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Article

Nano-Elicitation of Secondary Metabolism and Antioxidant Potential in Iraqi *Centaurea hyalolepis* using Silver Nanoparticles

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Abstract: The genus Centaurea contains many medicinal species such as *Centaurea hyalolepis* Iraqi with abundant potential secondary metabolites with antioxidant activities. The aim of this study was to assess the stimulation of secondary metabolism and increased the antioxidant capacity of *Centaurea hyalolepis* Iraqi under controlled environment at the presence of AgNPs. The methodology synthetizing silver nanoparticles (AgNPs) using *C. hyalolepis* extract is detailed the experimental section and the synthesized AgNPs were characterized using ultraviolet-visible spectroscopy, scanning electron microscope (SEM), dynamic light scattering (DLS), and X-Ray diffraction (XRD). Foliar spraying was performed with the different concentrations of nano-silver (10, 25, 50 and 100 mg/L) for two weeks. The total phenolic content (TPC) and free radical scavenging activity (DPPH) were then determined. The results showed that the prepared silver nanoparticles were spherical in shape with average diameter of 34.5±10.2 nm, characterized by a stable negative zeta charge. The total phenolic content (TPC) showed a significant increase with the concentration of AgNPs, resulting in an 89.1% increase in plants treated with 100 mg/L of AgNPs than untreated plants. The IC_{50} value (concentration at which 50% of the free radicals are scavenged) for antioxidant activity was also significantly lower at a concentration of 100 mg/L, demonstrating a noteworthy effectiveness of antioxidant property. This study concludes that nano-elicitation with 100 mg/L silver nanoparticles is optimal for enhancing the production of phenolic compounds and the antioxidant activity of *Centaurea hyalolepis*.

Keywords: Nano-Elicitation, *Centaurea hyalolepis*, Antioxidant, Silver nanoparticles

Introduction

The genus Centaurea is a large and important species for economic reasons in Asteraceae family which is distributed in many geographical areas such as flora of Iraq [1]. The extensive and intricate phytochemical makeup of these plants, featuring a wide range of secondary metabolites, is well documented. Among these are sesquiterpene lactones, flavonoids, phenolic acids and alkaloids, which all play their own roles for their biological activities [2]. Centaurea spp. have long been part of traditional medicine in which they have been used as herbal remedies for their reported medicinal properties, including anti-inflammatory, antioxidant, antimicrobial and cytotoxic activity [3]. In particular *C. hyalolepis*, an Iraqi native plant species, has received scientific focus due to its unique secondary metabolite profile and related prospects for a wide range of biological activities [4]. A key

highlight of these compounds is their antioxidant properties, which play a role in their ability to capture free radicals and reduce oxidative stress, a process associated with the development of various chronic diseases and cell damage [5].

Secondary metabolite production is an integral part of the defense strategies that naturally occur in plants as a complex response to various factors such as attack by pathogens, herbivores, and unfavorable climatic conditions. Although these compounds do not directly participate in the plant's basic metabolism, they are crucial for its survival, adaptation and ecological interactions [6]. This crucial enhancement of these valuable compounds can be done in several ways using biotechnology methods, one of them being elicitation. Elicitation is a process that uses specific agents known elicitors that trigger the plant defense response and result in increased secondary metabolite production [7]. Elicitors have been traditionally classified in two classes: biotic (comprising microbial extracts and polysaccharides) and abiotic (heavy metals, UV radiation and osmotic stress) [8]. Since then, however, advances in the nanotechnology have revolutionized the area and developed a very promising and efficient strategy, the nano-elicitation [9].

Silver nanoparticles (AgNPs) have proven to be very effective nano-elicitors, especially due to their distinct physiochemical properties. These are a high surface to volume ratio, high catalytic activities and the natural ability to cross the cell walls of plants into contact with intracellular components [10]. It is known that AgNPs can cause a controlled oxidative stress upon exposure to plant systems. This stress, in turn, acts as a signaling molecule, which subsequently activates key enzymes and intricate signaling mechanisms which are intrinsically connected in the biosynthesis of secondary metabolites [11]. This series of defense response results in the upregulation of genes involved in the production of a wide range of bioactive constituents and thus improves the phytochemical composition in plants and finally the antioxidant capacity [12]. In various medicinal plants, such as Caralluma tuberculata and Aerva sanguinolenta, the AgNPs and silver nanowires (AgNWs) have been reported to significantly enhance the accumulation of important phenolics and flavonoids, as well as of other antioxidant molecules [13]. It is often associated with the production of Reactive Oxygen Species (ROS) and the subsequent activation of the plant's endogen antioxidant defense and finally the substantial increase in secondary metabolites production [14].

