Preparation of low-toxic cadmium selenide nanoparticles using the plant extract: A comparative study on the extraction techniques, characterization, biological potentials

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Research Article

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Abstract

The present study reported a green approach for sonochemical-assisted synthesis (SAS) of cadmium selenide nanoparticles (CdSe NPs) by using the aqueous extract of *Leontice Leontopetalum* plant. The aqueous extract was obtained by ultrasonic- assisted extraction (UAE; 15 min, 50°C) and microwave-assisted extraction (MAE; 15 min, 180, and 270 W) as two instrumental techniques. Next, the as-prepared aqueous extracts were used in a plant-mediated synthetic approach for the synthesis of CdSe NPs by applying the SAS technique. The synthesized samples were characterized via different techniques; including transmission electron microscopy (TEM), scanning electron microscopy (SEM), X-ray diffraction (XRD), ultraviolet-visible absorption; photoluminescence, and Fourier transform infrared spectroscopy. The average particle size of the prepared CdSe NPs was estimated below 50 nm. UV-Vis absorption and fluorescence techniques indicate a wide absorption band around 360–420 nm and an intense emission peak around 460 nm, respectively. After green synthesis and characterization, antimicrobial, antioxidant, and antifungal properties of the aqueous plant extracts as well as their corresponded CdSe NPs were evaluated by different assays, comparatively. Moreover, the genotoxicity and toxicity potential of the samples were investigated. The results indicate the significant antimicrobial activity of the extracts as well as the CdSe NPs, with the negligible toxicity and genotoxicity impacts.

1. Introduction

Because of the unique characteristics of nanoparticles (NPs), they are increasingly used in various fields such as optoelectronics, biomedicine devices, biosensing, molecular recognition, and imaging (Prabha Dubeya et al. 2010). Due to the acceptable benefits of chemical and physical methods, usually, these methods have been performed for the synthesis of NPs; however, some disadvantages such as expensive, toxic, harmful waste, harsh reaction conditions, and poor biocompatibility can limit these synthetic methods. Recently, more efficient and green techniques named as biosynthesis has attended from the researchers as a favorite way for NPs production. Since synthesis by the plant extract is more cost-effective and more eco-friendly, this technique has been considered as an appropriate substitute for physical and chemical methods. However, preparation of herbal extract plays a critical role in the aqueous extract plant-mediated synthesis of metallic NPs. The common extraction techniques are mostly based on the correct choice of solvents and use of heat and agitation to increase the solubility of the desired compounds and to improve the mass transfer from the plant matrix to the extraction media (Ahmadi Shadmehri et al. 2020; Ahmad et al. 2012). Besides, these techniques usually require a long extraction time even up to several hours, thus running a long time severe prompt the possible thermal degradation of some phyto-constituents (Sahin et al. 2017). Therefore, due to these limitations, development of the new approaches with new characteristics such as shortened extraction time, low solvent volume, and increased pollution prevention concern with special care for thermolabile constituents is quite required.

Microwave-assisted extraction (MAE) and ultrasonic-assisted extraction (UAE) was attended by various researchers, in the last decade. MAE as a more efficient as the classical extraction methods can improve

the extraction condition by reducing the required extraction solvent volume with the enhanced extraction efficiency (Vinatoru et al. 2017). Ultrasonic-assisted synthesis methods have attracted a great deal of attention in a variety of chemistry research areas, such as organic synthesis and solid-state materials. According to the objective of sustainable “green” chemistry, ultrasonic-based process seems to be a key technology for the extraction of natural products. It was reported that the synthesis of NPs under ultrasonic irradiation has a prominent effect on the extraction time of the natural products, as well as the consumption of the extraction solvent (Chemat et al. 2017). Besides, high reproducibility, simplifying manipulation, work-up, and giving higher purity of the final product can be achieved by applying the ultrasonic irradiation. Meanwhile, this method is useful for preparing NPs with various particle sizes (Askari et al. 2019; Sina et al. 2020).

Leontice leontopetalum (*L. Leontopetalum*) is a member of the *Berberidaceae* family and grow mainly in the western side of the Middle East with the high content of quinolizidine alkaloids (Kolak et al. 2011; Greinwald et al. Greinwald). The existence of some secondary metabolites in this plant makes it suitable for green synthesis of NPs (Ahmad et al. 2012). Recently, numerous medicinal plants such as *L. Leontopetalum* due to synthetic antioxidants can be a part of human food and play an important role in neutralizing free radicals formation in the body. In the human body, there are several enzyme systems capable of inhibiting or eliminating the radical's formation. The consumption of antioxidant-rich foods through an alternative supply leads to increased activity of these enzymes in the body. Therefore, it is gaining importance to consume these foods due to their health concerns (Al-Dabbas. 2017). Cadmium selenide (CdSe) NPs are extensively investigated due to their high and tunable luminescence quantum yield, narrow band gap and a variety of optoelectronic conversion (Hamizi et al. 2012). Although synthesis of other NPs such as CdTe (Moradi Alvand et al. 2019), ZnTe (Moradi Alvand et al. 2019), Fe_3O_4 (Demirezen. et al.2019), Au (Sunayana et al. 2020), NiO (Iqbal et al. 2019.),ZnO (Rajabi et al. 2017), Ag (Rajabi et al. 2016.) have been carried out using the different extract plants; however, CdSe NPs usually synthesized by chemical synthetic approaches.

In this paper, sonochemical-assisted synthesis (SAS) of CdSe NPs was carried out in a plant-mediated approach using the aqueous extract of *L. Leontopetalum* plant obtained by UAE and MAE techniques. The optical and structural properties of the as-synthesized CdSe NPs were investigated by different techniques. Besides, the antimicrobial, antioxidant, antifungal, genotoxicity, and toxicity activities of the extracts as well as the as-synthesized CdSe NPs were examined by different assays, comparatively.

2. Experimental Methods

2.1. Materials and instruments

$Cd(NO_3)_2.7H_2O$ (99.9%), SeO_2 (99.9%), Ethylene glycol, Hydrazine hydrate, 2,4,6-tripyridyl-s-triazine (TPTZ) and $FeCl_3$,$6H_2O$, $FeSO_4$, $AlCl_3.6H_2O$, $NaC_2H_3O_2.3H_2O$, 1,1-diphenyl-2-picrylhrdrazyl, 2,2-azinobis (3-ethylbenzothiazoline-6- sulfonic acid), potassium persulfate, methanol, Gallic acid (GA), Nitric oxide (NO) radical, sodium carbonate were purchased from Merck.

All of the microwave extractions were carried out using a KOC-9N8T model, DAEWOO, Korea. An ultrasonic bath equipped with a heating system (KMH1-120W6501 model Pulse, Italy (40KHZ,120W) was used for ultrasonic-based procedures. UV-Vis absorption spectra of the samples were recorded on a Perkin-Elmer lambda 25 spectrophotometer. A Japanese JASCO FT-IR460 apparatus was used for infrared spectra recording. X-ray diffraction analysis was carried out using a Panalytical X pert PRO system. Transmission electron microscopy (TEM) images of the CdSe NPs were recorded on a Philips-CM30. All fluorescence spectra were obtained using a Cary Eclipse fluorescence spectrophotometer (Varian). Energy-dispersive X-ray spectroscopy (EDS) was taken on a Sirius SD energy spectrometer (EDS, United Kingdom).

