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Original Article

Antioxidant and antibacterial investigation of metal nanoparticle extract of whole components (root, leaf and shoot mixture) of *Actium lappa*

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ABSTRACT

In the past few years, many scientists and researchers have turned to the green synthesis method of nanoparticles from plant extracts in control and treatment of various cancers and infectious diseases. And there is no need to remove remaining organisms from synthesized materials. Then it is easier to control and inhibit growth of metal nanoparticles in extract. In this research, silver nanoparticles were synthesized by phytochemical method or green synthesis from Actium lappa plant extract. The characteristics of these silver nanoparticles were determined by TEM, DLS and UV-Vis absorption spectrophotometry. Obtained results from DPPH and ABTS methods showed strong and significant antioxidant effects ($P \leq 0.05$) for silver nanoparticles of this extract. As a result, the amount of inhibition of obtained free radicals from them was obtained at certain concentration of 0.72 mg/ml, equal to 86 and 71% respectively. And at high concentration, i.e. 1.4 mg/ml, free radical inhibition of artificial antioxidant (BHA) was obtained almost similar to this concentration. In diffusion disc method, the inhibition zone diameter increased with increase in concentration of silver nanoparticles of Actium lappa plant extract. And the values of MIC and MBC were obtained by this method.

1. Introduction

In prehistoric times, humans used natural products such as plants, fungi, animals and microorganisms to produce medicine to treat various diseases. (Yuan H and et al 2016) One of the most important and main diseases that cause the death of people on this planet is cancer. There are two main factors in the occurrence of cancer, which include the imbalance between proliferation and cell death and oxidative stress, which can be said about oxidative stress due to the production and lack of control and inhibition of reactive oxygen species that can ultimately cause become cell death. (Goodarzi V and et al 2014)Therefore, new perspectives have been created for the better and more effective use of natural antioxidants because they can easily be used as a food supplement and their favorable and beneficial effects can be used in the human body and human health. (Fernandes RPP and et al 2016) Nanotechnology has wide applications that are used in many fields of medicine such as cancer diagnosis and treatment. (Kim PS and et al 2011) The most important features that make plants candidates to be used in the synthesis of nanoparticles are factors such as biosecurity, cheapness and availability, non-toxicity, as well as a wide variety of metabolites that can interfere with ion reduction. (Roy N and et al 2010)One of the nanoparticles used in medicine is silver nanoparticles because it has a very small size and more contact surface. As a result, it increases the amount of adhesion to the cell surface and increases its efficiency and effectiveness. (Shah, V and et al 2009)Biological synthesis of metallic nanoparticles, or green synthesis of silver nanoparticles, is a method that utilizes plants and plant extracts. This approach is biocompatible and can easily replace physical and chemical methods for producing metallic nanoparticles. Therefore, extensive research has been conducted on the production of metal nanoparticles using green synthesis method with the use of plant extracts and various plants. (Narayanan, K and et al 2011, Shankar S and et al 2003, Song, J.Y and et al 2009) Because this method is very economic and does not need cell culture. (Mozafarian, V and et al 2012, Maruta Y and et al 1995.Arctium lappa is an herbaceous biennial plant from the Asteraceae family, which can grow up to 2 meters tall.one of the most important characteristics of this plant is its tall, brown roots and its round flowers with prickly bracts. The plant has spider web-like hairs on the underside of the leaves, on the stem, and on the flowers. The leaves on the lower part of the stem of this plant are very large and rough, with a lot of coarse, crackly covering. The under part of the stem of this plant has smaller leaves with less crackly covering. (Lin CH and et al 2002)The habitat of this plant is in Europe and northem Asia, and in Iran, it is found in humid