al*. 2018). Nanoparticles (NPs) have a number of advantages, compared to conventional materials, including improved catalytic efficiency, greater surface reaction sites, and high surface action (Wang *et al*. 2019), so, the use of NPs opens a new path for the improvement of soil cleanup (Liu *et al*. 2021). Selenium nanoparticles (SeNPs) are one of the numerous kinds of NPs that are gaining major attention because of their great bioavailability, antimicrobial and antioxidant potential, and low toxicity (Chockalingam *et al.* 2020). There is a need for eco-friendly and non-toxic approaches for the creations of SeNPs. Current attempts and investigation have focused on the biological creation of SeNPs (Qanash *et al*. 2023), due to unique properties if compared to chemical synthesis.

Plant-based approaches have gained attention for their simplicity, affordability, and avoidance of microbial cultures complexities (Abdelghany *et al*. 2023). Natural extracts of plant origin contain biological constituents including alkaloids, saponins, polyphenols, terpenoids, flavonoids, and antioxidants that function as reducing and stabilizing agents, making them appropriate for synthesis of NPs (Amin and Badawy 2017). Our investigation includes the green creation of SeNPs using *C. pendulus* extract which is used as reducing agent to create SeNPs. The extract of *C. pendulus* has positive medical benefits in the traditional medical system. According to Fahmeeda *et al*. (2017) *Shigella spp*., *Clostridium spp*., and *Klebsiella spp*. were sensitive while *E. coli* and *Salmonella spp*. were resistant to *C. pendulus* extract.

It is sufficient to consume meals in a normal manner to achieve the Recommended Daily Allowance of selenium (Se), an important nutrient. On the other hand, excessive daily dietary quantities of Se compounds can be detrimental. If excessive amounts of sodium selenite (Na_2SeO_4) or sodium selenite (Na_2SeO_3) are accidentally or purposefully swallowed (such as from a large number of Se supplement pills), it may be fatal if medical attention is not received right away. Deformed nails and brittle hair can occur even with moderately excessive Se consumption over an extended period of time (MacFarquhar *et al*. 2010). Interference of SeNPs with several fields such as medicinal, agricultural, food, and environmental has become the main target of numerous investigators. According to recent investigation, Hassan *et al*. (2022) reported the inhibitory potential of biosynthesized SeNPs towards fungi and bacteria such as *Fusarium oxysporum*, *Aspergillus niger*, *Staphylococcus epidermitis, E. coli, Klebsiella pneumonia,* and *Staphylococcus aureus*, besides its antioxidant activity. Also, Garza-García *et al*. (2023) indicated that the phytosynthesized SeNPs possess biological activities such as inhibition of *Alcaligenes faecalis, Serratia marcescens,* and *Enterobacter cloacae* growth. Moreover, it exhibited a promising antioxidant activity. Antibacterial activity of the synthesized SeNPs *via* the extract of *Bombax ceiba* flower against *S. aureus*, *K. pneumonia*, and *Pseudomonas aeruginosa* was reported by Safdar *et al*. (2023). In the agricultural field, SeNPs played a vital role as a biostimulator for enhancement of growth parameters of *Calendula officinalis* and *Catharanthus roseus* plants (Hernández-Díaz *et al*. 2021).

Heavy metals are considered one of the most critical abiotic stresses, because they have dangerous effects on plant development and public health through the food system (Gupta *et al*. 2019). One of nanoparticles used to clean soil from metals is Se, which is applied as a foliage application to lessen Cd and Pb stress of rice plants (Hussain *et al*. 2020). As a result of increased absorption and relocation, exposure of plants to various metals, such as cadmium (Cd), chromium (Cr), and lead (Pb) alters biochemical and physiological processes and impairs normal development and growth of plants. In addition, through binding to proteins, nucleic acids, and enzymes, these heavy metals disrupt the metabolic processes of the cell. However, under conditions of metal stress, Se metal can also benefit cellular processes such as membranes stability, mineral intake balance, antioxidant action, and photosynthesis (Hasanuzzaman *et al*. 2022). Furthermore, Se metal has different physiological functions, including immune system control, and antioxidant and anti-cancer properties (El-Batal *et al*. 2023).

Jute mallow (*Corchorus olitorius* L.) plant, a member of the Tiliaceae family, was used in this study and is one of the most important green vegetables in tropical regions. For example, Egypt farmed 887 hectares of jute mallow and produced approximately 2173 tonnes, with an annual yield of 2.45 tons/ha. Proteins, beta-carotene, vitamins (A, B, C, and E), and necessary minerals are all abundant in *C. olitorius*. Additionally, they are utilized in natural pharmacopoeia (Nowwar *et al*. 2022). In Egypt, and other countries more than one million of feddans are irrigated with heavy metal rich wastewater because of the shortage of fresh water. Therefore, the current study aimed to create an ecofriendly agent (SeNPs) to reduce the impact of heavy metals on plant development besides its utilization as antimicrobial and antioxidant agent.

EXPERIMENTAL

Materials Used

The following procedures required the acquisition of quantitative standard grade chemical compounds from Sigma-Aldrich, including sodium selenite and other reagents. Jute mallow (*Corchorus olitorius* L.) seeds were purchased from the Ministry of Agriculture's, Agricultural Research Center in Giza governorate, Egypt. *Cocculus pendulus* plant was obtained from Gebel Elba; it is located in the Eastern Desert, 20–25 kilometers from the Red Sea. The *C. pendulus* leaves were cleaned with distilled water to get rid of any dust, and then dried in the air.

High Performance Liquid Chromatography (HPLC) analysis of Phenolic Contents of *Cocculus pendulus* Extract

Agilent 1260 series HPLC equipment was used for the analysis. Using an Eclipse C18 column (4.6 mm x 250 mm i.d., 5 μm), the separation was performed. At a flow rate of 0.9 milliliters per min, the mobile phase was composed of water (A) and 0.05% trifluoroacetic acid in acetonitrile (B). The following was the sequential programming of the mobile phase using a linear gradient: 12 to 15 min (82% A); 15 to 16 min (82% A); 5 to 8 min (60% A); 8 to 12 min (60% A); 0 to 5 min (80% A); and 16 to 20 min (82% A). Monitoring of the multi-wavelength detector was place at 280 nm. For every sample solution, the injection volume was 5 μL. A constant 40 °C was maintained in the column.