2.2. Preparation of the aqueous extract by microwave and ultrasonic techniques

The *L. Leontopetalum* plant was collected in March and April, from Sekedeh -Alvand area in Souq, Kohgiluyeh and Boyer-Ahmad province, Iran. The collected plant samples were shade dried for four days, powdered, and then stored in a tightly closed container for further use. For the preparation of the plant extracts, 15 gr of the powdered flower of *L. Leontopetalum* plant was mixed with 200 ml double distilled water in a Meyer flask. Then, the vessel was kept in the ultrasonic device at 50°C, for 15 min for UAE approach. Also, MAE process was carried out by 15 min exposure of the extraction vessel under two different radiation powers (180 and 270 W). The obtained extract was filtered and placed in the refrigerator (Sherwani et al. 2012).

2.3. Preparation of CdSe NPs by SAS and chemical method

The preparation of CdSe NPs by SAS method was performed in the presence of the obtained aqueous extract according to the previous report, with some modification (Karimi et al. 2019; Moradi Alvand et al. 2019). For the plant-mediated synthesis of CdSe NPs combined with SAS method, 25 ml of the aqueous extract was placed in a two-naked flask. Then, 50 mL of the aqueous solution of $Cd(NO_3)_2.7H_2O$ (6.0 mM) was added dropwise to the solution. Next, 50 ml of SeO_2 (2.0 mM) was added to the above solution. The reaction was sonicated for 15 min, 40 °C (40 kHz, 120 W), under the Ar atmosphere (Scheme1**)**. The resulted solution of CdSe NPs was kept at 5 °C for further analyses. Meanwhile, in a control synthesis, CdSe NPs was synthesized by the chemical method at 40 °C. The reactants were treated according to the plant-mediated synthesis, however, in the absence of the aqueous extract. In the chemical synthesis, ethylene glycol (2.0 mM) and hydrazine hydrate (1.0 mM) was used as a capping agent and reducing agent, respectively. Then, the resulted solution was refluxed under vigorous stirring at 40°C for 5 h (Srivastava et al. 2012; Yen et al. 2005).

2.4. Assay of total phenol and flavonoid

Polyphenol content in the extract and NP samples were assessed by Folin–Ciocalteu reagent according to previous procedure (Mirzaei et al. 2014). In this method 100 µl of the sample mixed with 500 µl of the reagent. After 1 min, 400 µl of sodium carbonate solution (7.5%) was added, and the mixture was incubated at room temperature for 30 min. The absorbance intensity of the solution was measured at

765 nm using spectrophotometer apparatus. In this protocol, Gallic acid (GA) was used as the standard solution and the results were expressed as mg GA /g sample.

The total flavonoid in the samples was determined, according to the previous report (Mirzaei et al. 2014). To 1.0 ml of each sample, 1.0 mL of distilled water and 0.10 mL of 10% aluminum chloride was added. After six min, 0.10 mL of sodium nitrite (5%) was added to the above solution. After five min incubation and the addition of *sodium* hydroxide solution (1.0 ml; 1.0 M), the absorbance intensity of the sample was measured at 415 nm, against Rutin as the standard solution, spectrophotometrically. The results were expressed as mg Rutin /g sample.

2.5. Evaluation of biological activity of the samples

2.5.1. Antibacterial and antifungal potential

In this section, six microbial specimens including four bacterial species and two fungus strains were used to assay the antibacterial and antifungal potential of the samples. The bacteria were divided to gram-positive (*Staphylococcus aureus* (ATTCC6538), (*Bacillus subtilis* (ATCC6633)), and gram-negative (*Escherichia coli* (ATCC6538), *Pseudomonas aereuguinosa* (ATCC 9027)). The *Aspergillus oryzae* and *Candida albicans* (MTCC 227) were collected, too. The Bacteria were cultured in Mueller-Hinton agar for 24 at 37°C, while fungous samples were grown in Sabouraud dextrose agar and potato dextrose agar (PDA) media at 29°C for 72h (Montazerozohori et al. 2014). Three common methods including well diffusion method (WDM), minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) tests were performed for antibacterial experiments.

i. WDM assay

For the determination of the test organism's susceptibility, 10^7 colony-forming units CFU mL sterile saline overnight cultured of bacteria in Mueller-Hinton broth (MHB) (Oxoid, England) was collected, transferred, and treated with different concentrations of the samples (i.e. 6.25, 12.5, and 25 mg/ml) on a Petri plate incubated overnight (Hi-media) in 37°C for 18 h. The diameter of the inhibited area around each well was considered as an antibacterial activity (Ravikumar et al. 2012). Blank or negative Control, 100 µL of DMSO was used. Besides, the standard antibiotic disk contains amoxicillin (25 mg disk1), kanamycin (10 mg disk1), and cephalexin (30 mg disk) was used as controls, according to the protocols of the Clinical and Laboratory Standards Institute (Moradi Alvand et al. 2019).

ii. MIC test

Different amounts of samples prepared by serial tube dilution (0.0024 to 12.5 mg /mL) were inoculated in Mueller-Hinton broth which exposes to different test organism suspension (Hamedi et al. 2015). After an incubation period of 18 to 24 h, the number of macroscopic growth revealed MIC.

iii. MBC test

The MBC is defined as the least amount of the agent that is required for original bacteria-killing on an area of Muller-Hinton agar medium with an incubation period of 18 to 24 h. For assessment of MBC, a subculture of the extracts as well as CdSe NPs samples without growth prepared onto the Muller Hinton Agar medium plate in MIC procedure and incubated for 18 to 24 h at 37 °C (Montazerozohori et al. 2014).

iv. Antifungal activity test

The antifungal potential of the extracts and CdSe NPs assessed by *Candida albicans* and *Aspergillus oryzae* used well diffusion method for 72 h. The sporangial suspension was grown on potato dextrose agar (PDA) and the concentration of fungus was estimated by a cell-counting chamber. The inhibition zone was recorded as the antifungal potential of the samples (Montazerozohori et al. 2014).

2.5.2. Antioxidant Activity

The antioxidant activities of the extract and CdSe NPs were estimated using four popular 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2-azinobis-(3ethylbenzothiazoline-6-sulfonic acid) diammonium salt ($ABTS^+$), Ferric reducing antioxidant potential (FRAP), and NO tests, spectrophotometrically. It worth noting that Trolox was used as the standard reference in both ABTS and DPPH protocols.

i. DPPH method

1.0 ml of DPPH reagent, as a synthetic-free radical, was added to 20 µl of the samples and after 15 min, the inhibition percentage was calculated at 517 nm, as follow:

See equation 1 in the supplementary files.