and semi-arid regions such as Chalous, Alborz, Rudbar, Arak, Hamedan, Tafresh, khorasan, Kerman and Bakhtiari regions. (Kardosova A and et al 2006)This plant is among the herbal medicines in traditional medicine because its extract strengthens the immune system and improves metabolic function. (Ferracane R and et al 2010) In traditional Iranian medicine, this plant is used to treat respiratory conditions and respiratory infections. The extract of this plant has numerous properties, anti-inflammatory, antibacterial, ant diabetic and antioxidant properties. Due to the medicinal properties of all parts of this plant, but its one-year root has anti-cancer properties as it is a rich source of antioxidants and is used to treat skin diseases, HIV, and cancer. (Nashar, By Milka and et al 2019) in 2019, Dousti B and et al investigated the synthesis of silver nanoparticles from the aqueous extract of Fumaria Parviflora plant and its microbial and antioxidant properties. In conducted study, it was reported that the synthesized nanoparticles have antioxidant properties with a free radical inhibition power of 21 μg/ml. in conducted study of the antibacterial properties showed that the synthesized silver nanoparticles had more antibacterial activity against the gram-positive bacteria of Staphylococcus aureus than the gram negative bacteria of Escherichia coli. (Dousti B and et al 2019) In conducted research by Rajakumar G et al.(2018), which was conducted on the green synthesis of zinc oxide nanoparticles with Andrographis paniculata leaf extract. In conducted research by Rajakumar G et al. (2018), which was conducted on the green synthesis of zinc oxide nanoparticles with Andrographis paniculata leaf extract. The highest percentage of DPPH-radical inhibition at the concentration of 500 μg/ml for nanoparticles and leaf extract was expressed as 63.33 and 57.71%, respectively. (Rajakumar G et al. 2018) in 2024 year,Shabaani M and et al researched evaluation of Antibacterial and Antioxidant Activities of Biosynthesized Zinc oxide Nanoparticles using Aqueous Extract of Eriob otrya Japonica Seeds. Which reported that the antioxidant activity of extract and nanoparticles increases with increasing concentration. (Shabaani M and et al 2024) In another report in 2011 year, Dallas P et al showed that the contact time and concentration of silver nanoparticles and the type of bacteria are effective factors in finding antimicrobial properties. (Dallas P and et al 2011) Omid Aziziyan Sharmeh and et al researched the antimicrobial effect of biosynthesized silver nanoparticles using the aqueous extract of Mozaff odoratissima Kelussia leaves against some pathogenic microbes of food origin in 2019. The results of this research show that biosynthesized silver nanoparticles with an approximate size of 20-25 nm have high antimicrobial activity against all studied microorganisms and this activity is concentration dependent. (Omid Aziziyan Sharmeh and et al 2019) A. Hassanvand and et al in 2022 done effective of Antioxidant, Antimicrobial and Anticancer Potential of Silver Nanoparticles Synthesized by Viola tricolor L. Extract. Their evaluation results showed that the synthesized nanoparticles had a good antimicrobial effect in MIC. The results of minimum bacterial concentration (MBC) depended on the dose concentration of silver nanoparticles. Nanoparticles had a dose-dependent inhibitory effect on DDPH free radicals. (A. Hassanvand and et al 2022) Shahzadi I and et al in 2022 done Antioxidant, Cytotoxic, and Antimicrobial Potential of Silver Nanoparticles Synthesized using Tradescantia pallida Extract. (Shahzadi I and et al 2022) Sivasubramanian K and et al in 2023 done Antioxidant, antibacterial, and cytotoxicity potential of synthesized silver nanoparticles from the Cassia alata leaf aqueous extract. The findings showed that AgNPs have antioxidant properties at various concentrations (25–400 $\mu g \cdot mL^{-1}$) as determined using DPPH assay. The results demonstrated that the percentage inhibition increased as the concentrations of AgNPs, leaf extract, and ascorbic acid increased from 25 to 400 $\mu g \cdot mL^{-1}$ .the obtained AgNPs showed significant antibacterial activity for Staphylococcus aureus, Pseudomonas sp.(Sivasubramanian K and et al 2023) In this study, the green synthesis of silver nanoparticles of Actium lappa species was investigated. And its antioxidant and antibacterial properties were evaluated.