The use of AgNPs as an elicitor has a number of unique benefits compared to traditional methods of elicitation. Their nanoscopic size allows for efficient uptake and homogenous distribution into plant tissues, and thus more precise and strong elicitations response [15]. Also, the green synthesis of AgNPs typically involves biological matter such as extracts from various plants, and this could make the AgNPs biocompatible and decrease their environmental impact. This makes them to be sustainable and eco-friendly means for better production of plant derived pharmaceutical compounds. AgNP's ability to subtly influence plant metabolism and substantially increase yields of valuable secondary metabolites opens a new avenues of potential for a multitude of industrial sectors. This strategy is relevant for plants that have the ability to produce a wide spectrum of bioactive compounds, for example, *Centaurea hyalolepis*, that are not known to produce all these compounds in large quantities when growing naturally. Although the nano-elicitation is in the emerging phase and with encouraging outcomes, much more fundamental and systematic research is required. The aim of this study is to explore the effect of silver nanoparticles as elicitor of secondary metabolites and inducer of antioxidant potential in *Centaurea hyalolepis* from Iraq under in vitro conditions.

Materials and Methods

Collection and Preparation Plant

Specimens of *Centaurea hyalolepis* were collected from their natural habitat from Al-Mahawil District in Babylon Governorate during May 2025. One of the Professors in the Department of Biology, College of Science, University of Babylon taxonomically identified the plant species as *Centaurea hyalolepis* L. Collected plant material was thoroughly washed under running tap water to remove dust and debris, followed by rinsing with distilled water. The plant parts were then air-dried in the shade at room temperature (25±2 °C) until constant weight was achieved, typically within two weeks. The dried material was then mechanically milled into fine powder and kept in air tight containers at 4 °C for further use.

Synthesis of Silver Nanoparticles (AgNPs)

A green synthesis approach was used to synthesize silver nanoparticles (AgNPs) using an aqueous extract of *Centaurea hyalolepis* as a reducing and stabilizing agent. Dried and powdered material of *C. hyalolepis* (10 g) was added to 100 mL of deionized water and boiled for 15 min. The extract was filtered through Whatman No.1 filter paper and centrifuged at 5000 rpm for 10 minutes to obtain a clear supernatant. AgNPs synthesis involved the dropwise addition of 10 mL of the aqueous extract into 90 mL of 1mM $AgNO_3$ solution at room temperature (25±2 °C) under constant magnetic stirring (500 rpm). The reaction was performed in the dark, to avoid photo-reduction of the silver ions. The pH was monitored and was maintained at pH 8.2 for optimum reduction. The completion of the reaction was verified by the change in the color from pale yellow to reddish-brown and by scanning in the UV-Vis spectrum [16].

Characterization of Silver Nanoparticle (AgNPs)

1. Ultraviolet-Visible (UV-Vis) Spectroscopy

The successful synthesis of AgNPs was confirmed by using UV-Vis spectroscopy. A UV-Vis spectroscopy was performed colloidal solution of the synthesized nanoparticles over a wavelength range of 300-700 nm.

2. Scanning Electron Microscopy (SEM)

The overall morphology and surface structure of AgNPs was captured using Scanning Electron Microscopy (SEM) technique. Samples were prepared by coating a small amount of the dried AgNPs powder onto an aluminum stub with conductive tape and then sputter-coating with a thin layer of gold to enhance conductivity.

3. Zeta Potential Measurement and Dynamic Light Scattering (DLS)

The hydrodynamic size and polydispersity index (PDI) of the AgNPs in solution was determined using Dynamic Light Scattering (DLS) technique. The hydrodynamic size provides insight into the effective diameter of the nanoparticles, including any adsorbed layers of stabilizing agents. Zeta potential measurement was also performed to determine the surface charge of the nanoparticles, an important parameter to evaluate the system stability in colloidal suspension and its biological interactions. This measurement was carried out at 25 °C.

4. X-ray Diffraction (XRD)

The crystalline structure and phase purity of the synthesized AgNPs was studied by X-ray Diffraction (XRD). A dried sample of AgNPs powder was then placed on a glass slide and examined by X-ray diffractometer, Cu Kα radiation (λ = 1.5406 Å) at 40 kV and 30 mA. The diffraction was in the range of 2θ between 20 and 80 degrees. The Bragg reflection obtained was compared with the standard JCPDS (Joint Committee on Powder Diffraction Standards) file of silver to confirm that the synthesized AgNPs are crystalline in nature.