Where; A_0 and A_1 is the absorbance intensity of the control and the sample, respectively.

ii. ABTS method

1.0 ml of $ABTS^+$ radical reagent was mixed with 20 µl of the test samples and after 15 min, inhibition percentage was calculated at 534 nm, spectrophotometrically, according to Eq.1 (Mirzaei et al.2013).

iii. FRAP Assay

FRAP test is based on the ability of ferric iron reduction by sample (Fe^{3+} to Fe^{2+} ions) that is described by Benzie and strain (Wong et al. 2006). Blue color formation contrast to DPPH and ABTS is directly dependent on reduction potential as well as the antioxidant activity of the samples at 593 nm. An aqueous solution of $FeSO_4$ (1.0 ml; 0.37µM) was used as the standard. The results were reported as mmol Fe^{2+} / gr of samples.

iv. NO index

The scavenging potential of NO concentration of the samples was estimated by Griess Illosvoy procedure, colorimetrically (Kleinbongard , et al. 2002). In a test tube, 500 µl of the samples was added to 2.0 ml of sodium nitroprusside (10%) and 500 µl of phosphate buffer solution (PBS) and incubated at room temperature for 180 min. Then, 500 µl of the test tube content was transferred to 1.0 ml of Griess reagent (1:1 mixture of 1% sulfanilamide in 2.5% orthophosphoric acid and 0.1% N-(1-naphthylethylenediamine) in distilled water). After 30 min, radical inhibition was estimated from the absorbance intensity of the samples recorded at 540 nm against sodium nitrite, as a standard solution.

2.5.3. Toxicity and Genotoxicity

In this study, for toxicity and genotoxicity evaluation of the samples, six *A. cepa* bulbs were collected and placed in tap water, in dark condition, and room temperature. After 48 h, the best onion roots were selected for treated with different concentrations of the samples (500-3000µg/ml). For evaluation of toxicity, the half-maximal effective concentration (EC_{50}) was estimated and then 10 roots of each bulb in each sample was harvested and the length of each one was measured after 96 h. Tap water and ethyl methane sulfonate (EMS, 2.0×10^{-2} M) was used as negative and positive controls, respectively.

For assessed genotoxicity after 48 h, mitotic index (MI) and chromosomal abnormalities in the mitotic stage were estimated in each bulb. For the mitotic division and chromosomal anomalies, five or six root tips from each bulb were selected by random and treated with 1.0 M HCl, at 50°C for 10 min, then, 1-2 mm of each root tips were cut and stained with aceto-orcein 2%. For chromosome study in mitotic phases, 1000 cells screened by a light microscope at 40x magnification (Naithani et al. 2011). The MI values were estimated using Eq.2, as: **see equation 2 in the supplementary files.**

3. Result And Discussion

3.1. Characterization of the as-synthesized CdSe NPs

After green synthesis of CdSe NPs based on the plant-mediated approach, characterization of the as-synthesized samples was carried out using various techniques, including UV-visible absorption and fluorescence spectroscopy, XRD, FT-IR spectroscopy, EDS, TEM, and SEM.

To investigate the phase purity and average crystallite size of the synthesized CdSe NPs, XRD technique has been utilized. (Fig.1) illustrating crystallographic studies of CdSe NPs in the 2θ range from 10° to 80°. Several prominent Bragg peaks in XRD patterns can be seen at 2θ of 27.45, 34.06, 39.63, 44.72, 48.79, 54.63, 55.12, and 65.12 which corresponded to phase plane of (002), (101), (102), (110), (103), (112), (004), and (203). The intense and known peaks indicated the successful synthesis of CdSe NPs with a high crystalline single phase as well as the cubic structure (Srivastava et al. 2012; Choubey et al. 2018). Some additional peaks are also observed in the XRD pattern due to the presence which may be related to remained organic matter or amorphous impurities (Ramalingam et al. 2009). The average size of crystalline CdSe NPs using the Debye–Scherrer formula was obtained to be 37.3±0.4 nm.

Fig.2 shows the absorption and fluorescence spectra of the CdSe NPs prepared by SAS approach in the presence of *L. leontopetalum* aqueous extracts obtained by MAE and UAE techniques. As it can be seen (Fig.2 A,B,C), a broad absorption band from 360-420 nm was detected for all of CdSe NPs samples. Besides, an intense emission peak between 450-480 nm can be observed in PL spectra of the samples (Fig.2 G). The optical properties of CdSe NPs prepared by MAE and UAE extract is close, however, some spectral shift in both absorbance and emission spectra can be seen. Furthermore, the optical band gap energy (E_g) of the samples was calculated from absorbance data, based on the Tauc method (Fig.2 D,E,F). The E_g values of CdSe NPs prepared by UAE, MAE 180, and MAE 270 W were obtained to be 2.40, 2.20, and 2.35, respectively. The observed significant enhancement in the band gap energy as compared to the bulk CdSe (ca. 1.75 Ev). was attributed to the quantum size confinement effect (Xi et al. 2007). A similar E_g value of 2.61 was reported by (Das, et al. 2012).

FT-IR spectra of CdSe NPs prepared by aqueous extract of *L. Leontopetalum* plant were demonstrated in (Fig.2 H). Some of the intense peaks in all of the samples are as 3340-3400cm^{-1} which might correspond to an (-OH) or N-H stretching, 2850cm^{-1}(C-H alkane), 1639 cm^{-1} (C=O), 1400 (C=C aromatic stretching SP^2), 1050cm^{-1} (C-O alcohol or carboxylic acid). In some reports, the observed distinguish absorption band in finger print region, below 600 cm^{-1} was assigned to the prepared nanoparticles (Azizi et al. 2014; Azizi et al. 2013).

TEM images of CdSe NPs synthesized by SAS approach in the presence of *L. Leontopetalum* aqueous extract (MAE 180, 270W, and UAE) were provided in (Fig. 3). The images show the small particles with an average size below 50 nm. Besides, SEM images demonstrated the uniform and spherical nano-scaled particles with narrow size distribution and regular shape. Besides, the elemental analysis of the CdSe NPs was carried out using EDS As shown in (Fig.3 G), the strong signals of cadmium and selenium atoms can be observed in the results, which confirmed the successful synthesis of CdSe NPs.

3.2. Biological properties

Some experiments were performed to study and evaluate the biological potential of the extracts as well as CdSe NPs, in terms of antibacterial, antifungal and antioxidant activities by different assays. For safety and application of samples, toxicity, and genotoxicity were managed by allium cape.

3.2.1. Antimicrobial and antifungal activity

All extract samples and CdSe NPs were screened against four clinically important microorganisms by WDM. All bacteria especially *E. coli* and *P. aereuguinosa* at 25 mg/ml concentration exhibited acceptable and appropriate antimicrobial activities that reveal by zone inhibition (Fig 4). The plant extracts and CdSe NPs (UAE) and CdSe (MAE) showed a different levels of antibacterial activity. Among the screened samples, the antibacterial activity of the UAE samples was highest in all of the tested bacteria strains. As seen, the antibacterial activity of the samples was as the following orders:

CdSe NPs (UAE)> CdSe NPs (MAE; 270W)> CdSe NPs (MAE; 180W)> Aqueous extract (UAE) > Aqueous extract (MAE; 270W)> Aqueous extract (MAE; 180W).

Meanwhile, according to MIC assay (Table S1, S2), the maximum antimicrobial activity was taken for the aqueous extract obtained by UAE and its corresponded CdSe NPs. As observed, these exhibited the highest antibacterial efficacy against *P. Oeruginosa* and *E. coli* at 25 mg/ml concentration. The lowest antibacterial efficacy was observed against the gram-positive bacteria (*S. Aureus* and *B. Subtilis*); while it was inhibited at 6.25 mg/ml concentration of the samples (Table. S2). Accordingly, CdSe NPs synthesized by SAS approach waves showed more antibacterial activity than the extract samples against the tested bacteria.