2. Materials and Methods

In this research, all the chemicals used in the synthesis of silver nanoparticles are of high purity and manufactured by Merck. Actium lappa plant was obtained from one of the fields and gardens of Gilan province in Rudbar region. The evaluation of this plant was carried out and confirmed at the Herbarium Research Center of Science and Research Unit. The aerial organs, leaves and roots of this plant were washed with distilled water and then dried in room air and turned into powder by an electric mill and finally passed through a 6-mesh sieve.

2.1 Preparation of plant extract

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To extract the aqueous extract of the plant, we first weighed 12.5g of the powder of this plant with a scale. Then, the weighed amount of powder was added to 250 ml of double distilled water and mixed. The resulting mixture was placed in a bain marie for 90 min at a temperature of 80°C. After this step, it was centrifuged with a centrifuge at 4000 rpm for 15 min. the resulting extract was filtered through Whatman No. 1 filter paper. And finally, the obtained extract was stored in the refrigerator.

2.2 Biosynthesis of silver nanoparticles of plant extract

For this purpose, first, 20 ml of plant extract was mixed with 180 ml of $AgNO_3$ (1mM) solution for 70 min at a temperature of 25 °C. After observing the brown color, the colloidal solution was centrifuged for 30 min by the 11000 rpm centrifuge machine. In order to confirm formed silver nanoparticles of extract, a UV spectrophotometer was used in the range between 300 and 700 nm.

2.3 Transmission electron microscope (TEM) Method

In method TEM, a suspension of synthesized nanoparticles should be prepared in distilled water. And placed in an ultrasonic bath for 10 minutes to create better dispersion particles. Then a copper grid covered with carbon film is removed. And 2-3 drops of this suspension are poured on it to dry. Finally, an electron beam passes through the very thin surface of the sample. This beam, which has passed through the sample, has a special energy distribution that is specific only to that material. Which is observed and displayed on the fluorescence screen with very high magnification and resolution of 1 to 0.1 nm.

2.4 pH changes

In this study, 6 series of balloons were prepared, each of which contained 2.5 ml of Actium lappa extract and 4.5 ml of 1 mM silver nitrate solution. pH of its solution was adjusted by sodium chloride and 0.1 M hydrochloric acid by means of an pH-meter (Metrohm model 827) device. And finally, its spectrophotometric spectrum was determined in the range of 300 nm to 700 nm.

2.5 Volumetric amount of extract

In order to optimize this factor, the amount of 0.5 to 3 ml of the extract was added to 4 ml of 1 mM silver nitrate. And according to the optimal pH value, the pH value of the solution was adjusted. And based on the absorption of UV-Vis spectrometric spectrum, the optimal volume value was obtained.

2.6 Millimolar concentration of silver nitrate

In order to optimize this factor, the optimal volume of the extract was added to 10 ml of silver nitrate solution with different concentrations between 0.5 and 3 mM. At optimum pH, the absorbance of this solution was read by spectrophotometry. And the optimal concentration of this agent was obtained.

2.7 Temperature and reaction time

In order to optimize two other effective factors such as temperature and reaction time, separate solutions that have optimal conditions were prepared at different indirect temperatures (hot water bath) between 25 and 75 and different times from the moment of mixing the reagent up to 5.5 hours. And finally, spectrophotometric absorption was read from each of them separately. And the most optimal value of these two factors was obtained.

2.8 Estimation of antioxidant properties

There are many available methods to assess the antioxidant power of natural compounds. The two radical methods, ABTS and DPPH, are the most common methods for measuring antioxidant effects. The two main advantages of the ABTS method over the DPPH method are: Method ABTS reacts rapidly in aqueous buffer solutions. While the DPPH method reacts slowly with the sample. Second, the ABTS method has high flexibility and can be used in all different ranges of pH. While the DPPH method is sensitive to acidic pH ranges. (Shalaby EA and et al 2013).

2.9 Evaluation of DPPH-radical trapping power

5 ml of silver nanoparticle solution of Actium lappa extract in different concentrations were mixed with 2 ml of 0.3 mM DPPH solution. Then this solution was placed in a dark environment with an ambient temperature of 25°C for 35 min .All absorption of DPPH-containing samples after incubation in the wavelength range of 517 nm was read by a spectrophotometer. (Baharara.J and et al 2014).

