

Green synthesis, characterization, and potential biomedical applications of AgNPs using *Coriander sativum* and *Olea europaea*

ABSTRACT

Background: Over the last few decades, metallic nanoparticles, especially silver nanoparticles (AgNPs), have gained the focus of researchers globally due to their unique properties and a broad range of applications.

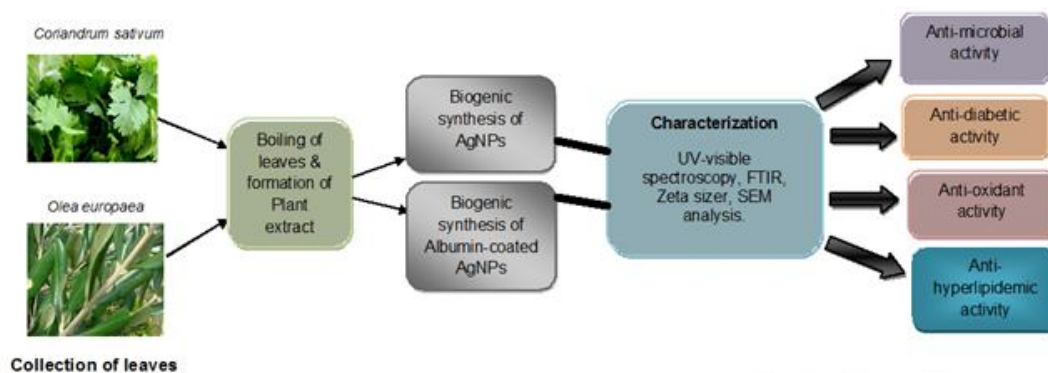
Aim: This research study focused on the green synthesis of AgNPs by using *Coriandrum sativum*, *Olea europaea* leaf extract, and their bovine serum albumin conjugates.

Materials and Methods: Biogenic AgNPs were characterized by UV-visible spectroscopy, Fourier transform infrared spectroscopy, dynamic light scattering, and scanning electron microscopy analysis. The potential biomedical applications of AgNPs and their conjugates were also evaluated through *in vitro* assays. AgNPs synthesis was confirmed by observing UV-visible absorption peaks at 380nm, 460nm (AgNPs derived from *C.sativum* and *O. europaea* respectively), 580nm, and 577nm (conjugates of particles from *C.sativum* and *O. europaea* respectively).

Results: FTIR analysis revealed the presence of various functional groups on the surface of AgNPs. The average diameters of *C. sativum* and *O. europaea* derived AgNPs were 1025 d.nm and 134 d.nm, whereas the average size of AgNPs was 500nm, 200nm, 100nm, and 300nm with uniform morphology. Results of biomedical activity showed that AgNPs and their albumin conjugates were potential antidiabetic, anti-oxidant, and anti-hyperlipidemic with significant IC50 values compared to standard. The antimicrobial potential of AgNPs and their conjugates were tested against gram-positive and gram-negative bacterial strains and the best zone of inhibition of *C.sativum* derived conjugated AgNPs was observed against *Salmonella enterica* i.e. 29mm.

Conclusion: The research project provides an ecofriendly green synthesis method of AgNPs and their conjugates as well as their potential for the treatment of different diseases.

Keywords: Green synthesis; Silver nanoparticles; Albumin conjugates; *Coriander sativum*; *Olea europaea*



Graphical Abstract

1. INTRODUCTION

Nanotechnology is a spectacular arena of various scientific methods that undergoes substantial development of nanosized materials with enhanced features [1]. Among all metal-based nanoparticles, silver nanoparticles (AgNPs) own the most unique and beneficial properties and can be used as an antimicrobial agent because of their inherent properties to kill both gram-positive and gram-negative bacteria [2]. These pharmaceutical agents can be used as targeted delivery systems and thus be applied for the treatment of human diseases. Therefore, AgNPs are employed as drug carriers for anticancer, antimicrobial, anti-inflammatory, and antioxidant agents [3]. AgNPs can be prepared through several ways like physical methods [4,5] that have various side effects such as the use of hazardous and expensive chemicals, high energy, and pressure [6]. Due to these reasons, alternative and safer methods such as “green or photosynthesis” mode are being preferred because it is cost-effective, eco-friendly, and do not require downstream processing. This research study was designed to fabricate *Coriandrum sativum* (*C. sativum*) and *Olea europaea* (*O. europaea*) for the synthesis of AgNPs. *C. sativum*, commonly known as coriander, is a Chinese plant that has another name “cilantro” and most commonly it is known as “Dhania”. It is an herbaceous plant and possesses many beneficial properties such as nerve relaxation, which helps to cure insomnia, headache, stomachic, and anorexia [7,8]. Another plant, *Olea europaea*, is commonly known as olive and has potential properties for the prevention of diabetes [9,10]. Conjugates of AgNPs were also analyzed using bovine serum albumin [11,12,13].

Currently, several research studies showed the potential role of nanoparticles in the treatment of various diseases. New investigations give evidence that few enzymes and transition metals have a special affinity that can be beneficial to treat certain disorders. For example, a strong antidiabetic effect can be generated by the α -amylase and α -glycosidase enzymes [14]. Green synthesized AgNPs are now being exploited as antidiabetic agents [1,15]. The antihyperlipidemic potential of green synthesized AgNPs has already been studied [1]. Microbial resistance to antibiotics requires alternative antimicrobial options and nanoparticles are being exploited for this purpose [16,17]. In the present research work, AgNPs along with their conjugates were synthesized from *C. sativum* and *O. europaea* leaf extracts based on environmentally benign and cost-effective method. Anti-hyperlipidemic, antioxidant, antimicrobial, and antidiabetic activities of AgNPs and their conjugates were analyzed. Nanoparticles have successfully modified the targeted drug delivery techniques.

2. MATERIALS AND METHODS

2.1 Collection of Plant samples and chemicals

Contamination-free leaves of olives and coriander were obtained from a local store. Silver nitrate (AgNO_3), dichloromethane (DCM), absolute ethanol, phosphate buffer saline (PBS), and bovine serum albumin (BSA) were used.