In addition, the antifungal activities of the as-prepared CdSe NPs and the aqueous extract samples were investigated in-vitro against *A. oryzae* and *C. albicans* fungal cultures, using the WDM procedure (Table S3). As can be observed, the antifungal activity of NPs as well as the extract samples enhanced by increasing their concentration. Among the screened samples, CdSe NPs synthesized by UAE extract showed significant inhibition against all of the tested fungal, however, with more susceptibility against *A. oryzae* at 25 mg/ml concentration. As obtained, the antifungal activity of CdSe NPs synthesized by the MAE-based extract is also higher than the extracts.

3.2.2. Assay the total phenol and flavonoid content, and antioxidant activity of the samples

As found, the high phenolic and flavonoid content was observed for the aqueous extract samples, especially for extract source. The total phenol content of the extract samples was highest in MAE and UAE samples (135-147 mg Gallic acid / g sample) and was significantly different from other samples (47-52mg Gallic acid / g sample) (Fig 5A). The highest flavonoid amount was observed for the extract samples obtained by MAE and UAE approaches (98-120mg Rutin / g sample), which was significantly different from the as-synthesized CdSe NPs (53-69 mg Rutin / g sample). (Fig 5B). The minimum content of total flavonoid was seen for CdSe at 180W (53 mg Rutin / g sample). As reported, some secondary metabolites were considered as responsible for the observed biological activities of the herbals such as antioxidant, antibacterial, and antifungal potential (Tungmunnithum et al. 2018). For example, flavonoids consist of a large group of polyphenolic compounds having a benzo-γ-pyrone structure and are ubiquitously present in plants (Kumar et al. 2013). They can simulate the biosynthesis of antioxidant molecules, metal chelating and scavenge free radical in-vivo or in biological systems (Babbar et al. 2015.). According to the previous epidemiological studies, another role of flavonoids has reported the decrease of cell proliferation and low-density lipoproteins (LDL) on biological systems (Zeka et al 2017). The reasonable use of the *L. Leontopetalum* in traditional medicine for the control of infectious disease may be related to total phenol and total flavonoids content of the plant which is revealed in the present study(Spiridon et al. 2011).

In addition, to determine the antioxidant capacity of the samples, two different mechanisms including radical scavenging (i.e. DPPH, ABTS) and reducing power mechanisms (FRAP) was applied. The DPPH,

ABTS, and FRAP methods are simple, suitable, and most commonly used practice to estimate the radical scavenging potential of plant derivatives in *vitr*o (Kaska, et al. 2019).

The DPPH and ABTS scavenging activities of all samples were estimated and the results are presented in (Fig.6A**)**. The mean antioxidant activity of the samples in DPPH assay was quite considerable (84-96%). However, no significant difference was observed between the antioxidant activities of CdSe NPs and the extract samples, at $p<0.05$.

The mean free radical scavenging activity based on ABTS test was similar to that of the DPPH test, however, the antioxidant activity of the extract (80- 88.3%) was higher than CdSe (68-77%). There was a significant difference between the antioxidant activity of extract and CdSe samples, at $p<0.05$. According to the results, the minimum and maximum antioxidant activity were observed for CdSe NPs (180 W) and the aqueous extract of MAE and UAE with the inhabitation percentage of 68.0 and 85.6 %, respectively (Fig 6B).

The highest and lowest mean radical scavenging activity of NO was observed for CdSe NPs (270W) and the extract with 60 and 48.3 % inhabitation percentage, respectively. As it can be seen in (Fig 6C), antioxidant activity based on NO assay was obey from the in the following order: CdSe (270 W)> CdSe (UAE)> CdSe (180 W)**>** Extract (UAE)> Extract (270 W) > Extract (180 W).

In addition, the FRAP assay was used to reducing the estimation of the samples in a redox reaction as an antioxidant activity index. The reducing potential of the samples was determined according to the FRAP approach and the results were shown in (Fig 6D). The values of antioxidant activity based on FRAP assay were in the following order: Extract (270 W) > Extract (180 W)> Extract (UAE)> CdSe (270 W) > CdSe (180 W)> CdSe (UAE). In this test, the maximum antioxidant activity was observed for the extract (270 W) sample with a value of 183.6 mmol Fe^{2+}/g of the sample. The high level of Fe^{2+} in the extract may be due to the presence of some important functional groups such as hydroxyl, carbonyl, and galloyl in the extract of *L. Leontopetalum* leaves (Afsar et al. 2018).

All samples revealed potent antioxidant activities using four different complementary systems in DPPH, ABTS, FRAP, and No assays. According to the present results, all samples were good candidates for scavenging of free-radical biosynthesis in organisms. Free radicals are can oxidized biomolecules and cause injury to the cell by a change in the DNA and RNA of the cell, resulting in disturbance in the function of the cell and may increase the risk of a lot of degenerative diseases, cancers, and finally cell death. (Nordberg et al.2001; McCord et al. 2000).

All studied samples in the present study revealed high scavenging activity against synthetic free radicals and this is an important subject in pharmacological practice for the treatment of degenerative diseases such as Alzheimer's and diabetes mellitus (Kolak et al. 2011). According to this finding, the potent antioxidant activity of *L. Leontopetalum* extract may be related to the high level of polyphenol and flavonoid content. These compounds in the *L. Leontopetalum* plant a tend to inhibit oxidative stress and lipid peroxidation stress in biological systems using different pathways (Al-Dabbas. et al. 2017).

The ability of the studied samples to scavenging nitric oxide free radicals by No Inhibition test has been demonstrated. It is useful for screening the anti-inflammatory potential of the material in vitro state in plant derivatives. In a recent study, *L. Leontopetalum* extracts showed higher anti-inflammatory activity than CdSe NPs NPs due to the presence of compounds such as total phenol, flavonoids, and tannins. According to some research reports, the anti-inflammatory activity of some plants can be due to total phenol, flavonoids, saponins, tannins, and cardiac glycosides (Govindappa et al. 2011).

3.2.3. Study of toxicity and genotoxicity

Usually, EC_{50} was used to estimate the toxicity in the experiments. It was considered as the mean effective concentration that inhibited 50 % root growth compared to the control. The morphological parameters such as length growth, shape, and color of the root in *A. cepa* for toxicity evaluation were considered. The inhibition of the root growth parameter is one of the most rapid responses to estimate the toxic effect of the samples by the root growth of *A. cepa*. The EC_{50} of the tested specimens was obtained in the range of 2420-2950 µg/ ml (Table S4, Table 1). The highest and lowest amount belonged to the CdSe NPs (270 W) and aqueous extract (180 W), after 48 hours, respectively. The percent of root growth retardation of *A. cepa* in the extract was the lowest (7.3%), while, CdSe 270 W was the highest amount in 96 h (31.7%).