Preparation of *Cocculus pendulus* Aqueous Extract

Fresh, young, and healthy *C. pendulus* leaves were gathered and cleaned with running water and then double-distilled water to get rid of any remaining solid dust particles. Then, the leaves were left to dry for two weeks in the dark at room temperature. Dry plant leaves were ground using a coffee grinder into a powder. They were then heated with sterile distilled water at a ratio of one to one hundred (w/v) at 60 ℃ for 45 min. Until they were used to create nanoparticles, dried leaves were stored at 4 ℃ in a sealed container.

Biosynthesis of SeNPs using an Aqueous Extract of *Cocculus pendulus*.

Under stirring, sodium selenite (0.02 mM) as a precursor was mixed with aqueous extract of *C. pendulus* (2g/L). To begin the reaction, the pH of the mixture was adjusted to 7.4 with 0.1 M potassium hydroxide. Under sonication, the reaction lasted 1 h and produced dark red NP Se (Vahdati and Tohidi 2020). After being centrifuged at 15000 g for 30 min (Hermle, Germany), the SeNPs that had formed were collected. They were then cleaned three times using deionized water and ethanol, and left to dry overnight. Before being subjected to additional testing and analysis, the nanoparticle suspension was made in sterile phosphate buffer (pH 7.4) and kept at 4 °C.

Characterization of the Synthesized Biogenic SeNPs

UV-Vis spectroscopy

An ultraviolet-visible (UV-Vis) spectrophotometer with a (JASCO V-560) double beam spectrophotometer was used to measure the optical behavior of the synthesized SeNPs using several wavelengths between 200 and 600 nm in order to determine the maximum peak surface plasmon resonance (SPR) and compare it to a combination of reactions lacking Se salt as a negative reference.

XRD spectroscopy

To investigate the structural properties (crystalline or amorphous) of the Se-NPs produced by extract of *C. pendulus*, XRD-6000, Shimadzu equipment, SSI, Japan were used. The intensity of the diffracted X-rays was computed as diffracted angle 2θ (El- Batal *et al*. 2023).

Transmission electron microscopy

A high-resolution transmission electron microscope (HRTEM, JEM2100, Jeol, Japan) was used to evaluate the microstructure, approximate shape, and average particle size of the biosynthesized SeNPs (Ash *et al.* 2018). To put it briefly, one milligram of biogenic-SeNPs was suspended in one milliliter of ethanol and sonicated for 15 min. After that, a drop of supernatant dispersion was gathered and put on the copper grid, and HR-TEM images were taken at various magnifications.

Fourier transform infrared (FT-IR) spectrometry

Using a Cary 630 FT-IR analyzer (Tokyo, Japan), the functional groups found in the biosynthesized SeNPs were described. 200 mg of the produced SeNPs were combined with potassium bromide (KBr), compressed onto a disc, and then scanned over a range of wavenumbers, from 500 to 4000 cm^{-1}, in order to do the analysis.

Antibacterial Activity *in vitro*

The antibacterial activity of both *C. pendulus* extract and *C. pendulus* extract mediated SeNPs against various bacterial and fungal species (*Staphylococcus aureus* (ATCC 23235), *Bacillus subtilis* (ATCC29211), *Escherichia coli* (ATCC25922), *Klebsiella pneumonia*(ATCC13883), *Candida albicans* (ATCC10231), and *Aspergillus niger*) were assessed using the agar well diffusion approach. To achieve this, the turbidity of bacterial strains was adjusted to a concentration of 10^6 CFU/mL, and evenly spread on Mueller-Hinton agar plates using a sterilized swab of cotton. Subsequently, wells with a 6-mm diameter were formed, and 100 µL of *C. pendulus* extract and *C. pendulus* extract mediated SeNPs at concentrations of 0.5 mg/mL) were individually added to these 6-mm diameter wells. Following a 24-h incubation period at 37 °C, the sizes of the inhibition zones were measured to assess the antibacterial activity. The positive control was applied using 5 mg/mL of Gentamycin (Abdelghany *et al*. 2021).

The minimal inhibitory concentration (MIC) (lowest doses of tested agent that could inhibit the growth of a microorganism) and minimal bactericidal concentration (MBC) (lowest doses of tested agent that could killing bacterial inoculums up to 99.99%) of both *C. pendulus* extract and *C. pendulus* extract mediated SeNPs at 1.95 to 250 µg/mL were estimated against *B. subtilis*, *S. aureus*, *E. coli*, *K. pneumoniae*, and *C. albicans*.

The MIC was determined using the broth microdilution technique in 96-well microplates. Mueller Hinton broth was used to create the twofold serial dilution (MHB). 100 µL of the bacterial suspension contains 2×10^8 CFU/mL was added to the wells of a microplate containing the twofold serial dilutions of the tested sample solution in the MHB. The cell density (2×10^8 CFU/mL) of McFarland Standard No. 0.5, which was used in this investigation, was ascertained by measuring the optical absorbance at 600 nm wavelength.

The minimum inhibitory concentration (MIC) was determined by recording the lowest concentration of the substance that inhibited bacterial growth during a 24-h aerobic incubation at 37 °C with 5% CO_2. The culture media without the bacteria and the bacteria put into the MHB without tested samples were regarded as the positive and negative controls, respectively. Ten microliters of the bacterial suspensions in the wells were added to the blood agar medium without turbidity in order to determine the MBC. The mixture was then incubated at 37 °C until there was enough growth. At each contact period, the MBC was defined as the lowest concentration that eliminated 99.9% of the original inoculum (French 2006).