Experimental Design and Nano-Elicitation Treatment

The cultivation of *Centaurea hyalolepis* was done under the controlled greenhouse conditions (16/8 h light/dark, 25±2 °C, (60-70) % relative humidity). Once the plants have reached an appropriate developmental phase (4-6 weeks old), they were split into several different experimental treatments with at least 10 replications in each treatment. The experimental groups consisted of included control (untreated plants sprayed with only deionized water) and plants treated with different concentration of AgNPs (10 mg/L, 25 mg/L, 50 mg/L and 100 mg/L). The prepared AgNPs solutions were prepared by diluting the stock AgNPs solution (that was characterized above) with deionized water. Treatments at 1/3 day intervals were applied as foliar sprays and then washed off a day after treatment for a period of 2 weeks [17]. Special efforts were made to provide ample, uniform coverage of plant foliage. Plant samples were harvested after the treatment period, washed and processed as described in Section 1.

Extraction of Secondary Metabolites

10 g of dried and powdered plant material of each experimental group was used for extraction. Commonly used 200 mL of 80% methanol in Soxhlet apparatus for (6-8) hours was employed for extraction at room temperature [18]. The extracts were then filtered using Whatman No. 1 filter paper, and the solvent was evaporated under reduced pressure using Rotary Evaporator at 40 °C to obtain

crude extracts. The dried crude extracts were weighed and dissolved in small volume of methanol and stored at -20 °C for further phytochemical characterization and antioxidant assays.

Total Phenolic Content (TPC)

The total phenolic contents of the plant extracts were estimated by Folin- Ciocalteu method. 2.5 mL of 10% Folin-Ciocalteu reagent and 2 mL of Na_2CO_3 7.5% was added to 0.5 mL of the extract solution. The mixture was incubated in the dark at room temperature for 30 minutes and absorbance was read spectrophotometrically at 765 nm. Gallic acid was used as the standard and the results obtained were expressed as milligrams of gallic acid equivalents per gram of dry extract (mg GAE/g DE) [19].

Antioxidant Potential Assay by DPPH Radical Scavenging

The plant extracts were tested for free radical scavenging activity by DPPH assay. Methanol solution of DPPH was prepared at the concentration of 0.1mM. Various concentrations of the plant extracts (10-500 μg/mL) were mixed with 2.5 mL of the DPPH solution. The mixtures were incubated under dark condition for 30 minutes at room temperature. The absorbance was measured and DPPH radical scavenging activity was calculated after which the IC_{50} value for was determined [20].

Statistical Analysis

Each experimental measurement was also repeated three times (n=3), the data were presented as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) in SPSS software (Version 26.0) was used to analyze the data. Tukey's post hoc test was used to identify significant differences between treatment group means at the 5% significance level. In all tables and figures, different lowercase letters (a, b, c...) indicate statistically significant differences between groups.

Results

UV-Vis Spectroscopic Analysis

The successful biosynthesis of silver nanoparticles (AgNPs) from the aqueous extract of *Centaurea hyalolepis* was first identified by visual observation of the color change from pale yellow to reddish brown after 30 minutes when the silver nitrate solution was mixed with the plant extract. This change of color is due to the generation of AgNPs through the surface plasmon resonance (SPR). The UV-Vis absorption spectra of the synthesized AgNPs showed a single SPR band at ~425 nm, indicating the formation of spherical silver nanoparticles (**Fig. 1**). The peak is narrow and symmetrical, indicating a relatively narrow particle size distribution. Similarly, plant extract did not show any significant absorbance in visible range as it was only the biosynthesized nanoparticles which showed the absorbances at the SPR band.