2.2 Preparation of leaf extract and salt solution

20g fresh leaves of *C. sativum* and *O. europaea* were taken and properly washed with distilled water about 4-5 times to remove dust particles. After proper washing, the leaves of both plants were boiled for 15-20 minutes in distilled water. Boiled leaf extract of both plants was then filtered with Whatman filter paper no.1 (125mm pore size) following syringe filtration (0.2 μm). Filtered extracts were kept in the refrigerator at 4 $^{\circ}\text{C}$ for further use (Khan et al., 2018). The stock solution of AgNO_3 was made by mixing 8.5g AgNO_3 in 50ml of distilled water.

2.3 Green synthesis of AgNPs

2.3.1 Synthesis of AgNPs from *C. sativum* and *O. europaea*

AgNPs were synthesized with some modifications [18]. Different concentrations (2mM-14mM) of silver nitrate were prepared from the stock solution. Silver nitrate of different molar concentrations was mixed with boiled leaf extract of *C. sativum* and *O.*

europaea in different ratios like 1:1, 1:2, and 1:4 (extract: salt). After proper mixing, falcon tubes were placed in a hot plate rotatory shaker for approximately 45-50 minutes (for *C. sativum*) and 2 hours (for *O. europaea*) at 70⁰C. Over time, a change in color was visualized and the initial incubation reaction mixture was placed in the incubator for 2 days at room temperature to get the maximum yield of nanoparticles [7].

2.3.2 Synthesis of Bovine serum albumin conjugates of AgNPs

The albumin conjugates of AgNPs were synthesized. For the biosynthesis of conjugates, two solutions including solution D and solution F were prepared. Organic solution was prepared to dissolve the BSA, which contains 40 μ l of absolute ethanol and 260 μ l of dichloromethane (DCM) and termed as solution D. Working solution F was prepared by adding 1980 μ l of PBS, 20 μ l DCM, 0.05g of BSA, and 500 μ l of AgNPs synthesized by *C. sativum* and *O. europaea*. After proper mixing of all chemicals, solution D was added into the falcon containing working solution F. It took 2-3 minutes to get amalgamated. A blank solution was prepared that contained all chemicals like solutions D and F except nanoparticles. Meanwhile, the falcon tubes were agitated for approximately 20 minutes, and after the formation of crude emulsion vortex for about 40 minutes. After the formation of milky suspension, the falcons were kept in a rotatory evaporator at 40⁰C for 15-20 minutes. In this step, DCM was evaporated. Falcon tubes were placed in the freezer at 4⁰C overnight, after 12 hours, thawed, and again with vortex for 5 minutes. Finally, it was ready to use for characterization and further analysis [11].

2.4 Characterization of AgNPs

2.4.1 UV-visible spectroscopy

The optical absorbance of biosynthesized AgNPs and their conjugates were examined with the help of a spectrophotometer (pg instruments T80) at 1nm resolution. The range was set at 300-700nm [19].

2.4.2 FTIR spectroscopic analysis

Fourier transform infrared spectroscopic analysis was carried out to analyze the functional groups of silver nanoparticles and their conjugates using Bruker Alpha FTIR spectrophotometer and the frequency was set in the range of 1000-3500 cm⁻¹ wavelength [20].

2.4.3 Dynamic light scattering analysis

DLS analysis was performed with the particle size instrument (Zetasizer nano series ZS, Malvern Instruments Ltd., U.K.). AgNPs and their conjugates were analyzed at 25⁰C to observe their polydispersity index (PDI) and hydrodynamic size [21].

2.4.5 Scanning electron microscope (SEM) analysis

To check the surface morphology and size of AgNPs, SEM analysis was carried out by using Philips XL-30S FEG. AgNPs were placed on a carbon-coated grid of copper to make a thin film of the sample. Extra water and solution were removed by blotting paper. The AgNPs solution was dried for 5 minutes. Images from the samples were captured at various magnifications [22].

2.5 Biomedical Applications of biosynthesized

2.5.1 Anti-bacterial potential of AgNPs and their conjugates

Antimicrobial activity of biogenic AgNPs and their albumin conjugates were evaluated against gram-negative bacterial strains, i.e., *Salmonella enterica* (*S. enterica* ATCC 14028), *Escherichia coli* (*E.coli* ATCC 25922 TM), and gram-positive bacteria including *Bacillus subtilis* (*B. subtilis* ATCC 6633 TM) and *Staphylococcus aureus* (*S. aureus* ATCC 25923). Inoculum of bacterial strains was prepared by adding these strains in nutrient broth. The falcons were incubated for 24 hours and they were supplemented with saline solution to get transparent cultures [23]. The Agar diffusion plate method was used to confirm the antibacterial potential of AgNPs and their albumin conjugates [24]. Ciprofloxacin was used as a standard drug. Wells were filled with samples. Plates were placed in the refrigerator for 5-10 minutes at 4⁰C to allow diffusion and then in the incubator for 24 hours at 37⁰C [25].

2.5.2 Alpha-amylase inhibitory activity of AgNPs and conjugates

The antidiabetic potential of *C. sativum* and *O. europaea* synthesized AgNPs and their conjugates were evaluated through α - amylase assay according to [26] with slight modifications. For this procedure, the reaction mixture was prepared by adding 100 μ l AgNPs and conjugates of different dilutions like (10-90 μ g/ml) in 200 μ l of phosphate buffer saline (0.1 M, pH 6.9). After that, 100 μ l enzyme solution was mixed in the reaction mixture and incubated at 37⁰C for 20 minutes. After completion of incubation, 100 μ l starch (1%) was added and placed in the water bath at room temperature. The reaction was stopped by adding

300µl dinitrosalicylic acid (DNS) and further incubated at 80°C for 10 minutes. The color change was observed and the absorbance of the samples and standard drug acarbose was measured at 540nm [12]. %age inhibition was calculated through the following equation:

$$\%age\ inhibition = 1 - (A_{(sample)} / A_{(control)}) \times 100$$

2.5.3 Antioxidant activity of AgNPs and their conjugates

The radical scavenging ability of biosynthesized AgNPs and their albumin conjugates were evaluated through DPPH assay. For this purpose, a 100µl, 0.1mM methanolic solution of 1,1 Diphenyl 2- picryl hydrazyl (DPPH) was prepared and added with an equal amount of AgNPs and conjugates of different dilutions [27]. After proper mixing, the working solution was incubated in the dark for approximately 30 minutes. After incubation, a color change was observed and absorbance was taken at 517nm. Ascorbic acid was used as a positive control and the assay was based on the reduction of DPPH over time [28]. %age inhibition was calculated by the following formula:

$$\%age\ inhibition = (\text{Control}_{(absorbance)} - \text{Sample}_{(absorbance)}) / \text{control}_{(absorbance)} \times 100$$