Estimation of Antioxidant Activity by DPPH Radical Scavenging Method

In the DPPH assay, both *C. pendulus* extract and *C. pendulus* extract mediated SeNPs, were subjected to testing. The estimation of free radical scavenging abilities involved stable DPPH and included standard ascorbic acid for comparison. A range of concentrations ranged from1.95 to 1000 μg/mL of tested samples were examined to understand their free radical scavenging capabilities across different concentrations (Alsalamah *et al*. 2023). After incubating the samples in the dark at room temperature (25 °C) for 30 min, the absorbance was measured at 517 nm. The percentage of scavenged free radicals was calculated by the following formula.

$$\%\text{ of scavenging} = \frac{\text{Absorbance of control} - \text{Absorbance of tested samble}}{\text{Absorbance of control}} \times 100 \quad (1)$$

The curve graph demonstrating the percentage of DPPH inhibition as a function of pollen grains extract concentration was applied to calculate the dose of each sample required to scavenge 50% of DPPH (IC_{50}).

Plant Cultivation and Treatment Procedure

Jute mallow seeds were sown in pots (30 cm in diameter) with 6.0 kg of clay soil. The pots were separated into 4 groups, each one representing a different type of treatment: I- Control without treatment; II- Heavy metals (5 mg/L of lead and cadmium chloride); III- Heavy metals +15 mg/L of SeNPs and IV- Heavy metals+ 200 mg/L of an aqueous extract of *C. pendulus*. Plants in each group were treated (SeNPs or plant extract) twice (as a soil drench) with the aforementioned treatments at 15 and 30 days after sowing. At 37 days following planting, plant samples from the various treatments and the control were taken for growth traits (shoot length and root length), chlorophyll a, b, a+b and carotenoids and heavy metal analysis in jute mallow leaves.

Analyses of Heavy Metals in Shoot System in Plants

The heavy metal (Cd and Pb) contents in the various samples of the researched plant leaves (edible plant parts) were assessed in accordance with Parkinson and Allen (1975).

Estimation of Pigments and Carotenoids

Vernon and Selly (1966) found a way to measure the pigments found in green plants. In this manner, green tissues were weighed in one-gram aliquots and chopped into tiny pieces. The tissue pieces were ground in a blender for 2 min in 100 milliliters of 80% acetone in order to remove the plant dyes. A Buchner filter fitted with man No. 1 filter paper was used to quantitatively transfer and filter the mixture. After the filtrate was moved to a 100 mL volumetric flask, 80% acetone was added to the volume to make 100 mL. Using a Carl Zeiss spectrometer, the optical density of the extract was determined at three different wavelengths (649, 665, and 470 nm). These wavelengths are in the region where chlorophyll "a" "b" and carotenoids absorb the most light. The following formulas were used to determine the amounts of chlorophylls a, b, and their sum in plant tissues.

Chlorophyll a/g in tissue = 2.39 (A 649) - 11.63 (A 665) mg (2)

Tissue's mg of chlorophyll b/g = 20.11 (A649) - 5.18 (A665) (3)

Tissue's mg of chlorophyll a+b = 6.45 (A 665) + 17.72 (A 649) (4)

The concentration of carotenoid pigments was determined using the equation developed by Lichtenthaler, H. K. (1987),

Car. = 1000x (A470) – 1.82 Ca - 85.02 Cb\198= mg\g fresh wt. (5)

Where the optical density is indicated by (A).

Statistical Analysis

The computer applications SPSS version 25, Minitab version 19, and Microsoft Excel version 365 were used to perform statistical computations at the 0.05 level of probability. Quantitative data having a parametric distribution were analyzed using the analysis of variance, one-way ANOVA, and post hoc Tukey's test. The allowed margin of error was set at 5% with a 95% confidence interval as outlined in Houk *et al.* (1995).

RESULTS AND DISCUSSION

HPLC Analysis of *Cocculus pendulus* Extract

Table 1 shows the phenolic and flavonoid compounds of *C. pendulus* extract. Rutin, gallic acid, chlorogenic acid, catechin and rosmarinic acid were the most detected compounds with the highest concentrations (3989.44, 2884.03, 2711.07, 1168.17 and 1143.33 µg/g) respectively, while methyl gallate, coumaric acid, cinnamic acid, daidzein, kaempferol, ellagic acid, hesperetin and quercetin were detected with the lowest concentrations of 3.37, 10.45, 13.90, 17.73, 38.26, 44.50, 45.06 and 80.20 µg/g, respectively in *C. pendulus* extract. Using the leaves of *C. pendulus*, the indigenous system of medicine to treat internal parasites, rheumatoid arthritis, biliousness, intermittent fever, febrifuge, vermifuge, diuretic, menstrual cycle issues, and discomfort (Naglaa *et al*. 2014), this may be due to the high content of phenolic and flavonoids compounds in Table 1.

Table 1. Identified Flavonoid and Phenolic Flavonoid Compounds in *C. pendulus* Extract

Component	Retention Time	Area (mAU)	Area (%)	Concentration (µg/g)
Gallic acid	3.594	658.159	23.89	2884.03
Chlorogenic acid	4.380	394.95	14.33	2711.07
Catechin	4.554	101.19	3.67	1168.17
Methyl gallate	5.605	1.28	0.04	3.37
Caffeic acid	5.830	30.17	1.09	124.38
Syringic acid	6.364	145.75	5.29	555.72
Pyro catechol	6.649	0.00	0.00	0.00
Rutin	6.915	492.44	17.87	3989.44
Ellagic acid	7.316	10.31	0.37	44.50
Coumaric acid	8.704	5.61	0.20	10.45
Vanillin	8.905	93.43	3.39	178.34
Ferulic acid	9.722	520.06	18.88	1592.06
Naringenin	10.585	31.62	1.14	152.38
Rosmarinic acid	11.997	206.84	7.50	1143.33
Daidzein	15.691	6.02	0.21	17.73
Querectin	17.620	12.84	0.46	80.20
Cinnamic acid	19.267	14.90	0.54	13.90
Kaempferol	20.844	11.54	0.41	38.26
Hesperetin	21.401	17.36	0.63	45.06