Table 1:Mitotic index of *A. cepa* root tips exposed to different samples after 48 hours

MI (%)	Dividing cells	cells	Concentration	Treatment groups	
31.5	315 ±26	1000	----	Tap water	
6.2	62 ± 17	1000	12 mM	EMS	
27.6	276 ± 22	1000	300 µg/ml	180W	Aqueous
24.6	246 ± 25	1000	300 µg/ml	270W	Extract
27.3	273 ± 18	1000	300 µg/ml	UAE	
24.1	241 ± 23	1000	300 µg/ml	180W	CdSe NPs(SAS)
23.1	231 ± 24	1000	300 µg/ml	270W	
25	250 ± 31	1000	300 µg/ml	UAE	

Ethyl methanesulfonate (EMS, (MI): Mitotic index. Data are expressed as Mean ± S.D

In addition, mitotic index values (MI) was performed to assess the chromosome damage and cell division disturbance in term of genotoxicity. As observed, the lowest (23.1 %) and highest (27.6%) MI values were recorded for CdSe 270W and the aqueous extract (180W), compared to the control. There was no significant difference was observed through the samples in terms of MI over 48 h. (Table 2). According to

the results, there was a positive correlation between MI values with the root tips length and EC_{50}. In contrast to the mitotic index, there was a significant difference in root growth inhibition and EC_{50} in the extract and NP groups (Table 2 and Fig7,8).

Table 2: The root length and EC_{50} of *A. cepa* root tips exposed to different concentrations of samples at 96 hours

EC50 (µg/ml)	% Inhibition	Growth compared to control (%)	Root length Growth(cm)	Treatment groups	
-	0	100.0	4.1± 0.23	Tap water	
-	66	34	1.4 ± 0.11	EMS	Control
2950	12.2	87.8	3.6 ± 0.25	180W	Aqueous Extract
2640	17	83.0	3.4 ± .27	270W	
2820	7.3	92.7	3.8 ± 0.31	UAE	
2600	24.4	75.6	3.1 ± .24	180W	CdSe NPs(SAS)
2420	31.7	68.3	2.8 ± 0.26	270W	
2640	22	78	3.2 ± 0.38	UAE	

Ethyl methanesulfonate (EMS, (MI): Mitotic index. Data are expressed as Mean ± S.D

According to the analysis of the chromosomes, no abnormalities were observed in morphological parameters in all of the samples exposed to *A. Cepa* over 96 h. Very low toxicity manifested in CdSe samples as reduced MI may be due to the inhibitory role of NPs on *A. Cepa* growth. Due to the high EC_{50} (2420-2950 µg/ ml) and low growth inhibition of root tips in *A. Cepa* (7.3-31.7) and the normality of mitotic indexes (no toxicity) and no chromosomal abnormalities, it can be considered the tested samples as lack toxicity and genotoxicity samples. According to the previous reports, the growth inhibition of *A. Cepa* root more than 45 % indicates the presence of toxic material (Konuk et al. 2007). Meanwhile, the MI normal level indicates that the NPs as well as the extracts lack mitodepressive agent and consequently, DNA and microtubule synthesis was at normal (Fig 7,8).

4. Conclusion

In this paper a green, facile and nontoxic route was developed for the sonochemical synthesis of CdSe NPs, using the aqueous extract of *L. leontopetalum* plant, prior to extraction with ultrasonic, and microwave approaches. The current study suggests that SAS can be considered as an efficient, safe, quick, economic, and eco-friendly route for the synthesis of CdSe NPs. The aqueous extract of *L.*

leontopetalum obtained by the MAE can be provided as a suitable and natural medium for the synthesis and stabilizing of the CdSe NPs. According to the biological studies, it can be supposed that *L. leontopetalum* is considered as an alternative source of natural antioxidant or food additive. This plant can be useful in preventing and treating of many human diseases. Further research such as; isolation and detection of the constituents of *L. leontopetalum* can be useful in future research. The 96-hour exposure of *A. Cepa* to different NPs concentrations (CdSe and …), did not cause significant toxicity. In addition, the exposure to NPs caused a normal value of MI and EC_{50} and low growth inhibition of root tips in *allum cepa*. The low toxicity of the NPs in the present study, is likely imparted due to the green synthesis coating, which has been earlier described to efficiently reduced cytotoxicity.

Declarations

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N.A
Consent to Participate:

All authors contributed, read, and approved the final manuscript.
Consent to Publish:

All listed authors have approved the manuscript before submission, including the names and order of authors.
Authors Contributions:

Dr. Hamid Reza Rajabi proceed with the conceptualization, writing-reviewing, and editing, Zinab Moradi Alvand and Farideh Sajadiasl did experiments, data collection, and draft preparation, Dr. Ali Mirzaeib provides the material and equipment facilities.

Availability of data and materials:

N.A
Declaration of interests:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
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Figures