2.10 Evaluation of ABTS-radical trapping power

The basis of this method is that ABTS-radicals should be created in the form of cations (ABTS•+). When the sample containing the synthesized silver nanoparticle is added to it in a period of time, a decrease in absorption can be seen in the solution. In this method, first, 4 ml of ABTS solution (7 mM) was mixed with 2 ml of 45.2 mM potassium per sulfate solution. Then it was placed in a dark place at a temperature of 25°C for 16 hours to complete the reaction. The obtained solution was diluted with distilled water to achieve absorption of 0.756 at 743 nm wavelength. Finally, to 1.5 ml of fresh ABTS solution, was add 1.5 ml of samples (including silver nanoparticles, extract and BHA) with different concentrations. And after 7 minutes in the dark environment, the absorbance of the samples could be read by the spectrophotometer at a wavelength of 734 nm, and the inhibition percentage of the samples was obtained. (Mehata, M. S and et al 2022). In both methods, DPPH and ABTS, synthetic antioxidant BHA was used as the positive control and ethanol was used as the negative control. (Baharara.J and et al 2014, Mehata, M. S and et al 2022)

2.11 Estimation of antimicrobial effect of silver nanoparticle extract

In agar diffusion disk method, 5 paper disk in around and one disk in center of plate were used as a negative control. 25 microliters of different concentrations of silver nanoparticles of plant extract were slowly placed on surface of disks. And it was poured on surface of culture medium containing each of standard strains. After that, cultured Petri dishes were placed at a temperature of 25 °C for 17 minutes. Then Petri dishes were placed in an incubator at temperature of 37°C for 24 hours. When time of incubator was over, the microbial plates were removed from it. And inhibition zone diameter was measured in mm by a ruler. (Saffari S E and et al 2020, Gholami A and et al 2016).

2.12 MIC and MBC method

In this study, the tube dilution method was used to estimate MIC. For this purpose, 2 mL of Mueller Hinton Brat culture medium was poured into each of 10 sterile tubes. In first tube, 2 mL of nanoparticles was poured and serial dilution was prepared. Then, according to standard of 0.5 McFarland, 100 µL of standard bacteria suspension was inoculated into each tube individually. Here, three tubes were considered as controls. Which included tube number 1 containing primary culture medium and synthesized silver nanoparticles; Tube number 2 containing bacteria and culture medium; Tube number 3 only contained culture medium. The tubes were incubated at 37 °C for 24 hours. The turbidity of the tubes was read visually. The growth or non-growth of bacteria in the tubes was investigated. After preparing a single colony, a portion of the colony was dissolved in physiological serum to obtain a concentration of 10 CFU/ml. To determine MBC, non-turbidity tubes in which bacterial growth was inhibited were cultured on agar mueller Hinton medium. And incubated for 24 hours at 37°C. The lowest concentration of silver nanoparticles which no bacteria survived was considered as MBC. (Hashemi B and et ah 2018, Ghaderi RS and et al 2021).

2.13 Statistical analysis

Data analysis was performed using software Spss 23. All experiments were repeated three times. Values was presented as mean ± standard deviation. Data analysis was performed using one-way analysis of variance method (one way ANOVA). Using the T-test, a comparison of means was conducted at probability level ($p \leq 0.05$). For drawing the graphs, software Excel was used.

3. Results and Discussion

Fig.1 shows UV-visible spectrophotometric spectrum of the extract.

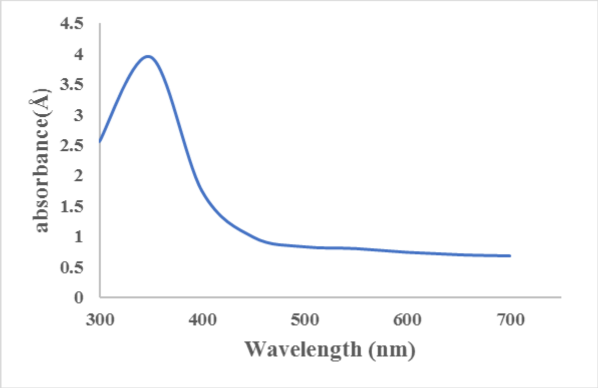

Fig.1. UV-Vis spectrophotometric spectrum of extract of Actium lappa plant.