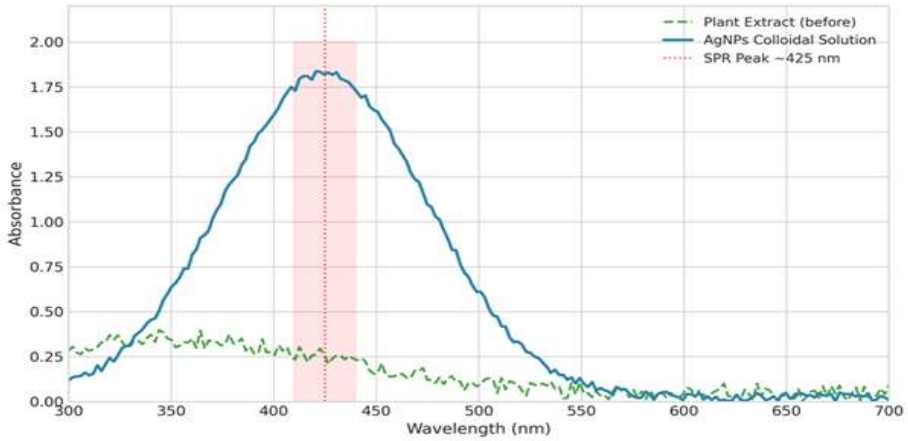

Fig. 1. UV-Vis absorption spectrum of the biosynthesized silver nanoparticles showing a characteristic surface plasmon resonance peak at ~425 nm

Morphological Analysis by Scanning Electron Microscopy (SEM)

The scanning electron microscopy of biosynthesized AgNPs was used to characterize their morphology and sizes. The SEM micrographs indicated that the nanoparticles had a predominantly spherical shape with a smooth surface (**Fig. 2**), and that the individual nanoparticles were well dispersed and there was no sign of aggregation, this confirms the effective stabilization of the nanoparticles using plant extract. The histogram analyses on the particle sizes obtained SEMs revealed that the mean particle diameter was 34.5±10.2 nm, and most of the particles were in the range from 20 to 55 nm. This nanoscale dimension would also be beneficial in elicitation applications, due to yet another added benefit compared to larger particles – they have a greater surface area to volume ratio, hence more interactions with the cell components of plants.

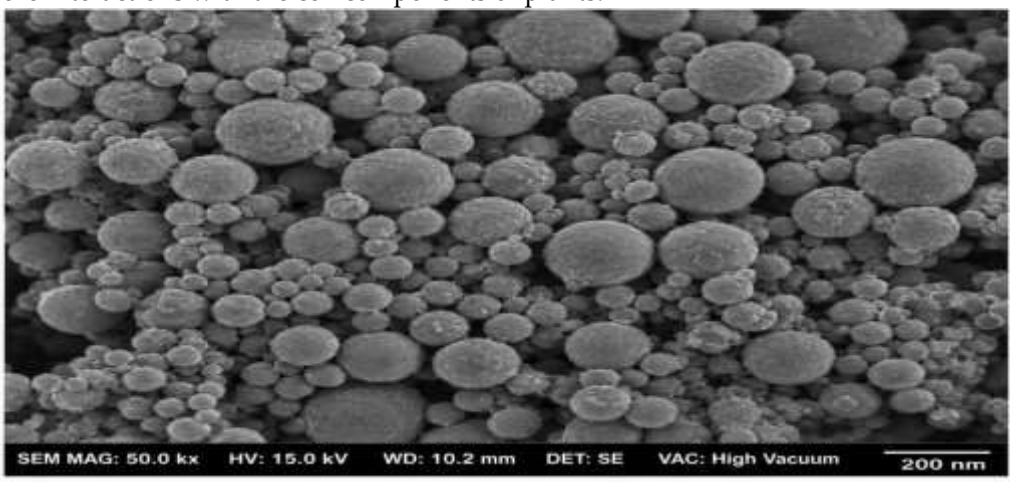

Fig. 2. SEM micrographs of the biosynthesized silver nanoparticles at different magnifications, showing spherical morphology and size distribution

Dynamic Light Scattering (DLS) and Zeta Potential

Dynamic light scattering (DLS) was used to determine the hydrodynamic size and polydispersity of the AgNPs. DLS analysis showed that the hydrodynamic diameter of the particles is 48.3 ± 5.6 nm, indicating that the diameter of the core is slightly less than that observed by SEM. The reason for this difference is believed to be due to the existence of a hydration shell and planted extracted biomolecules in addition to the nanoparticle core. A polydispersity index (PDI) of 0.24±0.03 was obtained, and was considered to be moderately monodisperse. (**Fig. 3 & Table 1**) indicated that the zeta potential readings were strongly negative with values between −26.8 and −31.5 mV regardless of the concentrations of treatments, values above the ±30 mV value range usually required for the colloidal stability. The adsorption of negatively charged phytochemicals, including the phenolic acids and flavonoids, on the nanoparticle surface is reported to give an electrostatic repulsion and hinder aggregation of the nanoparticles in aqueous suspension accounting for the negative zeta potential.