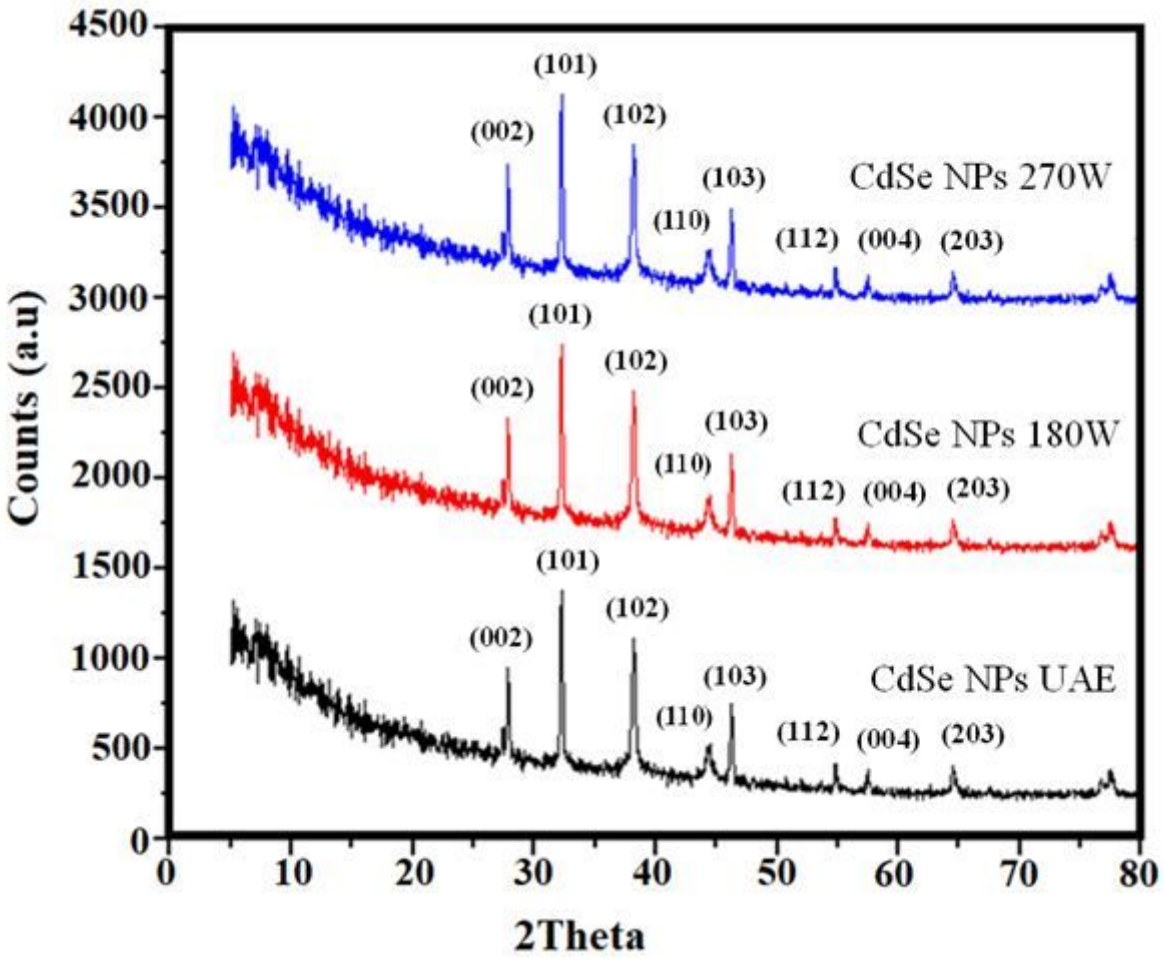

Figure 1

XRD patterns of as-prepared CdSe NPs by MAE and UAE

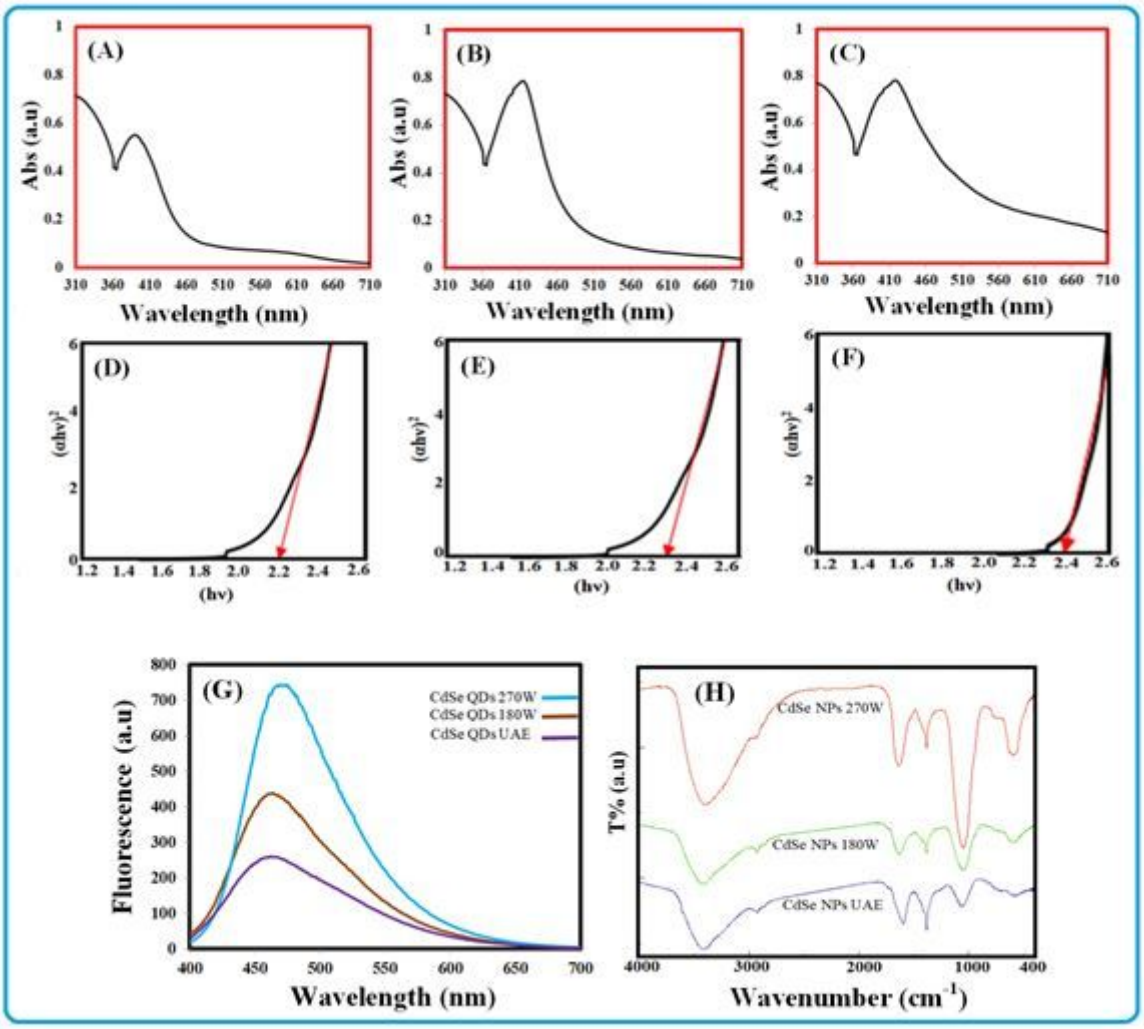

Figure 2

UV–Vis absorption spectra, The insets are the Tauc's plots for the calculation of band gap energy. of the CdSe NPs prepared by MAE at (A,D) 180W (B,E) 270 W and (C,F) UAE, fluorescence, and FT-IR spectra of CdSe NPs prepared by MAE and UAE.