3.1 Investigating factors to optimize silver nanoparticle synthesis

3.1.1 pH optimization

Based on this Figure, it can be seen at range of different pH that silver nanoparticles are not formed at a pH lower than 4, because in this pH range, absorption changes in the solution are not observed. However, with increase in amount of pH, the formation of silver nanoparticles increased due to increase in amount of absorption. According to Fig.2, the optimal value of pH is obtained. After optimum amount of pH, further increase in pH is associated with decrease in amount of adsorption in solution. (Fig.2) Here, the most optimal adsorption was obtained at pH 6.7. Because the highest absorption was observed at this pH. Also, nanoparticles was not produced at a pH lower than 4. Because absorption changes have not seen at pH less than 4.

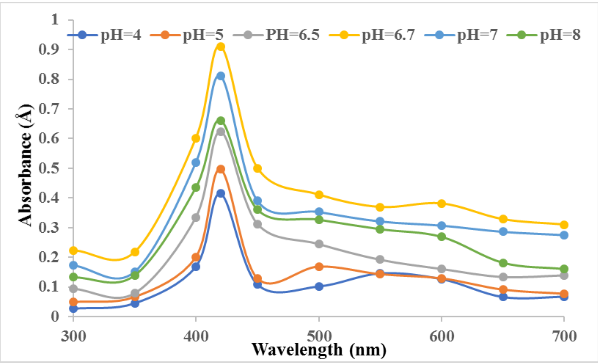

Fig.2. UV-Vis spectra of solutions containing silver nanoparticles in different pH

3.1.2 Optimization of volume of Extract plant

In the evaluation of this factor on synthesis of silver nanoparticles, it was observed that increasing amount of extract increased amount of absorption. And as a result, it increased the formation of silver nanoparticles. According to the Fig.2, it was evaluated that the volume amount of 2.5 ml of the extract is the optimal volume for the synthesis of silver nanoparticles. Because according to amount of absorption in volume values higher than 2.5

ml, a very noticeable drop in the amount of absorption was observed. (Fig.3)

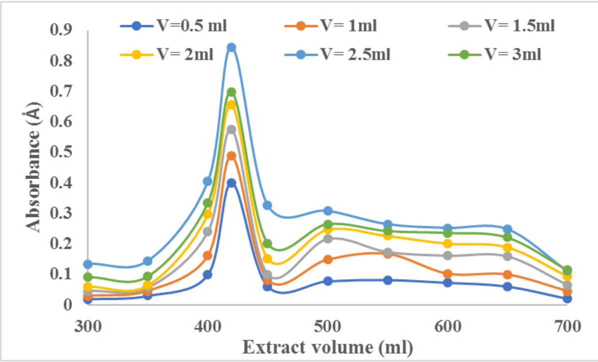

Fig.3. UV-Vis spectra of solutions containing silver nanoparticles in different volumes of extract

3.1.3 The optimal amount of silver nitrate concentration

Another affecting factor in formation of silver nanoparticles was concentration of silver nitrate. In analysis of this factor, according to Fig.4, when the concentration of silver metal ion increased, an increase in amount of absorption was observed due to formation of silver nanoparticles. That the most optimal amount of silver nitrate concentration was obtained 1.5 mM. After the optimal amount of silver nitrate, increasing the concentration of silver metal ions caused a decrease in absorption. (Fig.4)

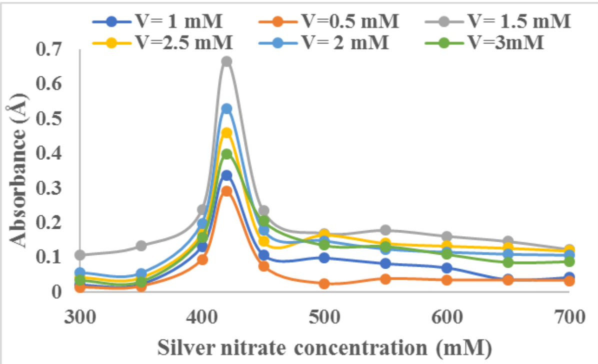

Fig.4. UV-Vis spectra of solutions containing silver nanoparticles in in different concentrations of silver nitrate