2.5.4 Anti-hyperlipidemic activity

The potential of AgNPs and their albumin conjugates as an antihyperlipidemic agent were evaluated with the HMG-CoA (β-Hydroxy β-methylglutaryl-CoA) reductase assay kit (SIGMA-ALDRICH (Catalog Number CS1090)). First of all, a blank solution was prepared that consisted of all reagents of the kit except pravastatin and HMGR. Then the activity and inhibition solution was prepared. The working solution was made by mixing 455µl 1x assay buffer in 2.5µl of AgNPs of coriander, olives, and their conjugates as well as 2.5µl of the drug. After that, 10µl NADPH was mixed following the addition of 30µl of substrate solution to the inhibition and working solution. Finally, for inhibition and activity, the solution was mixed with 2.5µl of HMG-CoA reductase. The whole process was done on ice because the enzymes are less stable. Every sample of AgNPs was read for 5 seconds till 5 minutes and reading was taken on a spectrophotometer at 340nm wavelength. %age inhibition was calculated as per formula:

$$\%age\ inhibition = 1 - (A_{(sample)} / A_{(control)}) \times 100$$

3. RESULTS

3.1 Biosynthesis of AgNPs from *C. sativum* and *O. europaea*

For different molar concentrations (2-14mM), the nanoparticles were obtained at 8mM of *C. sativum*) and 14mM of *O. europaea*. At low molar concentration, slight changes and very few NPs were formed after incubation. However, at 8mM and 14mM a significant change in color was observed and a good quantity of nanoparticles was produced after 2 days of incubation. For *C. sativum* and *O. europaea*, a change in color from pale yellow to reddish-brown and grayish-black were observed, respectively, indicating the formation of nanoparticles. After proper mixing of the organic solution with the reaction mixture, we observed a milky suspension indicating the formation of albumin conjugates of silver nanoparticles (Fig. 1).

3.2 Characterization of AgNPs and conjugates

3.2.1 UV-Vis spectroscopic analysis

The stability and formation of AgNPs and their conjugates were evaluated at 300-700nm. Due to surface plasmon resonance, the sharp spectral peak of *C. sativum* and *O. europaea* nanoparticles was observed at 380 and 460nm, respectively. While albumin conjugates showed absorption at 577nm (*C. sativum*) and 580nm (*O. europaea*) (Fig. 2). The phenomenon of SPR happens when NPs and their conjugates absorb light and silver reduced from Ag^{+2} to Ag^0 .

3.2.2 Fourier transform infrared spectroscopic analysis

FTIR showed the functional groups responsible for the stabilization and capping of nanoparticles. For *C. sativum* NPs, three absorption peaks at 3252.60cm^{-1} , 2125.18cm^{-1} , 1637.60cm^{-1} were observed presenting the presence of N-H bond, $-\text{C}=\text{C}-$ stretching and alkynes respectively. For *O. europaea*, N-H bond at 3255.53cm^{-1} , alkyne stretching at 2125.18cm^{-1} and $-\text{C}-\text{H}-$ bond stretching at 1637.04cm^{-1} . Albumin conjugates of *C. sativum* derived AgNPs showed carboxylic acid at 3331.26cm^{-1} , and C=O stretching at 1638.02cm^{-1} while conjugates of *O. europaea* AgNPs presented spectral peaks at 3325.79cm^{-1} , 1637cm^{-1} , and 1100cm^{-1} indicating the presence of N-H, C=O and protein stretching (Fig. 3).

3.2.3 Dynamic light scattering and SEM analysis

DLS analysis revealed the size, charge, and polydispersity index (PDI) of AgNPs (Fig. 4). The analysis revealed the average size of AgNPs synthesized from *C. sativum*

1025d.nm and the average diameter of *O. europaea* nanoparticles 134d.nm. PDI value was 0.423 and 0.434 for coriander and olive-derived AgNPs at 25⁰C and 90⁰ angles, indicating the polydispersity of nanopartilces. The scanning electron microscopy technique was used to identify the morphology and size of the AgNPs. Fig. 5 showed the nano sizes with aggregated and uniform shapes.

3.3. Biomedical applications of AgNPs

3.3.1 Anti-bacterial activity

The antibacterial activity of AgNPs and their albumin conjugates were examined through diffusion method against four different bacterial strains including *B. subtilis* ATCC 6633, TM, *S. aureus* ATCC 25923, *E.coli* ATCC 25922 TM, and *S. enterica* ATCC 14028. Ciprofloxacin drug was used as standard. AgNPs and conjugates showed antibacterial potential with a zone of inhibition (ZOI) ranging from 15-29mm (Table 1). AgNPs produced from conjugates showed a 29mm zone of inhibition against indicator strains. It was found that a substantial increase in ZOI with an increase in the concentration of NPs and conjugates. The ZOI of standard drug was 40mm.

3.3.2 Radical scavenging activity of AgNPs

IC₅₀ of AgNPs from *C. sativum* and *O. europaea* at 90µg/ml concentration exhibited significant antioxidant activity of 9.66µg/ml and 9.02µg/ml respectively. While their albumin conjugates at 40µg/ml concentration showed radical scavenging ability with IC₅₀ of 4.43µg/ml and 4.42µg/ml for *C. sativum* and *O. europaea* particles. Ascorbic acid was used as a positive control in this assay (Fig. 6).

3.3.3 Alpha-amylase and HMG-CoA reductase activity

Silver nanoparticles showed significant alpha-amylase inhibitory activity. Compared to acarbose, it was observed that with the gradual increase in the concentration of samples (10-90µg/ml), substantial inhibition of enzyme activity was observed indicating the antidiabetic effect (Fig. 7). AgNPs and albumin conjugates indicated significant antihyperlipidemic activity with IC₅₀ value of 2.95µg (*C. sativum*) and 1.61µg of (*O. europaea*) compared to pravastatin as control (IC₅₀: 1.02µg) (Fig. 8).