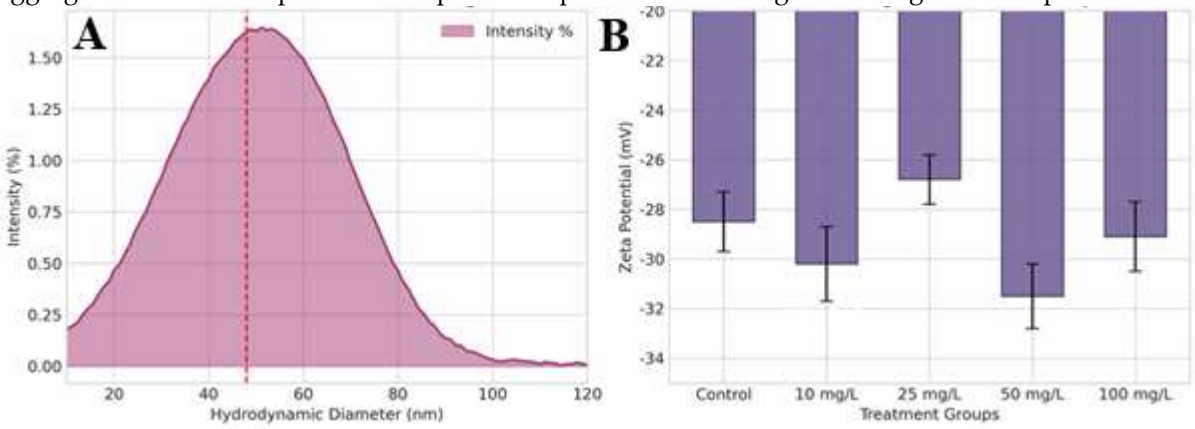

Fig. 3. DLS particle size distribution (A) and zeta potential measurements (B) of the biosynthesized silver nanoparticles

Table 1. Hydrodynamic size, polydispersity index (PDI), and zeta potential of biosynthesized silver nanoparticles at different treatment concentrations

Treatment Group	Hydrodynamic Diameter (nm)	PDI	Zeta Potential (mV)
Control (stock)	48.3 ± 5.6	0.24 ± 0.03	−28.5 ± 1.2
10 mg/L	47.8 ± 4.9	0.22 ± 0.02	−30.2 ± 1.5
25 mg/L	49.1 ± 5.3	0.25 ± 0.03	−26.8 ± 1.0
50 mg/L	46.5 ± 4.2	0.21 ± 0.02	−31.5 ± 1.3
100 mg/L	47.2 ± 4.8	0.23 ± 0.03	−29.1 ± 1.4

X-ray Diffraction (XRD) Analysis

The biosynthesized AgNPs has a crystalline structure, along with being pure in phase, which was confirmed by X-ray analysis. The XRD pattern displayed four prominent diffraction peaks at the angle 2θ of 38.12°, 44.28°, 64.44° and 77.38° that can be indexed as (111), (200), (220) and (311) crystallographic planes of face centered cubic (FCC) silver respectively (**Fig. 4**). The peaks obtained have very good matching with the standard JCPDS card no. 04-0783 for metallic silver, which confirm the crystalline in nature of the synthesized nanoparticles. The crystallite size was estimated using Debye-Scherrer equation from the full width at half maximum (FWHM) of (111) peak and the average crystallite size obtained was around 28.7 nm, which is in reasonable agreement with SEM and DLS results. The biosynthesized AgNPs were found to be highly pure, as no impurity phases like AgO were detected in the XRD plot.