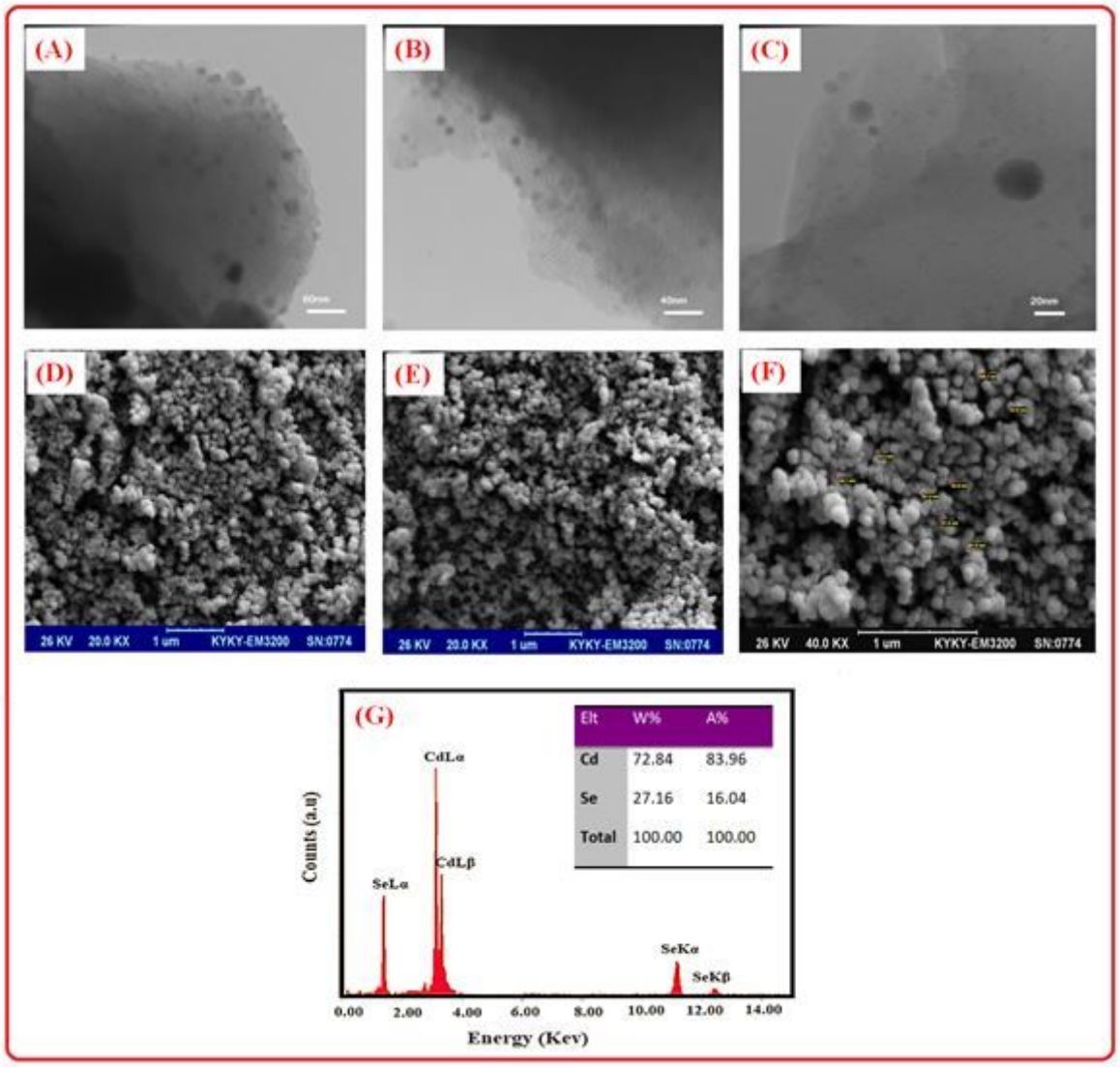

Figure 3

TEM , SEM images of the CdSe NPs prepared by MAE at (A,D) 180W (B,E) 270 W and (C,F) UAE and EDS pattern of as-prepared CdSe NPs by UAE.

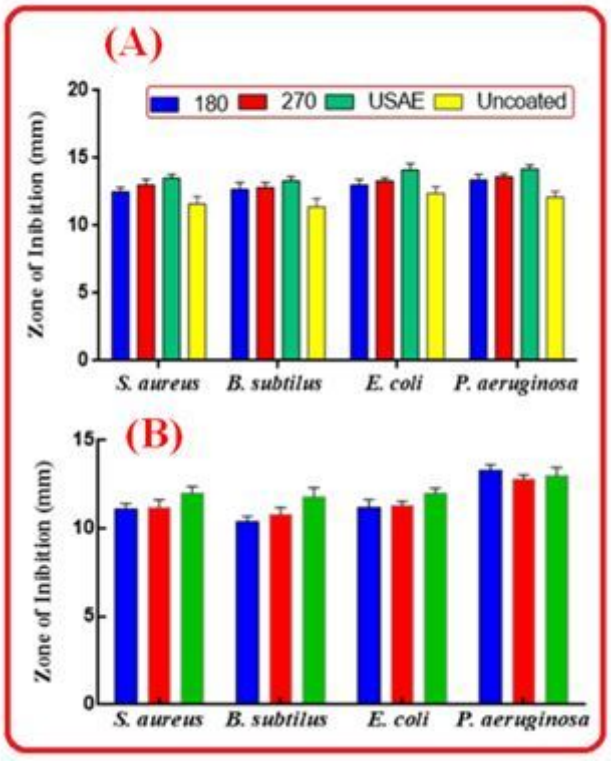

Figure 4

Antibacterial results of CdSe NPs (A) and the extract samples (B) by well diffusion method.

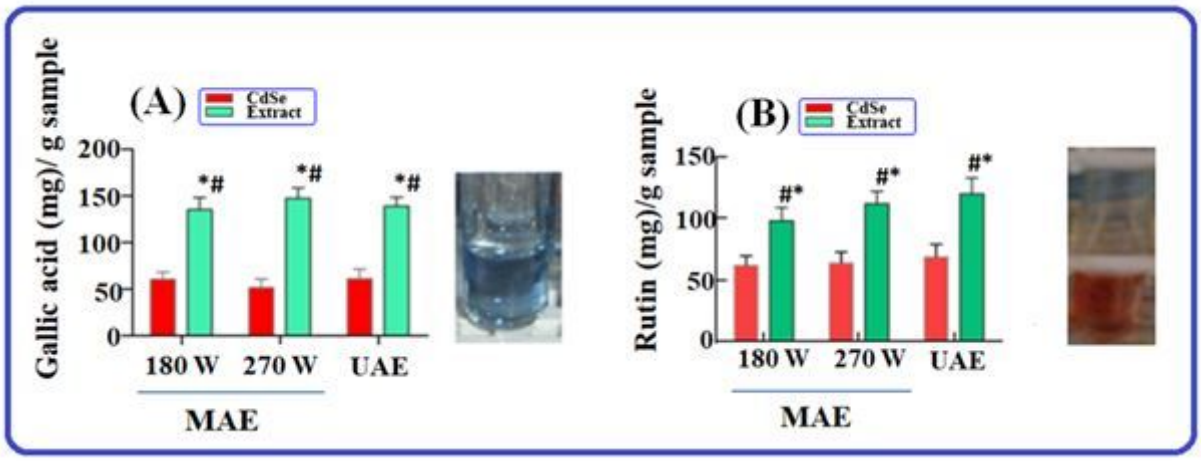

Figure 5

Total phenolic (A) and flavonoid (B) content of samples and typical photographic images. Values are given as mean ± SD. The data were analyzed using one-way ANOVA and post hoc Tukey's tests. A significant difference was considered at. $p < 0.001$ # compared to CdSe and * compared to extract.