4. DISCUSSION

Green synthesis of NPs is an economical, rapid, and contamination-free mode, does not require any extra capping or reducing agents, and it has solved several bottom neck problems related to other techniques [18,29]. The purpose of this research project was a green synthesis of AgNPs through *C. sativum* and *O. europaea* plant extracts and synthesis of their albumin conjugates in optimized conditions. Senthilkumar and his research colleagues have already reported AgNPs synthesis from *C. sativum* [7,30]. While in this study, NPs were prepared at 1:4 and 8mM concentration. Similarly, fabricated olives for the synthesis of AgNPs were used at 1:20 containing 20ml salt and 1ml leaf extract [31], but we observed that AgNPs were produced using 1ml extract and 2ml salt solution. Albumin conjugates of AgNPs were prepared by co-centrifugation of bovine serum albumin with already synthesized AgNPs. Azizi and his co-workers also performed a similar experiment on chemically synthesized CdNPs. Therefore, following their work, we successfully prepared conjugates of AgNPs [11].

UV-visible spectroscopic analysis was carried out to ascertain the formation and stability of AgNPs at 300nm-700nm wavelength. It has been observed that AgNPs showed the absorption peak at 430nm from Olives [31], while our biosynthesized AgNPs exhibited a sharp peak at 460nm in the case of *O. europaea* indicating the formation of NPs. We have seen significant outcomes [32] at 380nm. Conjugates of AgNPs showed absorption spectrum at 580nm and 577nm [33]. FTIR analysis was carried out in the range of 1000cm^{-1} - 3500cm^{-1} to find the functional groups and biomolecules attached to AgNPs and their conjugates. Three absorption peaks were observed for AgNPs of coriander at 3252.60cm^{-1} , 2125.18cm^{-1} and 1637.60cm^{-1} indicating the presence of N-H, alkynes, and -C=C- stretching respectively, and similar functional groups were reported by [34]. FTIR analysis of *O. europaea* derived AgNPs showed the presence of N-H, C-N, and C-H stretching [31], while in the case of conjugates showed C-N stretching which was due to vibration of protein confirming the presence of amine, alcohol, alkenes, aliphatic amines, and alkynes functional groups.

The bactericidal potential of AgNPs and their albumin conjugates were evaluated and they proved as potential antibacterial agents with a zone of inhibition (ZOI) in the range 15-29mm. Bacterial sensitivity of AgNPs against *E. coli*, *P. aeruginosa*, and *S. aureus* with zone of inhibition was 12mm, 14mm, and 11mm, respectively [35,36]. Therefore, in comparison with Jagtap *et al.* and Nithya *et al.*, our phytosynthesized AgNPs and their conjugates showed significant ZOI against bacterial strains. Nanosized particles due to the larger surface to

volume ratio contain more antibacterial potential due to more absorption in the cell membrane [25].

Diabetes mellitus is the most burning disorder that has affected billions of people globally. Biosynthesized AgNPs and their conjugates strongly inhibited α -amylase enzyme, indicating the therapeutic effect of AgNPs as the best antidiabetic agents. Ganesh Dattatreya already studied the alpha-amylase inhibitory activity of AgNPs with IC₅₀ value of 55.5 μ g/ml [12,26]. Alpha-amylase results of the current research work are more significant for *C. sativum*, *O. europaea*, and their conjugates with IC₅₀ values: 21.34 μ g/ml, 6.40 μ g/ml, 3.43 μ g/ml, and 12.6 μ g/ml respectively. The radical scavenging ability of those AgNPs was improved with an increase in the concentration of particles. Our findings showed that these AgNPs showed significant activity with IC₅₀ values of 9.66 μ g/ml (*C. sativum*) and 9.02 μ g/ml (*O. europaea*) [28]. Brajesh Kumar along with his colleagues studied the radical scavenging activity of AgNPs from *Ficus carica* and their results indicated 36.86 μ g/ml and 21.59 μ g/ml IC₅₀ values for different concentrations of NPs [37,38]. Plants are a vital source of sterols, flavonoids, and vitamins which lower lipid content in the blood. We analyzed the anti-hyperlipidemic activity with significant outcomes [39].

5. CONCLUSION

Green synthesis of AgNPs offers an economical, environmentally benign, faster, and single-step method with no downstream processing. These biogenic AgNPs and their albumin conjugates are effective biomedical agents that can potentially retain these applications.

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UNDER PEER REVIEW

FIGURE LEGENDS

Fig. 1. Biosynthesis of AgNPs from (i) *Coriandrum sativum*, (ii) *Olea europaea* (iii) conjugates, (iv, v) synthesis of AgNPs after proper incubation change in color from pale to reddish-brown, (vi) formation of milky suspension confirm the synthesis of conjugates.

Fig. 2. UV spectral analysis of AgNPs (a) *C. sativum*, (b) *O. europaea*, (c) albumin conjugates.

Fig. 3. FTIR spectroscopic analysis displays the functional groups (a) N-H bond, C=C, and alkynes stretching in AgNPs of coriander (b) N-H, C-N, and C-H functional groups in olive NPs, (c & d) shows protein stretching in albumin conjugates.

Fig. 4. Size distribution of AgNPs from (a) *C. sativum* (b) *O. europaea* (c) Conjugated nanoparticles using *C. sativum* (d) Conjugated nanoparticles using *O. europaea*

Fig. 5. SEM analysis at various magnifications

Fig. 6. DPPH assay of AgNPs from *Coriandrum sativum* and *Olea europaea*.

Fig. 7. Alpha-amylase activity of AgNPs from coriander, olives, and albumin conjugates as compared to acarbose.

Fig. 8. Antihyperlipidemic activity of AgNPs from coriander, olives, and their albumin conjugates