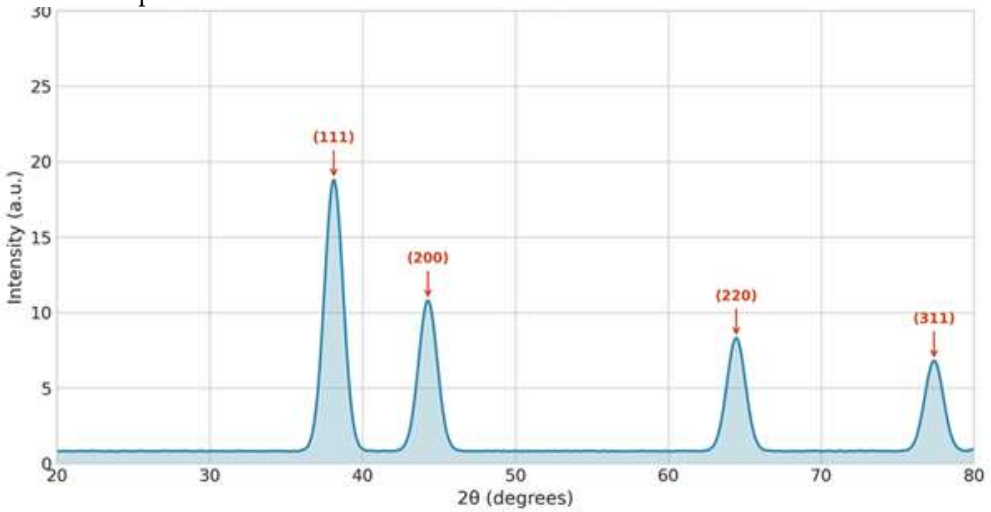

Fig. 4. X-ray diffraction pattern of the biosynthesized silver nanoparticles showing characteristic peaks of face-centered cubic silver

Effect of AgNPs on Total Phenolic Content (TPC)

The concentration of the AgNPs treatment had a significant effect on the total phenolic content of the extracts of *C. hyalolepis*. Results showed a definite trend of dose dependent increase in the TPC with an increase in concentration of AgNPs (**Fig. 5 & Table 2**). The normal (control) plants had a TPC of 32.45±1.82 mg GAE/g DE. The lowest concentration of AgNPs (10 mg/L) caused a slight (38.72±2.15 mg GAE/g DE) but significant increase (19.3%). The TPC for 25 mg/L was 45.18±2.48 mgGAE/g DE, which increased by 39.2% compared to the control. The highest improvement was seen at concentration 50 mg/L and 100 mg/L, with the highest TPC obtained as 53.64±2.73 mg GAE/g DE and 61.37±3.05 mg GAE/g DE which represents 65.3% and 89.1% increase, respectively. The difference between the 50 mg/L and 100 mg/L treatments was significant (at 100 mg/L was statistically differential from the 50

mg/L treatment, $p<0.05$); hence the elicitation effect continued to increase even up to the highest concentration treated.

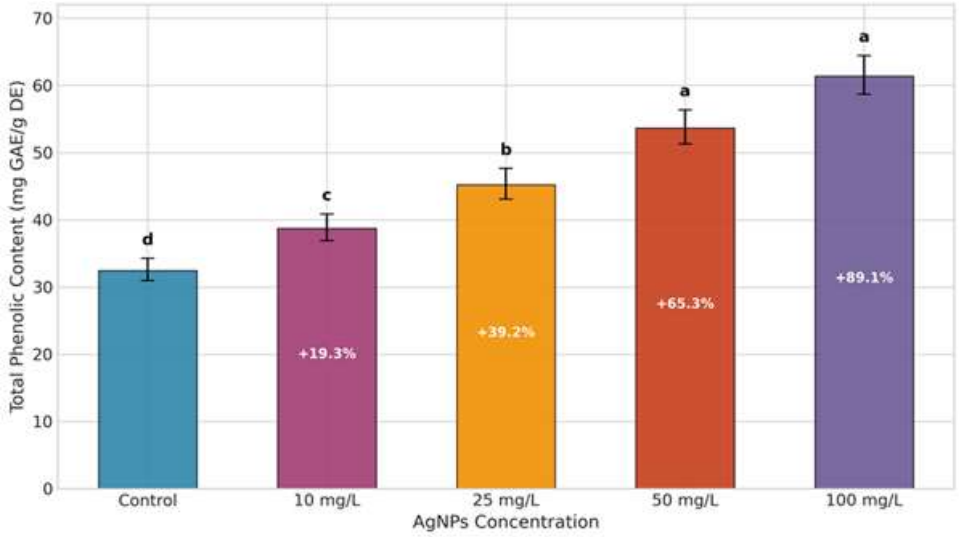

Fig. 5. Effect of different AgNPs concentrations on the total phenolic content of *C. hyalolepis* extracts. Different letters indicate statistically significant differences ($p<0.05$) by Tukey's post-hoc test

Table 2. Total phenolic content of *C. hyalolepis* extracts under different AgNPs treatment concentrations

Treatment	TPC (mg GAE/g DE)	% Increase over Control
Control (0 mg/L)	32.45 ± 1.82^{d}	—
10 mg/L	38.72 ± 2.15^{c}	19.3
25 mg/L	45.18 ± 2.48^{b}	39.2
50 mg/L	53.64 ± 2.73^{a}	65.3
100 mg/L	61.37 ± 3.05^{a}	89.1

Note: Different lowercase letters (a, b, c, d) indicate statistically significant differences between groups ($p<0.05$) based on Tukey's post-hoc test.