3.1.4 The optimal numerical value of the reaction time factor and the reaction temperature

According to results, by increasing of temperature, absorption values also increased. So the most optimal amount of temperature occurred at 45°C, and after the optimal temperature, values of absorption decreased. Also, the interaction time between reactants increased as a result the amount of absorption increased. So the most optimal numerical value occurred at 15 min. After this optimal time, a noticeable decrease in amount of absorption was seen, that was a measure of stability of synthesized silver nanoparticles.

3.2 Morphology of synthesized nanoparticles

In this study, transmission electron microscope was used to determine the morphology, structure of materials and particle size. This test was performed by transmission electron microscope model Philips EM208s made in the Netherlands. That According to obtained TEM micrograph from this research, the average distribution of synthesized biological nanoparticles was a completely spherical shape. (Fig.5) the distribution and average size of silver nanoparticles were measured by DLS device. According to results, the size distribution of synthesized silver nanoparticles was obtained between 8 and 100 nm. And their average size was 27.15 nm. (Fig.6)

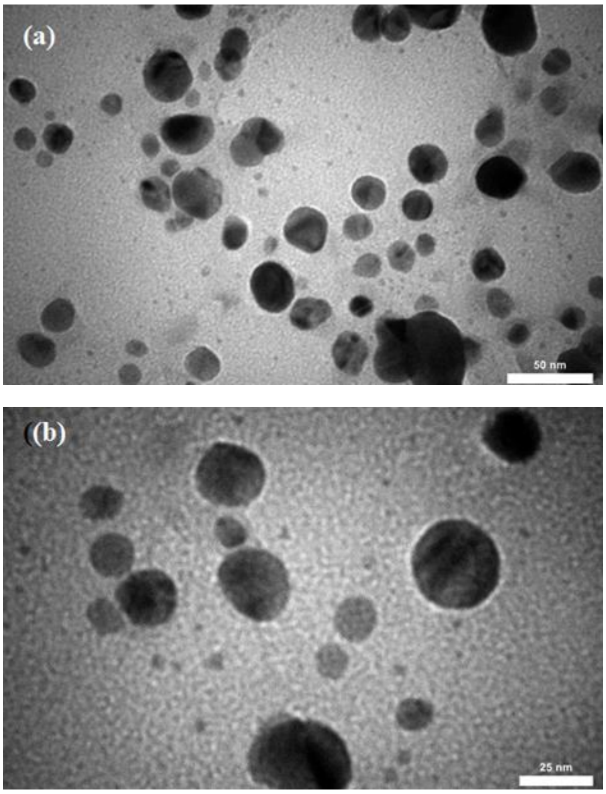

Fig.5.TEM micrograph spectrum of silver nanoparticles at different magnifications :(a) 50nm (b) 25nm

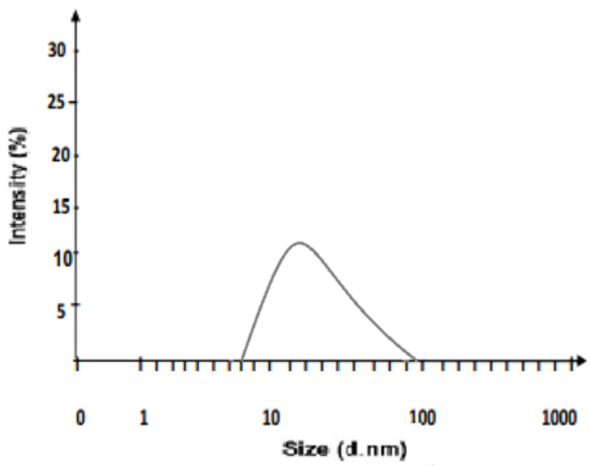

Fig.6.Size distribution of silver nanoparticles by dynamic light subtraction method using DLS device