Characterization of Se Nanoparticles

In this study, the watery extract of *C. pendulus* was used as reducing agent to convert sodium selenite to SeNPs and stabilize them in colloidal form. In green synthesis, natural, non-toxic, affordable, and ecologically friendly materials are used as reducing, capping, and stabilizing agents. This reduces the impact of the synthesized nanoparticles on the environment, improves their biocompatibility, and opens up new applications for them in a variety of fields (Ying et *al* 2022). The metabolites released by *C. pendulus* serve as the capping and reducing agent in green approaches for the creation of nanoparticles. The synthesis of SeNPs was observed by a change in solution color to dark red. The spherical shapes with considerably mono-dispersed SeNPs, which ranged in size from 45.48 to 24.72 nm, were depicted by the HRTEM image. According to the calculation, the mean diameter was 36.19 nm, as seen in Fig. 1A. The generated red color served as a reliable spectroscopic indicator of their emergence and was attributed to the stimulation of biogenic SeNPs surface Plasmon changes (El-Ghazaly *et al*. 2017). The strength of the red color created matched the prepared *C. pendulus* extract capacity for biological synthesis SeNPs. HTEM pictures were taken to look into the biosynthesized SeNPs' intended shape and average particle size (Elkodous *et al*. 2019). The UV-Vis investigations revealed that the SeNPs were tiny, as indicated by the practical peak in the spectra (Fig. 1B), which was produced by the O. D. (0.5453; diluted 10 times).

The XRD pattern of the crystalline metallic SeNPs showed four noteworthy peaks. Figure 2 is the XRD pattern of the SeNPs powder synthesized by *C. pendulus* extracts. There is a specific diffraction peak in the range of 25 to 35° at the angle of 2θ, which is basically consistent with the diffraction peak of JCPDS card number 05-001-0210 and 01-086-2246. It can be inferred that the obtained particles are Se. The diffraction peak in the pattern is very wide, indicating that the synthesized SeNPs particles are very small in size, poor in crystallinity, and amorphous. The possible reason for the formation of amorphous particles is that there is a biomolecular coating of polyphenols, flavonoids, vitamins, and other biological macromolecules in *C. pendulus* extracts (as the analysis presented in Table 1 and Fig. 1). Because it reveals the atomic state of the exposed atoms, XRD analysis was used to check the crystal arrangement and average crystal dimensions of the produced SeNPs (Pal *et al*. 2019). The results from the Joint Committee on Powdered Diffraction Standards (JCPDS) of SeNPs with a standard card like JCPDS File No. 05-001-0210 were all matched by the XRD results' peaks (Bai *et al*. 2017).

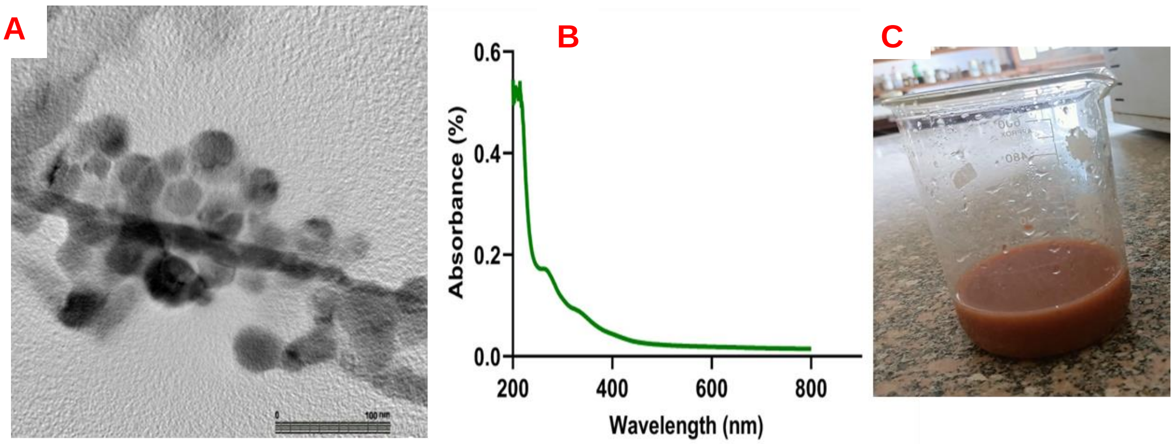

Fig. 1. (A) HRTEM image; (B) UV-Vis spectroscopy, which reveals the SPR peak at 200 nm (C) The final product Se NPS