Effect of AgNPs on DPPH Radical Scavenging Activity

The antioxidant activity of the *C. hyalolepis* extracts was determined by DPPH radical scavenging assay. The results indicated that the antioxidant activity was increased with the increase in the dose of AgNPs (**Fig. 6 & Table 3**). The scavenging activity of the control extract was found to be 54.8%, and the extracts of plants exposed to 10, 25, 50 and 100 mg/L AgNPs were 69.2%, 79.1%, 86.9% and 91.2%, respectively. With the increase of AgNPs treatment, the values of IC_{50}, defined as the concentration of extract that scavenges 50% of the DPPH radicals, decreased accordingly (**Table 3**). The IC_{50} value for the control extract was 428.5±12.3 μg/mL, and the IC_{50} for the 100 mg/L AgNPs-treated extract was 132.4±7.1 μg/mL, which showed 3.2 fold improvement in antioxidant potency of the 100 mg/L AgNPs-treated extract than the control extract.

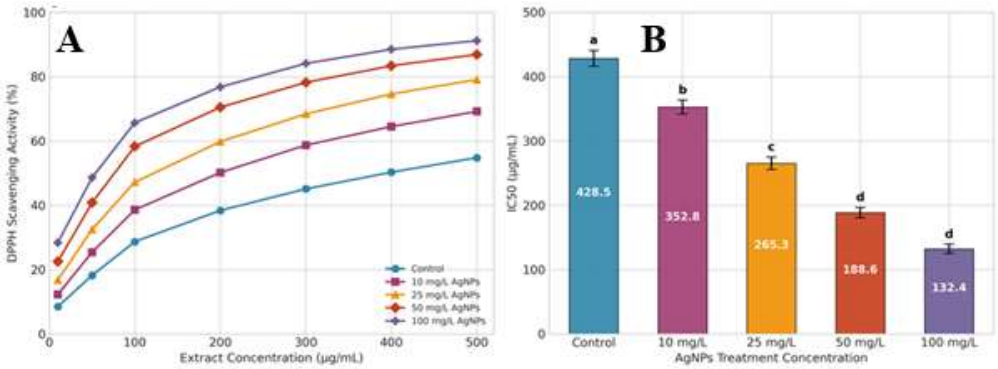

Fig. 6. (A) Dose-response curves of DPPH radical scavenging activity at different extract concentrations. (B) IC_{50} values for each treatment group. Different letters indicate statistically significant differences ($p<0.05$)

Table 3. DPPH radical scavenging activity and IC_{50} values of *C. hyalolepis* extracts under different AgNPs treatment concentrations

Treatment	IC_{50} (µg/mL)	Max Scavenging at 500 µg/mL (%)	% Improvement over Control
Control (0 mg/L)	428.5 ± 12.3[a]	54.8	—
10 mg/L	352.8 ± 10.8[b]	69.2	17.7
25 mg/L	265.3 ± 9.5[c]	79.1	38.1
50 mg/L	188.6 ± 8.2[d]	86.9	56.0
100 mg/L	132.4 ± 7.1[d]	91.2	69.1

Note: Different lowercase letters (a, b, c, d) indicate statistically significant differences between groups ($p<0.05$) based on Tukey's post-hoc test.

Discussion

The nano-elicitation process based on silver nanoparticles (AgNPs) is one of the cutting-edge and most effective approaches to modify and improve the secondary metabolism in medicinal plants such as Iraqi *Centaurea hyalolepis* as confirmed in the results of the present work. Results showed that the plants when exposed to exposure of biosynthesized AgNPs caused significant physiological and biochemical changes, which included significant increase in total phenolic content (TPC) and a marked improvement in antioxidant activity of the plants. Firstly, the characterization of the biogenically synthesized nanoparticles was carried out using UV-Vis spectrometer which revealed that the synthesized nanoparticles exhibited a well-defined surface plasmon resonance (SPR) band with its peak at 425 nm. The presence of this peak has been taken as a characteristic peak in the spherical formation of silver nanoparticles, therefore the phytochemical presents in the Centaurea extract flavonoids and phenolic acids is considered to act as a effective reducing and stabilizing agent to transform the silver ions to their stable nano-form [21]. Moreover, SEM and DLS analysis results showed that the synthesized particles were in the optimal nanometric range with an average diameter size of 34.5 nm. This is important for the use in bioapplications, because plants with thin membranes of cell walls are amenable to penetration with the nanoparticles, enabling effective binding to the intracellular components that in turn alert the plant's defense signaling mechanisms [22].