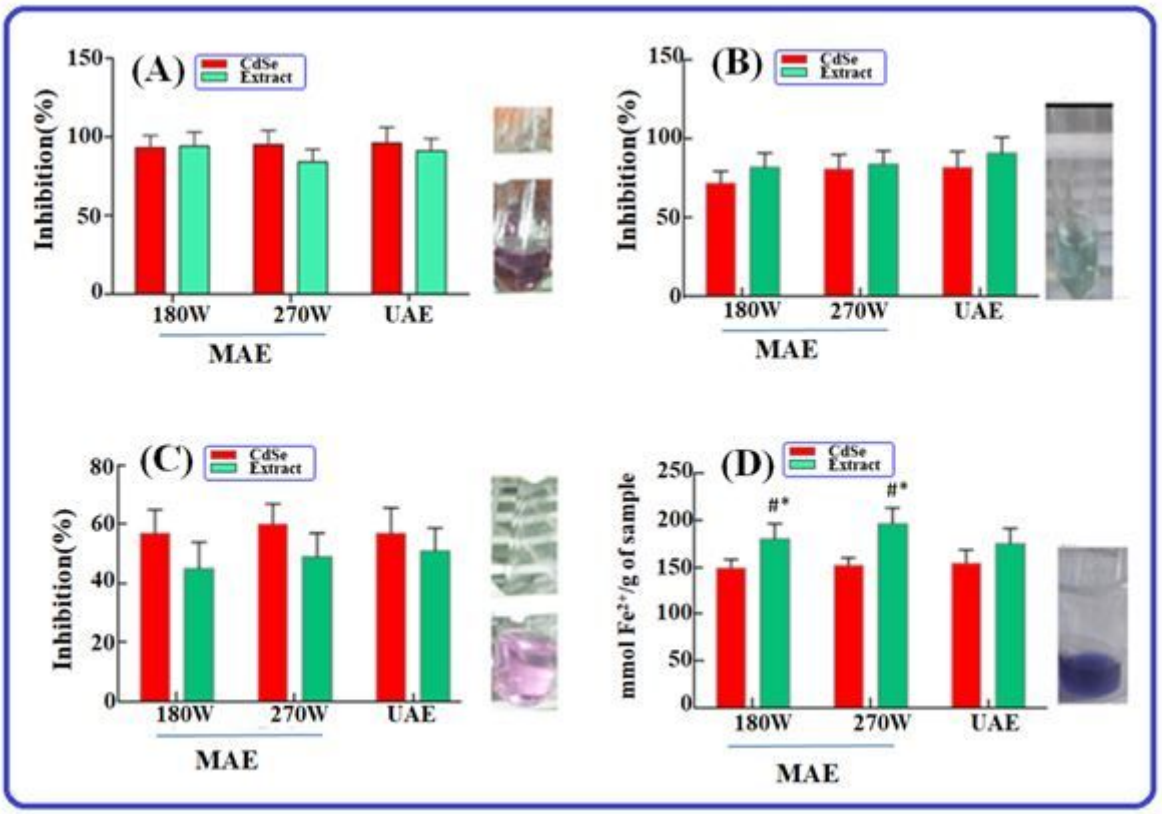

Figure 6

Antioxidant activity of samples by DPPH(A), ABTS(B), No (C) and FRAP(D) with photographic images. Values are given as mean ± SD. The data were analyzed using one-way ANOVA and post hoc Tukey's tests. A significant difference was considered at $p < 0.05$ * compared to CdSe and # compared to extract.

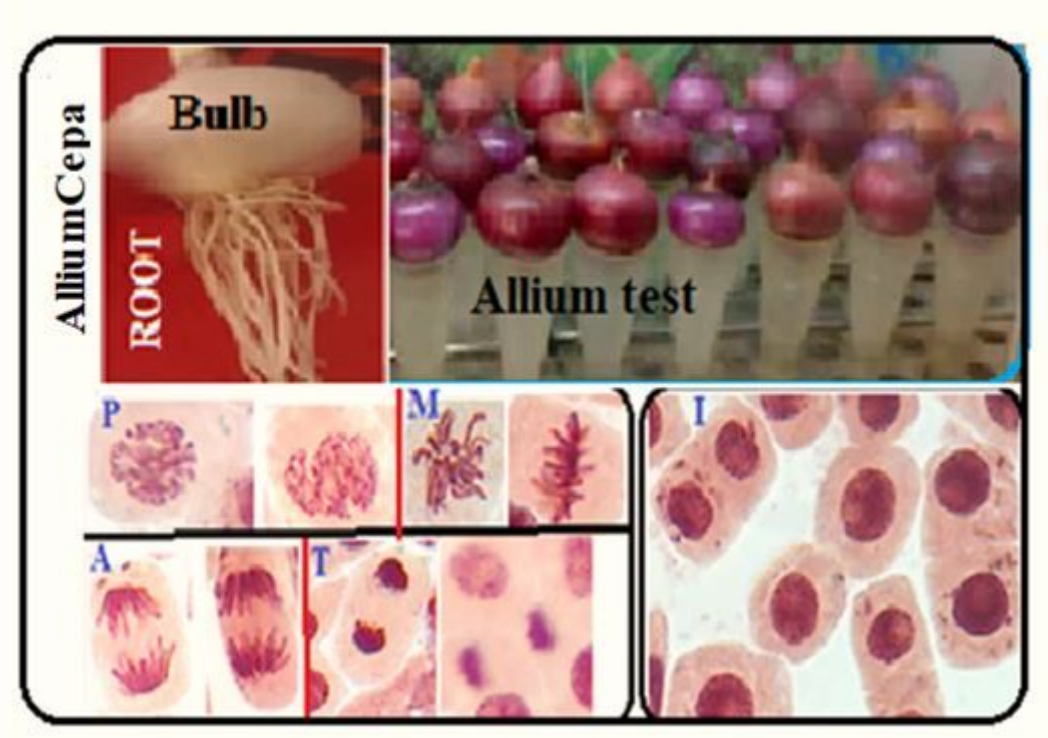

Figure 7

Chromosomes analysis, with normal morphological mitosis in A. Cepa root tips exposed to all samples in 48 h. P = Prophase, M = metaphase, A = anaphase, T = telophase and I = Interphase

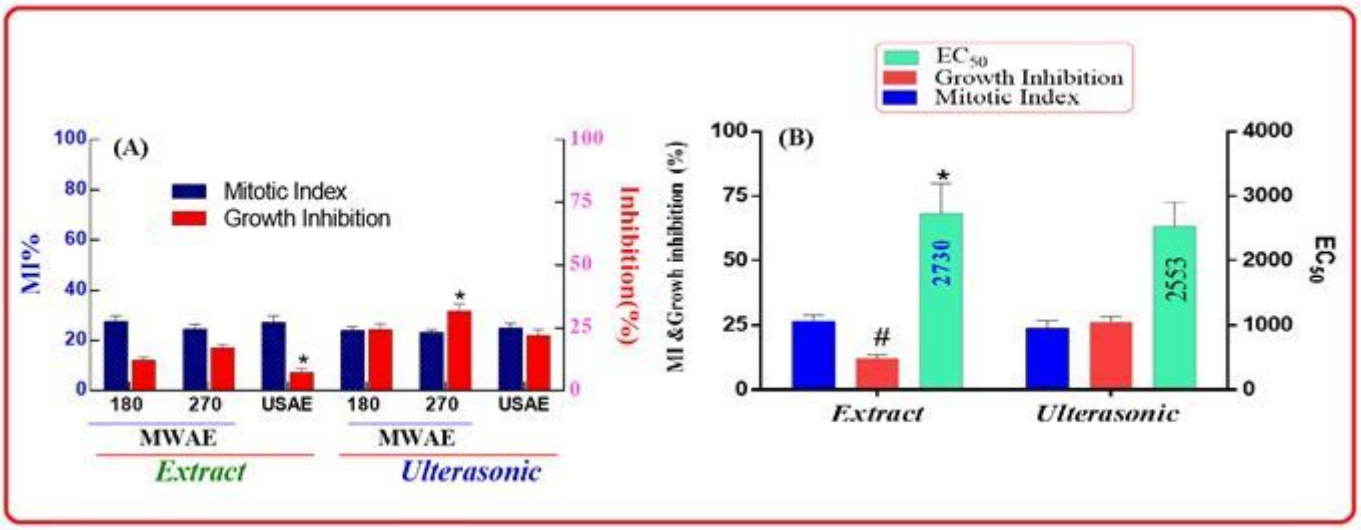

Figure 8

Comparison of root growth inhibition, Mitotic index (A) and Comparison of EC50, root growth inhibition, and Mitotic index (B) of A. Cepa exposed to all samples in 48 h.

Supplementary Files

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- ESM.docx