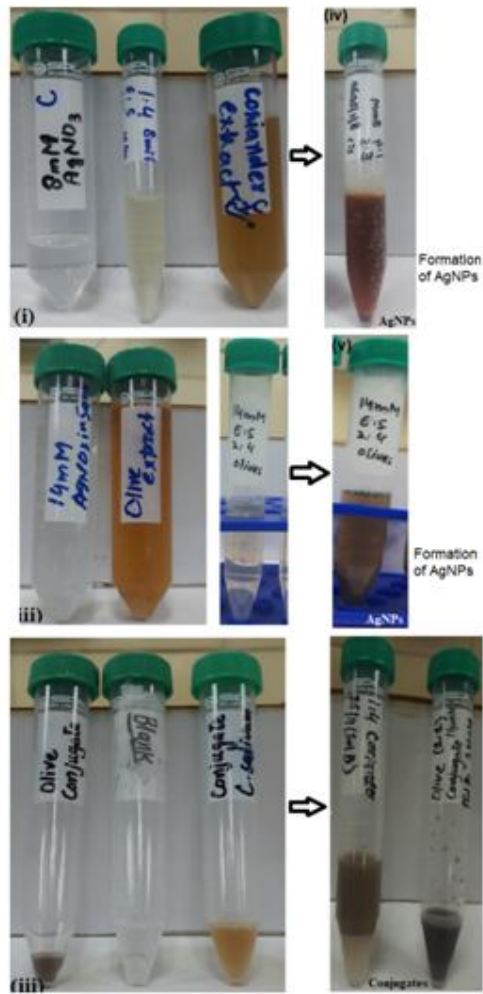


Fig. 1

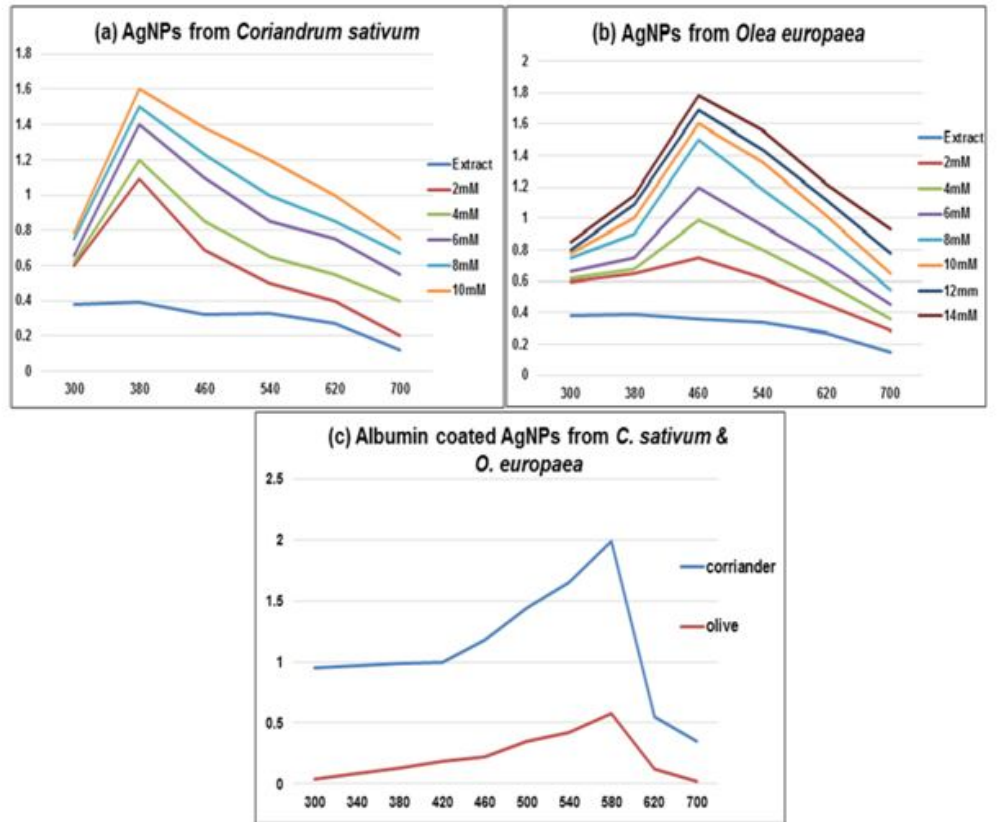


Fig. 2

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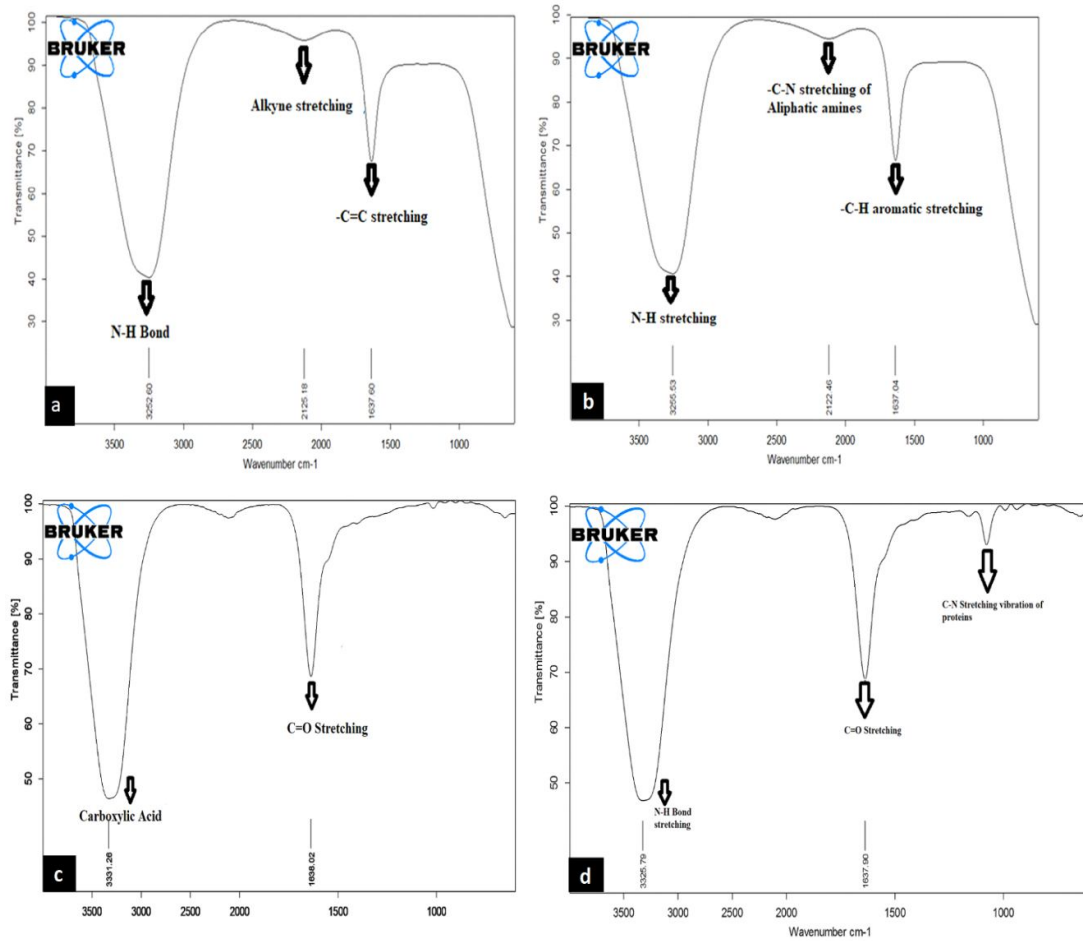