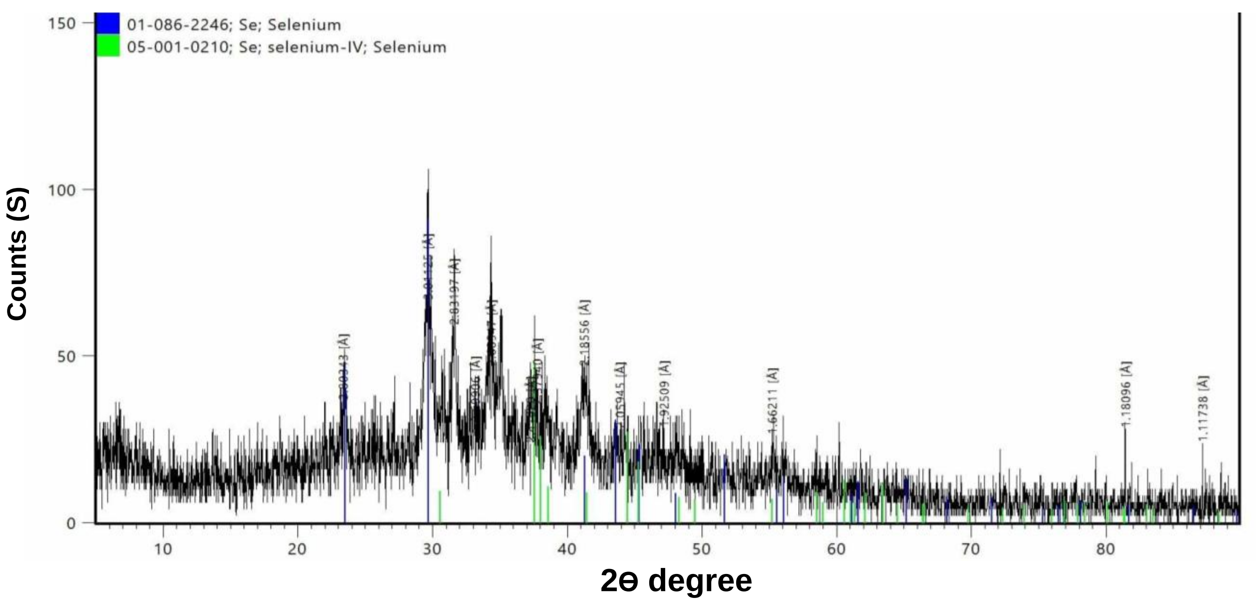

Fig. 2. Crystallinity analysis of the biogenic SeNPs by XRD spectrum

FTIR was conducted for biogenic SeNPs to verify the viability of *C. pendulus* extract in the biosynthesis of SeNPs, FTIR spectroscopy investigation was carried out by analyzing the vibrational frequencies of chemical bonds; FTIR makes it possible to identify the functional groups that are present on the surface of nanoparticles. FTIR analysis was conducted across a variety of wavenumbers, from 4000 to 500 cm^{-1} (Fig. 3). As shown the spectra of SeNPs containing six peaks at wavenumbers of 3299.87, 2160.04, 1632.74, 1381.17, 425.14, and 409.84 cm^{-1}. The absorption band at 409 to 425 cm^{-1} corresponds to stretching vibration motion of Se metal, strong broadband at 3299.87 cm^{-1} was observed due to the presence of O-H stretching vibration of alcohol (Malachová *et al* 2020). An extremely narrow band that emerged at 2160.04 cm^{-1} is indicative of C≡H stretching vibration mode. The peak at 1632.74 cm^{-1} refer signify the vibration of C=C, this confirm by appearance weak band at 1036 cm^{-1} which attributed characteristic vibration of C=H (Britto *et al*. 2021). Peak 1381 cm^{-1} is corresponded to binding CH_3 vibration (methyl group). The FT-IR data suggest that a green technique was used to successfully synthesis - SeNPs using bio-compounds found in the leaf aqueous extract of *C. pendulus*.

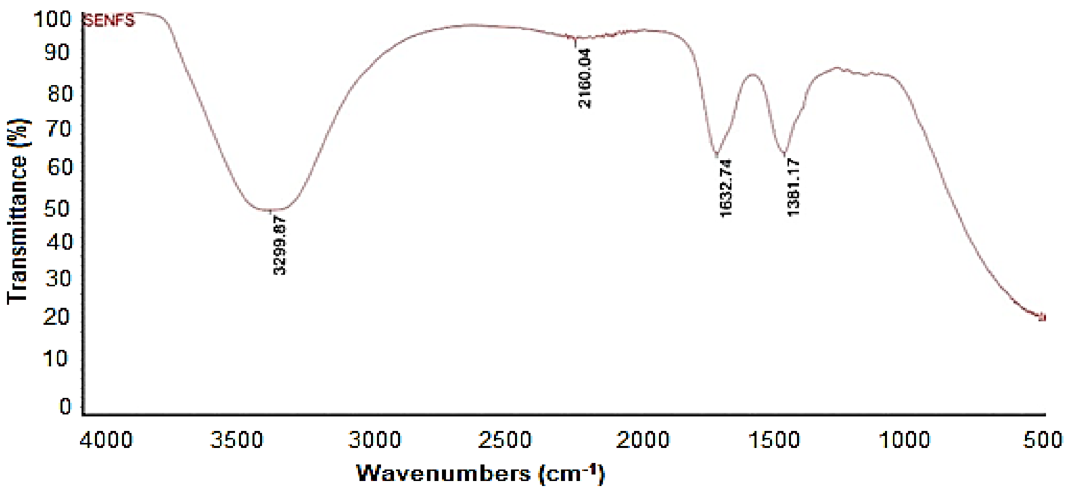

Fig. 3. FTIR analysis for SeNPs

Antimicrobial Activity

The obtained results indicated that the created SeNPs exhibited more antimicrobial activity than *C. pendulus* extract with different inhibition potential depending on the tested microorganism (Table 2 and Fig. 4). The inhibition zone was 38±0.3, 18±0.2, 18±0.1, 31±0.2, 27±0.1 mm using *C. pendulus* extract, while it was 40±0.2, 20±0.1, 19±0.4, 32±0.1, and 28±0.4 mm using SeNPs against *B. subtilis*, *S. aureus*, *E. coli*, *K. pneumoniae*, and *C. albicans*, respectively. The used antibiotic/antifungal showed less inhibition zones if compared with *C. pendulus* extract/created SeNPs. Weak inhibitory action of SeNPs was observed on *A. niger* with inhibition zone 13±0.4 mm, while *C. pendulus* extract didn't inhibited the fungus. According to Nafees *et al*. (2019), both ethanolic extracts of stem and root of *C. pendulus* showed antibacterial activity against *Xanthomonas* sp. (17 mm) against *Proteus* sp. with inhibition zones ranged from 17-18 mm. Recently, Hassan *et al*. (2022) reported that *Staphylococcus epidermitis*, *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumonia* were inhibited using biogenic SeNPs with inhibition zones of 18.5, 21, 20, and 20.5 mm, respectively. The current study outcomes are well correlated with the recent investigation (Garza-García *et al*. 2023) in which the *Amphipterygium glaucum* extract mediated-SeNPs showed antibacterial activity against *Alcaligenes faecalis Enterobacter cloacae*, and *Serratia marcescens*.