3.3 The inhibition percentage of DPPH and ABTS radicals

From analysis of DPPH and ABTS data, it is evaluated that concentration of synthesized nanoparticles of plant extract had a significant effect ($p \leq 0.05$) on inhibition of

DPPH and ABTS radicals. According to ABTS figure information, it was observed that with increase in concentration of synthesized silver nanoparticles of plant extract, ABTS free radical inhibition percentage increased significantly. So that at concentration of 0.045 mg/ml, 58% inhibition was observed and by increasing the concentration to 1.4 mg/ml, it reached 89%. (Fig.7)

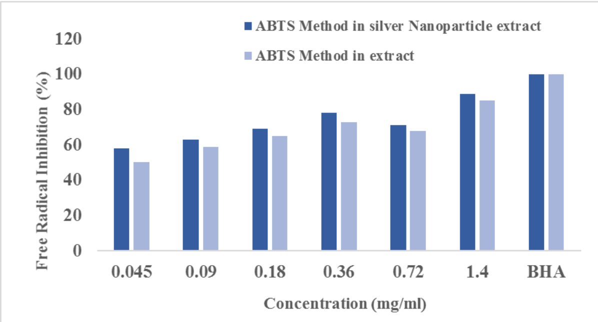

Fig.7. the percentage of ABTS-radical inhibition in different concentrations: (a) silver nanoparticles in plant extracts (b) extract

However, regarding the percent inhibition of DPPH free radical, inhibitory effect of synthesized silver nanoparticles of plant extract on free radical was completely dependent on concentration. So that at 0.045 mg/ml, the rate of inhibition was obtained 37% and at 0.18 mg/ml was 61% and the rate of inhibition at 1.4 mg/ml was about 93 %.(Fig.8)

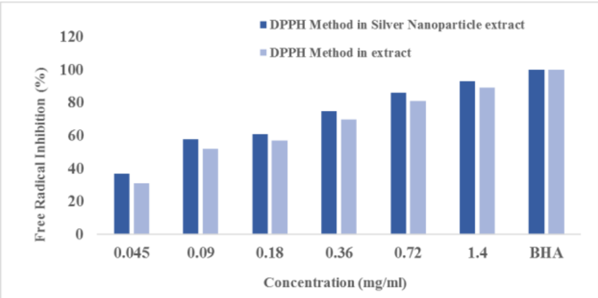

Fig.8. the percentage of DPPH-radical inhibition in different concentrations: (a) silver nanoparticles in plant extracts (b) extract

In measuring and comparing inhibitory effect of synthesized silver nanoparticle of plant extract in both methods according to Figure 9, the biosynthesis of silver nanoparticle of plant extract had created a great ability to inhibit free radicals. Therefore, compared to BHA, both methods had nearly equal inhibitory power to BHA. Another result is that the low concentration of synthesized silver nanoparticle of plant extract was inhibit DPPH free radicals much better than ABTS free radicals. With increasing of concentration, the inhibition percentage of ABTS method was higher than that of DPPH. Which was seen in Fig.9.

The results of evaluating microbial activity of silver nanoparticles of plant extracts were seen in Table .1. According to Table 1, it was found that the inhibition zone diameter around the disc was larger for gram-positive bacteria than for gram-negative bacteria. By increasing concentration of silver nanoparticles of plant extract in both pathogens, the inhibition zone diameter increased. Results of statistical test showed that there was a significant difference in silver nanoparticle concentrations of studied extract. ($P < 0.05$)

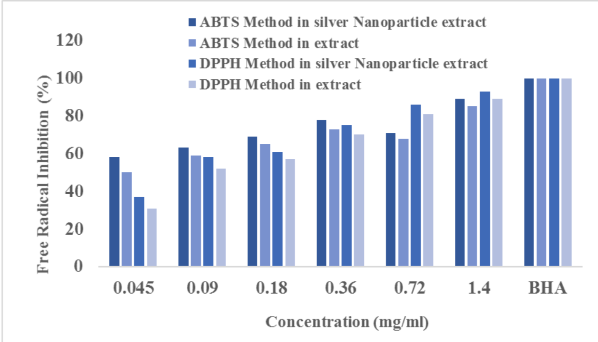

Fig.9. Comparison of free radical inhibition percentage in different concentrations: (a) silver nanoparticle in plant extract (b) extract