Fig. 3

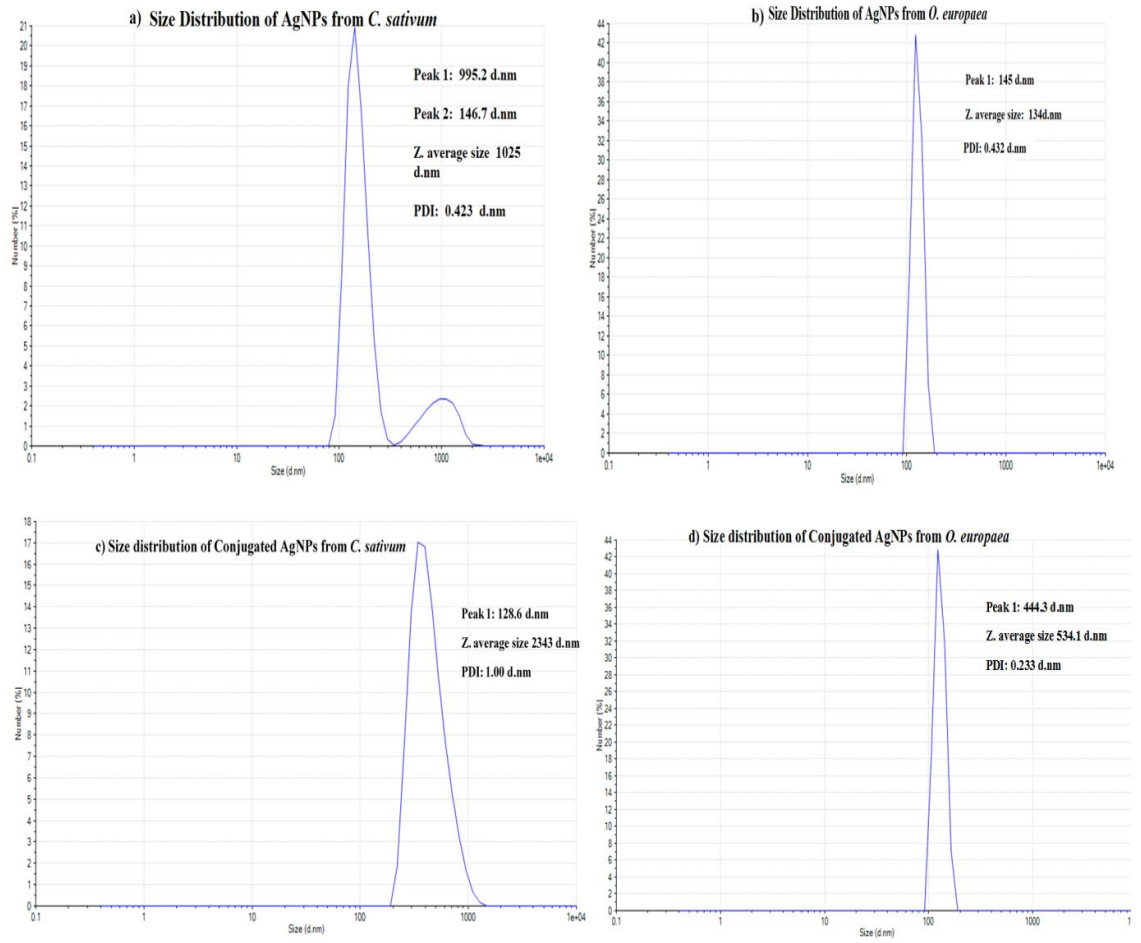


Fig. 4

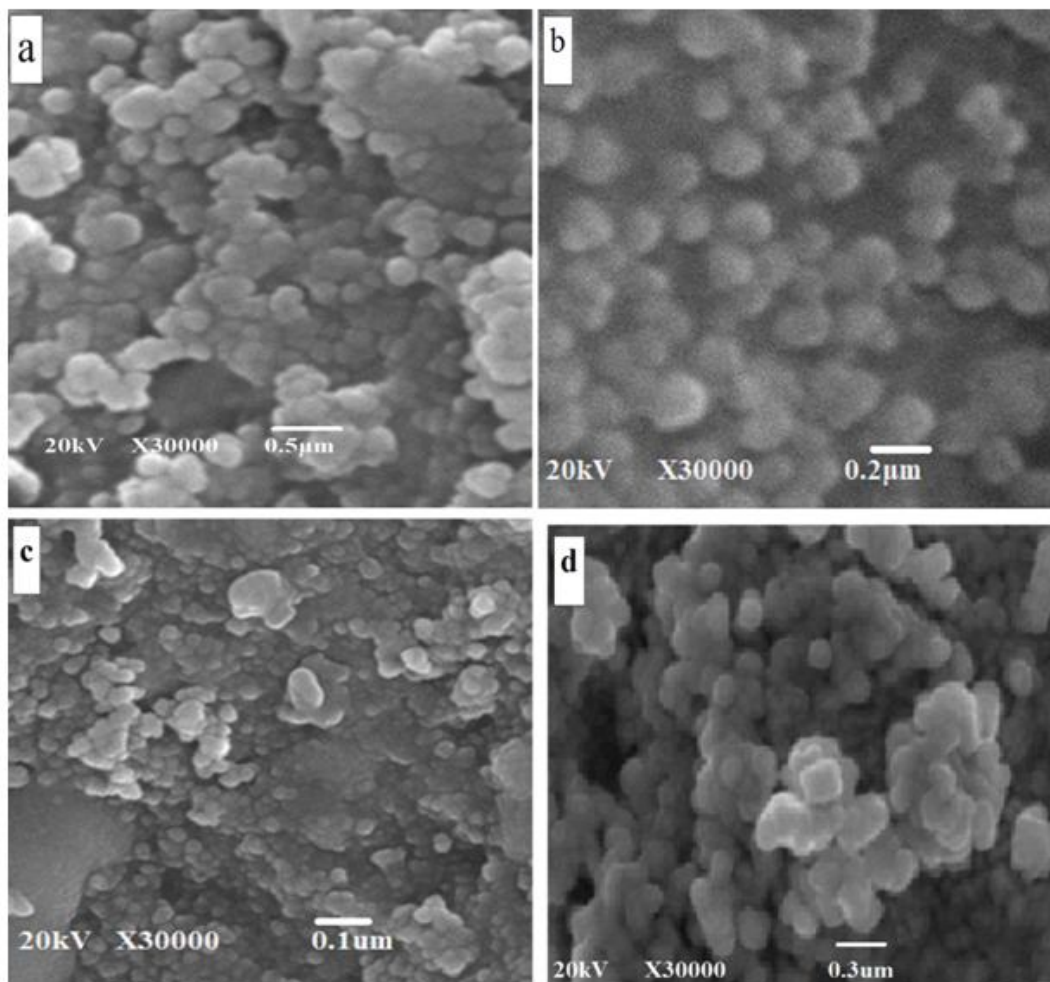