The inhibition of *S. aureus* and *K. pneumonia* was recorded as a result of exposure to 100 µg/mL of phytosynthesized SeNPs with inhibition zones of 20 mm and 28 mm, respectively (Safdar *et al*. 2023). In another study, different inhibition zones were observed against *Staphylococcus epidermitis* (18.5 mm), *Escherichia coli* (20 mm), *Klebsiella pneumonia* 920.5 mm), and *Staphylococcus aureus* (21 mm), *Aspergillus niger* (17.5 mm) and *Fusarium oxysporum* (21 mm) (Hassan *et al*. 2022). Table 3 shows that the value of *C. pendulus* extract MIC was equal to the value of SeNPs MBC in case *S. aureus* (31.25 µg/mL), *E. coli* (62.5 µg/mL), and *K. pneumoniae* (7.8 µg/mL), while MIC value of SeNPs was less (1.95 and 15.62 µg/mL) than MIC of *C. pendulus* extract (3.9 and 31.25 µg/mL) against *B. subtilis* and *C. albicans*, respectively. Moreover, MBC value of *C. pendulus* extract was the similar value of SeNPs against tested microorganisms except *S. aureus* and *E. coli*. All values of MBC/MIC index of *C. pendulus* extract and SeNPs were less than 4, indicating its cidial properties. Proposed antimicrobial mechanisms of SeNPs were reported previously and include disruption and depolarization of bacterial membrane and repression of biofilm development (Hernández-Díaz *et al*. 2021). A number of mechanisms were attributed to the inhibitory action of SeNPs against bacteria including damaging cell membranes and walls, interference with the functions of cell active molecules, and raising oxidative stress (Escobar-Ramírez *et al*. 2021).

Table 2. Efficacy of *C. pendulus* Extract and SeNPs Tested Microorganisms

Tested Microorganisms	**Mean Inhibition Zone (mm)**				**P-value**	**HSD**
	Extract	SeNPs	**+ve C***	**-ve C***		
B. subtilis	38±0.3	40±0.2	33±0.2	0.0	0.01	1.67
S. aureus	18±0.2	20±0.1	16±0.2	0.0	0.05	2.00
E. coli	18±0.1	19±0.4	16±0.2	0.0	0.04	0.87
K. pneumoniae	31±0.2	32±0.1	28±0.1	0.0	0.02	2.25
C. albicans	27±0.1	28±0.4	19±0.2	0.0	0.00	3.21
A. niger	0.0	13±0.4	22±0.1	0.0	0.00	5.36

*** +ve C**, positive control (Gentamycin/Nystatin) and **-ve C,** negative control (solvent of extraction used). Each value is mean of 3 replicates ± standard error of means. Honestly Significant Difference (HSD) at $P \leq 0.05$ by Post hoc-Tukey's test.

Table 3. MIC, MBC, and MBC/MIC Index Detection of *C. pendulus* Extract and SeNPs Tested Microorganisms

Tested Microorganisms	MIC (µg/mL)		P-value	HSD	MBC (µg/mL)		P-value	HSD	MBC/MIC Index		P-value	HSD
	Ext.	SeNPs			**Ext.**	SeNPs			**Ext.**	SeNPs		
B. subtilis	3.9	1.95	0.00	0.963	3.9	3.9	1.00	2.21	1	2	0.00	0.25
S. aureus	31.25	31.25	1.00	2.54	62.5	31.25	0.00	11.21	1	2	0.00	0.36
E. coli	62.5	62.5	1.00	3.52	125	62.5	0.00	20.35	1	2	0.00	0.16
K. pneumoniae	7.8	7.8	1.00	2.21	7.8	7.8	1.00	1.54	1	1	1.00	2.65
C. albicans	31.25	15.62	0.00	5.52	31.25	31.25	1.00	2.25	1	2	0.00	0.31

Each value is mean of 3 replicates ± standard error of means. Honestly Significant Difference (HSD) at $P \leq 0.05$ by Post hoc-Tukey's test.
Ext. = plant extract of *C. pendulus*

Table 4. Antioxidant Activity of *C. pendulus* Extract and SeNPs

Concentration (µg/mL)	DPPH Scavenging (%)			P-value	HSD
	***C. pendulus* extract**	**SeNPs**	**Ascorbic acid**		
1000	82.4±0.21	97.9±1.23	99.3±0.87	0.00	4.68
500	74.9±1.32	94.4±3.45	96.3±1.25	0.01	6.27
250	67.3±2.43	85.7±1.32	94.8±2.92	0.00	5.94
125	59.5±1.34	78.0±2.45	91.9±4.65	0.00	8.38
62.50	51.8±2.54	70.1±2.67	84.2±2.50	0.00	11.35
31.25	43.9±1.87	62.8±0.76	76.1±1.67	0.00	9.247
15.63	35.3±2.65	54.5±3.76	67.6±1.54	0.00	10.25
7.81	27.6±4.56	46.6±2.34	60.4±3.54	0.00	8.81
3.90	19.9±4.12	39.5±2.89	52.1±1.67	0.00	12.32
1.95	11.6±2.56	30.9±1.90	43.7±2.56	0.00	7.25
0.0	0.0	0.0	0.0	00	00
IC_{50}	55.54 µg/mL	10.31 µg/mL	2.52 µg/mL	0.00	7.21

Each value is mean of 3 replicates ± standard error of means. Honestly Significant Difference (HSD) at $P \leq 0.05$ by Post hoc-Tukey's test.