The high stability of synthesized AgNPs is indicated by a zeta potential of more than -30 mV, which is attributed to the formation of the 'Bio-corona' due to the adsorption of the secondary metabolites. It is an organic coating which avoids aggregation and increases their efficacy as abiotic elicitors to be sprayed through the leaves [5]. This chemical-physical stability guarantees a uniform and complete distribution on the leaf surface and the effective penetration into the plant tissues by stomatal

pores, resulting in the induction of a regulated nonlethal oxidative stress in the plant tissues [23]. Concerning secondary metabolism, the results indicated that TPC linearly increased with an increasing AgNP**s** concentration where the highest concentration of AgNP**s** (100 mg/L) resulted in 89.1% increase in the TPC compared to the other groups. The above mentioned dose dependent increase in the TPC may be attributed to the fact that AgNPs function as potent abiotic stressor which when exposed to plant triggers up-regulation of enzymes playing pivotal roles in the phenylpropanoid pathway, such as PAL and subsequently causes over production of antioxidant phenolics as protective mechanism against ROS induced oxidative damage. These particles stimulate the production of reactive oxygen species (ROS) inside cells, which act as secondary signalling molecules that activate genes involved in phenylpropanoids biosynthesis [24]. This is a main pathway in the biosynthesis of phenolics and flavonoids, which act as a chemical defense mechanism in the plant and prevent oxidative damage in the presence of metallic nanoparticles [2].

Recent studies showed that the nano-elicitation not only enhances the quantity of existing compounds but also promotes the synthesis of novel secondary metabolites, that were not detected during normal growth. For Centaurea, nano-elicitation has shown to help increase the content of sesquiterpene lactones and polyoxygenated flavonoids which are compounds of high medical value in the Asteraceae family [8]. AgNPs penetrate the plasma membrane, interact with membrane transporters and enzymes, temporarily disrupting ion balance, which in turn stimulated the activity of PAL, the rate-limiting enzyme in phenolic biosynthesis [9]. Regarding the antioxidant potential, the results from DPPH assay indicated that all the extracts exhibited a significant increase in their free radical scavenging activity. The highest nano-elicitation concentration in the plants resulted in a decreased in the IC_{50} value from 428.5 μg/mL in the control to 132.4 μg/mL. This 3.2-fold increase is clearly related to the higher fractions of phenols. The phenols and flavonoids, induced by nano, play a crucial role as potent antioxidants, which have the capacity of donating a hydrogen proton to the free radical and consequent reduction of free radical to a stable, harmless molecule [7]. The complementary work of the phenolic types that the seeds elicit, when combined, gives them a full spectrum protection for the plant, as evidenced by the quality of medicinal extracts obtained from these seeds [6].

In addition, silver nanoparticles can be used as elicitors with a greener approach as compared to the conventional methods of chemical elicitors. Because of the high surface-area-to-volume ratio, very low concentrations can yield significantly greater results than conventional metal salts do [14]. Comparative studies are confirming, that nano-elicitation is more effective in inducing durable defense response means that induction of plants defense system stays in "alert" state for a longer time, leading to the gradual accumulation of secondary metabolites [15]. The mechanism of action believed is the interaction between AgNPs and the DNA and regulatory proteins in the nucleus, resulting in modifications in the gene expression of redox enzymes like superoxide dismutase (SOD) and peroxidase (POD) [1]. Together with the phenolic compounds, these enzymes are used to maintain oxidative homeostasis inside the cell. This response has demonstrated high effectiveness Iraqi *Centaurea hyalolepis*, reflecting the species' high adaptive ability to metallic stress, through strengthening the biochemical arsenal [11].

Conclusion

Based on the results obtained from this study, nano-elicitation by the use of biogenically synthesized silver nano particles (AgNPs) represents a revolutionary approach to improve the biological value of Iraqi *Centaurea hyalolepis*. The results showed that accumulation of phenolic compounds and improved antioxidant activity is maximum at 100 mg/L of AgNPs, indicating a strong and controlled plant defense mechanism to the nano stress. These findings pave the way for broad industrial application, particularly in pharmacy and nutraceuticals, where this technology can be used to obtain extracts from plants with better therapeutic efficiency. Study advocates the use of nano-elicitation techniques as an alternative sustainable and effective way of enhancing the quality of medicinal plants, and underscores the need for future studies to comprehend the long-range impacts of such nanoparticles on the layer of soil, and the ecosystem.

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