Fig. 5

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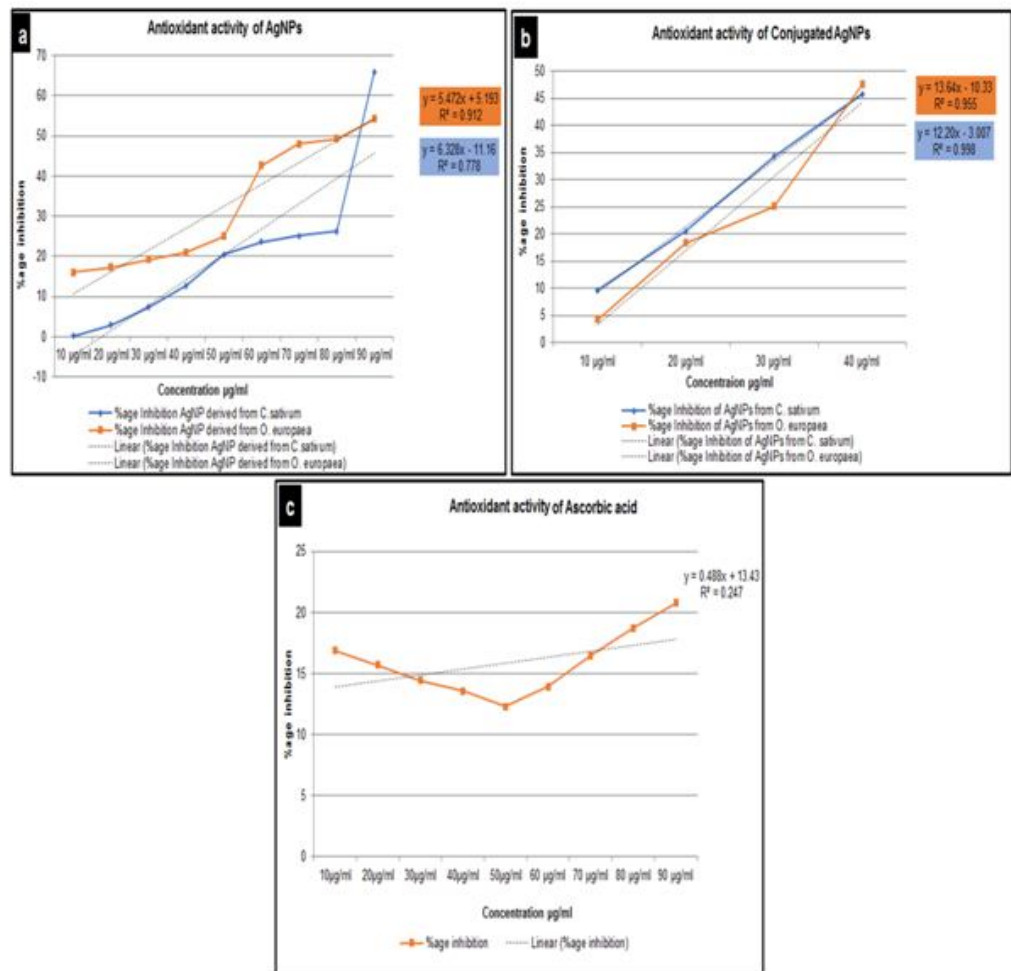