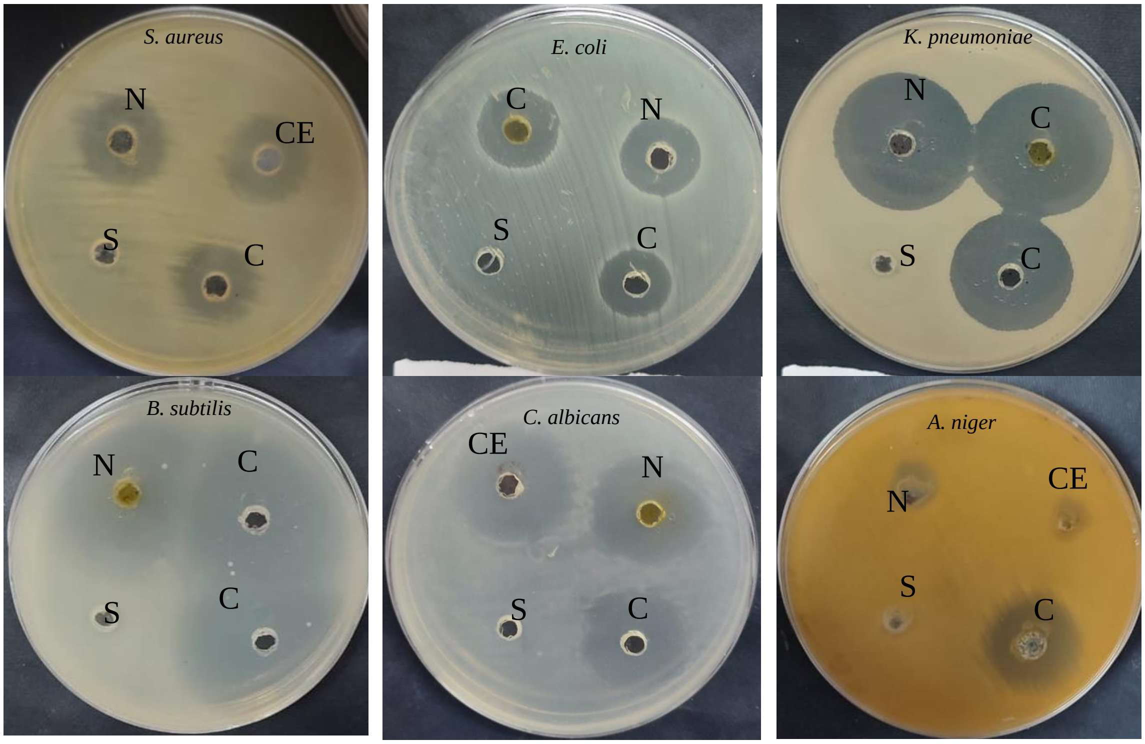

Fig. 4. Efficacy of *C. pendulus* **e**xtract and SeNPs against tested microorganisms (CE, *C. pendulus* extract; N, SeNPs; C, positive control; S, solvent used as negative control)

Antioxidant Activity

Table 4 shows that *C. pendulus* extract and SeNPs possess antioxidant activity that led to increasing of DPPH scavenging with the increment of concentration. However, SeNPs exhibited a promising antioxidant potential compared to *C. pendulus* extract at all tested concentrations. At 1.95, 62.50 and 500 µg/mL, DPPH scavenging% was 30.9±1.90, 70.1±2.67, and 94.4±3.45% using SeNPs but it was 11.6±2.56, 51.8±2.54, and 74.9±1.32% using *C. pendulus* extract, respectively. The results of the DPPH assay revealed that SeNPs had effectively scavenged the free radicals of DPPH with IC_{50} value (10.31 µg/mL) less than the IC_{50} value (55.54 µg/mL) of *C. pendulus* extract compared to the IC_{50} value (2.52 µg/mL) of standard (ascorbic acid). The result is consistent with earlier reports utilizing SeNPs created from other natural sources such as *Spirulina platensis*, which exhibits a DPPH scavenging potential of 89% at 500 µg/mL (Abdel-Moneim *et al*. 2021).

Growth and Biochemical Traits in Response to Treatments Under Pb and Cd Stress

Figure 5 shows the positive effects of SeNPs on shoot and root lengths under cadmium and lead pollution compared to other groups. One of the key processes for stress tolerance is metal uptake by roots and translocation to shoots, which can be restricted by Se metal supplementation to improve plant growth and development under metal stress (Hasanuzzaman *et al.* 2022). Additionally, SeNPs have been linked to improvements in Cd-induced oxidative damage in *Brassica napus* (Qi *et al*. 2021). In Cd-stressed tomato seedlings, such Se-induced lower Cd absorption by roots combined with reduced translocation into shoots and leaves has been described (Alyemeni *et al*. 2018). Crop plants cultivated in contaminated soils benefit from SeNPs; adding SeNPs to rice plants growing in Pb and Cd-contaminated soil enhances plant growth and photosynthesis as well as the concentration of associated genes, proteins, and chlorophyll (Wang *et al*. 2019).

Although consuming cereal grains is the main source of Se metal, most rice-producing nations in Asia and Africa have low levels of the mineral. According to the World Health Organization (WHO), Se metal deficiency affects 15% of the world's population (Tan *et al*. 2018). Therefore, it is crucial for human health to increase the Se metal levels in crops using fertilizer that contains Se metal. In many terrestrial ecosystems around the world, stress caused by heavy metals was previously recognized as a serious problem. Industrialization has decreased soil and crop production by collecting heavy metals (Bashandy *et al*. 2020). Plant extracts act as capping agents, keeping NPs from aggregating and changing their biological activity (Paiva-Santos *et al*. 2021). Under greenhouse conditions, SeNPs increase the number of flowers, fresh and dry weight of stems, roots, and leaves, and the length of *C. roseus* and *C. officinalis* (Safdar *et al*. 2023).

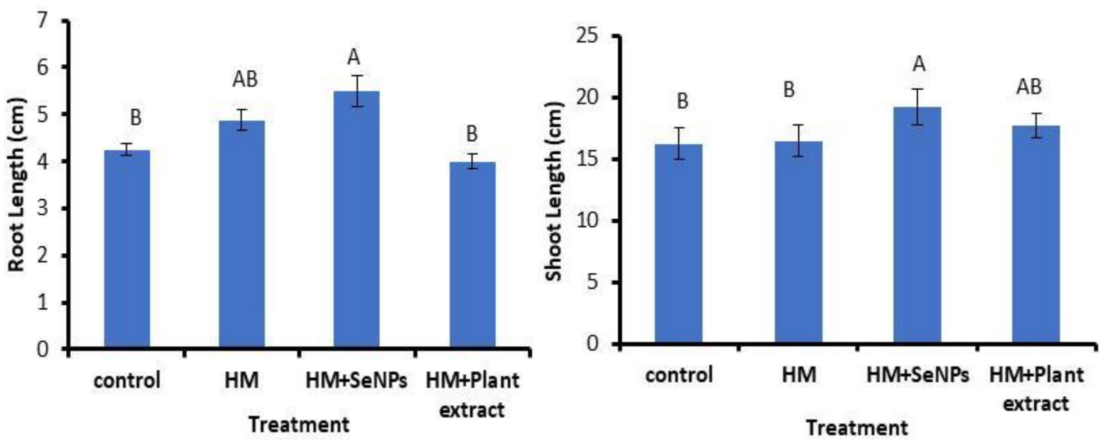

Fig. 5. Growth traits of jute mallow; (A) shoot lengths, (B) root lengths under different treatments (control), Heavy metals (treated with 5 mg/L of HM), HM+ SeNPs (15 mg/kg of soil) and HM+ Plant extract (200 mg/kg of soil)). Each value is mean of 10 replicates ± standard deviation of means. Different lower-case-letters in the same column are significantly different by post hoc-Tukey´s test at P ≤ 0.05; values of the same column with the same letter are not significantly different.