Table 1. The diameter of halo of no microbial growth in different concentrations of synthesized nanoparticles by disc diffusion method (in mm)

	Concentration(mg/ml)			
Bacterial pathogen	10	30	60	120
Enterococcus faecalis ATCC 11700	8.63 ∓ 0.17	14.91 ∓ 0.61	17.25 ∓ 0.35	18.99 ∓ 0.83
Salmonella enterica ATTC 13076	7.40 ∓ 0.33	10.97 ∓ 0.72	11.76 ∓ 0.25	12.94 ∓ 0.12

The results of minimum inhibitory and lethal concentration of silver nanoparticle of plant extract were shown in Table.2. The results showed that minimum inhibitory concentration (MIC) for Enterococcus faecalis and salmonella enterica bacteria was equal to 7.5 and 63 mg/ml. The minimum inhibitory concentration was observed in Gram-negative bacteria more than Gram-positive bacteria.

Table 2. The results of MIC and MBC of synthesized nanoparticles with aqueous extract of plant on two pathogen.

Bacterial pathogen	MBC of silver nanoparticle of extract (mg/ml)	MIC of silver nanoparticle of extract (mg/ml)
Enterococcus faecalis ATCC 11700	30	7.5
Salmonella enterica ATTC 1307	126	63

4. Conclusion

The effect of size and morphology on the cellular mechanisms of particles creates very diverse effects for different nanoparticles because there is an inverse relationship between the amount of nanoparticle absorption by the cell and the size of the nanoparticles. (Kang K and et al 2011, Zhang XF and et al 2016) According to the scope of the research conducted with different methods on the synthesized nanoparticles and also the studies that have been conducted on the anticancer properties of metal nanoparticles. Therefore, in this study, we investigated the antioxidant properties of silver nanoparticle in a mixture of the root, leaf, and aerial parts of Actium lappa extract, and the results are comparable with previous studies. (Kalishwaralal K and et al 2011, Shin SW and et al 2015) The evaluated results of this research show very high power and ability of this extract in creating silver nanoparticles by the green synthesis method. So that the completion and end of process is done in short period of time. The very high power and potential of biosynthesized silver nanoparticles regenerates metal ions and changes them to metal atoms in Nano dimensions. The results of free radical estimation in both DPPH and ABTS methods show that with increasing concentration of silver nanoparticle of Actium lappa plant extract, the rate of free radical inhibition increases. So that the concentration of 1.4 mg/ml in ABTS and DPPH is 89 and 93%, respectively. Which is almost equal to the inhibition percentage of BHA free radical. Also, the optimal conditions for the synthesis of silver nanoparticles of this plant extract were considered in various factors such as pH, volume of extract, volume of silver nitrate, temperature and reaction completion time. Because these factors can have a great effect on the range of changes in the size of nanoparticles. (Mehata 2022) The results of this evaluation are 6.7, 2.5 ml, 1.5 ml, 45°C and 15 min respectively. That the best conditions for producing silver nanoparticles extract from Actium lappa plant are. Thus both studied antioxidant methods with optimal conditions can have a degree of free radical inhibition. So that in the concentration of 0.18 mg/ml it is (ABTS= 69%) and (DPPH=60%) respectively. The results of measuring zone of inhibition by agar diffusion disk method show that inhibitory effect decreases with decrease of concentration of silver nanoparticles of plant extract. So that the lowest average zone of inhibition in gram positive and gram negative bacteria corresponds to concentration of 10 mg/ml. Values of MIC and MBC for Enterococcus faecalis and Salmonella enterica bacteria are equal to 7.5, 63 (MIC), 30 and 126 (MBC) respectively.

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