Fig. 6

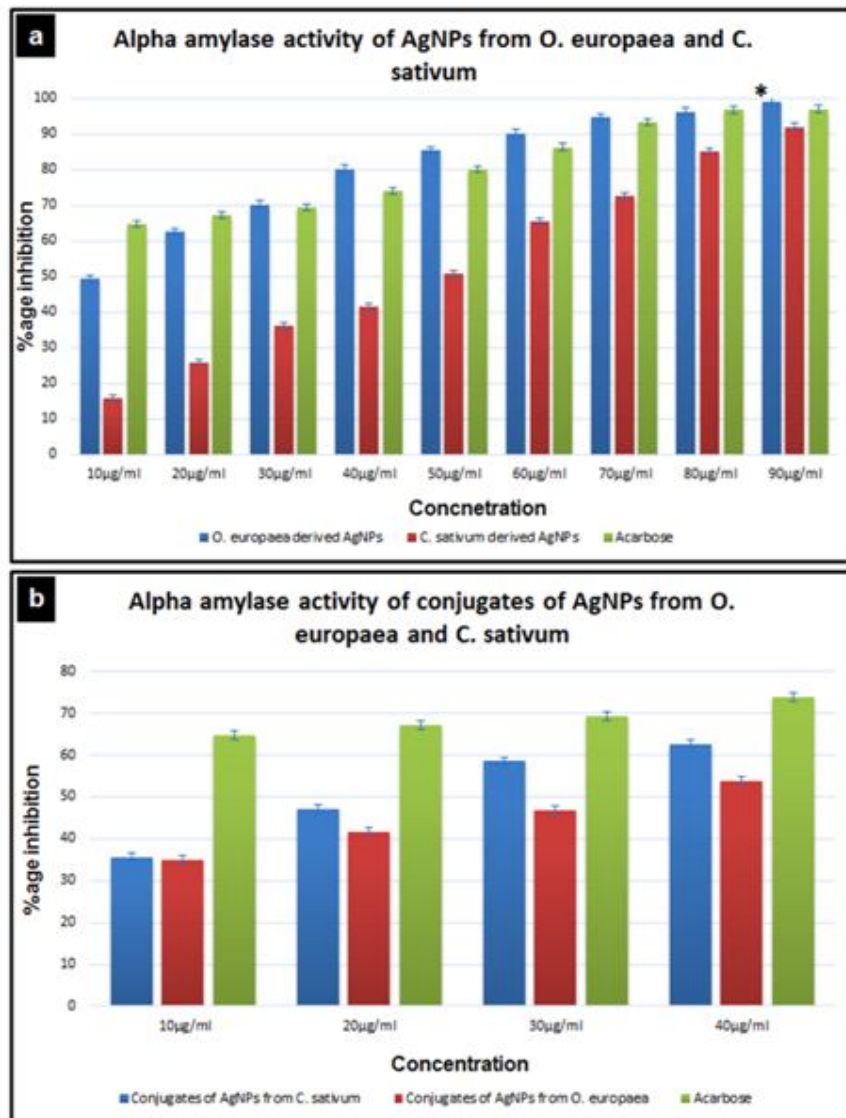


Fig. 7

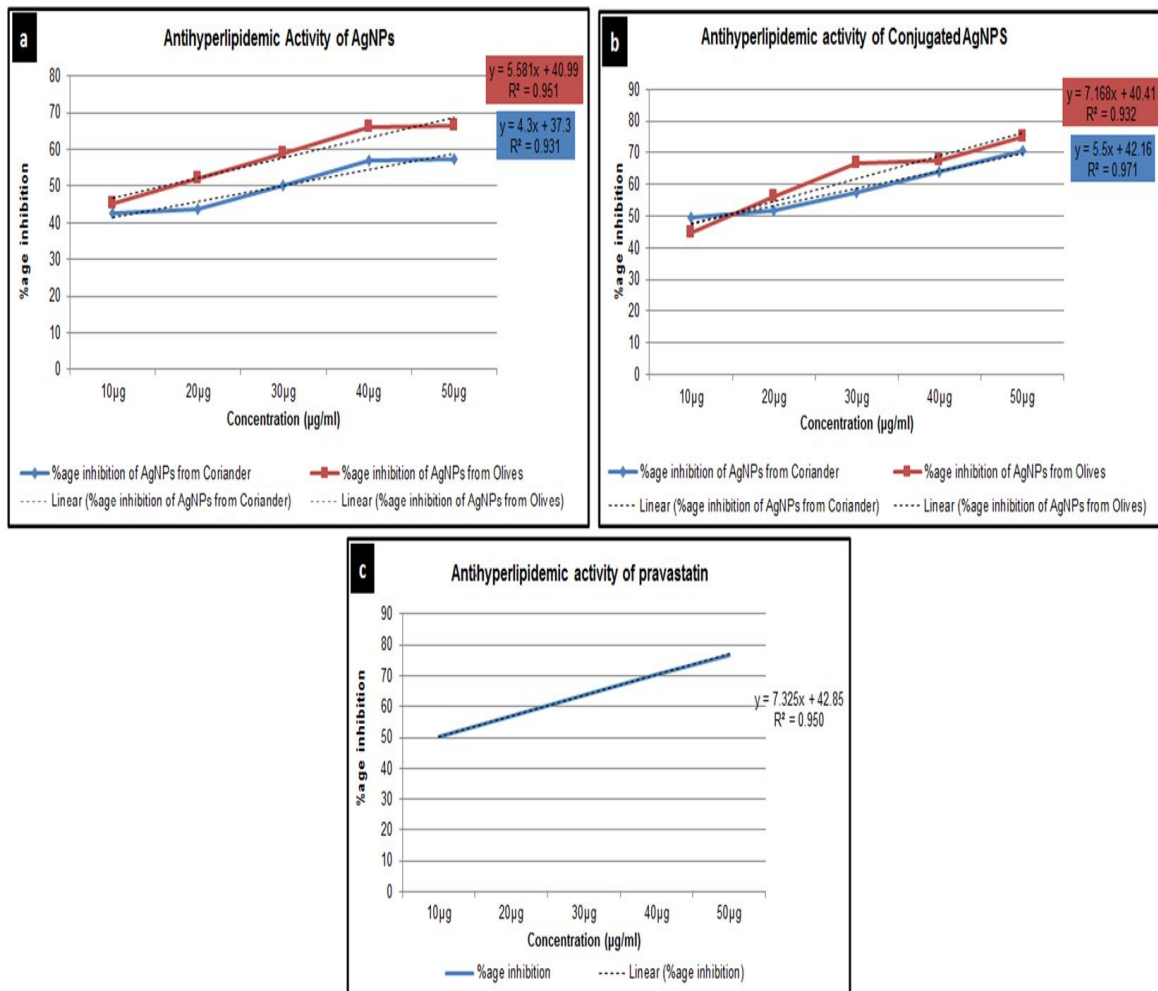


Fig. 8

Table 1 . SAntibacterial potential of AgNPs and conjugates from *Coriandrum sativum* & *Olea europaea*.

Samples	Zone of Inhibitions against bacteria (mm)				
	Replicates	<i>E.coli</i>	<i>B.subtilis</i>	<i>S.aureus</i>	<i>S.enterica</i>
Standard	R1	36	41	37	42
	R2	35	40	38	41
	R3	37	42	39	40
	Mean±STD	36±1.391	41±1.144	38±1.143	41±1.144
AgNPs from <i>C. sativum</i>	R1	18	19	21.5	17
	R2	17	20	20	21
	R3	19	21	18.5	19
	Mean±STD	19±1.432	20±1.432	21±1.444	22±1.441
AgNPs from <i>O. europaea</i>	R1	17.5	22	17.5	23
	R2	20	18	23	17
	R3	19	21	19.5	20
	Mean±STD	19±1.432	20±1.442	21±1.432	22±1.444
Conjugates of AgNPs (<i>C. sativum</i>)	R1	22	23	16	26
	R2	23	21	12	31
	R3	18	25	17	30
	Mean±STD	21±1.422	23±1.432	15±1.332	*29±1.444
Conjugates of AgNPs (<i>O. europaea</i>)	R1	22	24	21	22
	R2	20	28	25	20
	R3	18	26	24	25
	Mean±STD	20±1.432	26±1.444	23±1.431	22±1.429

*significance p<0.05