Table 5. Photosynthetic Contents (mg/g Fresh Weight) in Response to Different Treatments

Treatments	Chlorophyll **a**	Chlorophyll **b**	Chlorophyll **a+b**	**Carotenoids**
Control	8.58±0.229A	3.758±0.292B	12.339±0.065B	6.41±0.072B
HM	7.594±0.106C	3.743±0.144B	11.068±0.248C	5.71±0.013D
HM+ SeNPs	11.969±0.099A	5.467±0.147A	17.437±0.245A	6.89±0.014A
HM+ Plant Extract	8.54±0.152B	3.946±0.025B	12.490±0.106B	5.95±0.159C
HSD	0.806	0.293	0.947	0.617
P. value	0.000	0.023	0.000	0.000

Each value is mean of 3 replicates ± standard deviation of means. Different lower-case-letters in the same column are significantly different by post hoc-Tukey´s test at P ≤ 0.05; values of the same column with the same letter are not significantly different. Control (free HM), HM = Heavy metals (Pb+Cd 5 mg/L), HM+ SeNPs = Heavy metals (Pb+Cd 5 mg/L) + SeNPs at 15ppm and HM+ Plant Extract= Heavy metals (Pb+Cd 5mg/L)+plant extract at 200mg /kg soil.

Plant development and yield characteristics are influenced by photosynthetic pigments, which are critical elements of photosynthesis. In the present investigation in Table 5, the toxicity of heavy metals led to a considerable drop in the levels of the carotenoid, chlorophyll a, b and a+b pigments in the leaves of jute mallow. All treatments appeared to significantly increase the contents of carotenoids, chlorophyll a, b, and a+b when compared to the HM group. Heavy metals decrease photosynthesis by damaging the ultrastructure of plastids; prohibiting the building of essential pigments; and decreasing the activity of the Calvin cycle (in which heavy metals act as inhibitors to many enzymes in the Calvin cycle) (Giannakoula *et al*. 2021). Under conditions of metal stress, the prevention of pigment oxidation, sustained enzymatic activity, enhanced stomatal function, and increased photosystem activity have all contributed to an improvement in photosynthesis. Se reduces oxidative stress by regulating the antioxidant defense system. Plant quality and yield are enhanced by Se metal. Se metal, however, has harmful effects on plants when present in excess. Crop yield is decreased by metal toxicity, and metal intake through the food chain poses health risks. Low levels of Se can have a variety of beneficial effects on plant tolerance, including reducing metal toxicity. The manufacture of hormones is stimulated by Se metal, which changes the architecture of the roots and reduces metal intake. Se metal has been found to have a growth-promoting role, which is the result of improved physiological characteristics (Hasanuzzaman *et al.* 2022). Jute mallow plants' capacity to remove hazardous compounds from contaminated soil was improved by SeNPs and plant extract. These findings support those of other researchers (Table 6). When jute mallow plants were exposed to HM stress conditions, their concentrations of Cd and Pb rose considerably in comparison to other groups (Table 6). However, the application of SeNPs or plant extract resulted in a significant reduction in these heavy metal levels when compared to stressed carrot plants. The ability of different plant species to collect heavy metals varies greatly. These results imply that carrot plants irrigated with heavy metals have larger concentrations of metals and plant allocation of the metal substrate, together with enhanced internal plant mobility. SeNPs and plant extract improved the ability of jute mallow plants to eliminate toxic materials from contaminated soil. In this concept, SeNPs and plant extract (which contain many biological compounds and functional groups in the plant extract) have the ability to adsorb substances, which makes them useful for the remediation of soil and water contaminated with heavy metals.

Table 6. Heavy Metals (mg/kg dry weight) in response to Different Treatments

Treatments	Heavy Metals (mg/kg dry weight)	
	Cd	Pb
Control	0.015±0.01C	0.042±0.005C
HM	0.387±0.06A	0.431±0.08A
HM+ SeNPs	0.096±0.01B	0.085±0.01B
HM+ Plant Extract	0.016±0.004C	0.013±0.004C
SD	0.032	0.041
Value	0.003	0.006
*Permissible limit	0.10	0.20

Each value represents the average of three replicates plus the standard error of the means. Values of the same column with the same letter are not substantially different; different lower-case letters in the same column are significantly different by post hoc Tukey's test at $P < 0.05$. Control (free HM), HM = Heavy metals (Pb+Cd 5 mg/L), HM+ SeNPs = Heavy metals (Pb+Cd 5 mg/L) + SeNPs at 15ppm and HM+ Plant Extract= Heavy metals (Pb+Cd 5mg/L)+plant extract at 200mg /kg soil. *Permissible limits are according to FAO/ WHO (2019, 2020).

CONCLUSIONS

1. The phytogenerated SeNPs presented mono-dispersed SeNPs with spherical shape and mean diameter of 36.19 nm were documented.
2. SeNPs demonstrated antimicrobial activity against *B. subtilis, S. aureus, E. coli, K. pneumoniae,* and *C. albicans.*
3. A high antioxidant potential with the IC_{50} value of 10.31 µg/mL was associated with SeNPs.
4. *C. pendulus* extract alone or in combination with SeNPs ameliorated the negative impact of heavy metal problems generated by irrigation with lead and cadmium contaminated water at 5 mg/kg, resulting in significant increases in jute mallow plant growth traits, leaf pigments and a decrease of Pb and Cd contents to a safe dose according to FAO.
5. The antibacterial and antioxidant potential of green synthesized SeNPs *via* extract of *C. pendulus* extract demonstrated in this investigation support their use as a promising tool in the pharmacological field.

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