Development and comparative evaluation of anti-microbial activity of silver nanoparticles prepared from red and yellow onions (*Allium cepa L.*) bulb extracts

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Abstract:

The goal of present work to develop silver nanoparticle utilizing red and yellow onions (*Allium cepa L.*) extract. Prepared AgNPs were expected to have antimicrobial activity. Biological synthesis method used to developed silver nanoparticles by using silver nitrate solution and extract of two different types' red and yellow onions (*Allium cepa L.*). Developed AgNPs were characterized by UV-Vis spectroscopy, Fourier Transmission Infrared Microscopy (FTIR), Scanning Electron Microscopy (SEM) and X-Ray Diffraction (XRD). Antimicrobial activity was carried out in agar well diffusion method, against four pathogenic bacterial strains *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Klebsiella pneumoniae.*UV-Vis spectroscopy showed peaks at 418 nm for red onions AgNPs and 399 nm for yellow onions AgNPs. FTIR evaluated that amines and hydrocarbons reduction involved in silver nanoparticle stabilization. Results of SEM image demonstrated that particles were cubical and spherical in shape. The particle size distribution of red onions AgNPs were ranged in 2-9 nm, and that of yellow onions AgNPs were 3-8 nm. Further, XRD revealed different intense peaks positions at 2θ. Average grain size of red onions AgNPs was 9.65 nm and yellow onions AgNPs average grain size was 7.48 nm. SEM and XRD confirmed formation of silver nanoparticles. Silver nanoparticles were successfully synthesized from both types red and yellow onions (*Allium cepa L.*) bulb extract. From the study, both types of biosynthesized AgNPs red and yellow onions (*Allium cepa L.*) were observed to showed antimicrobial potential. However, yellow onions AgNPs exhibited maximum antimicrobial activity due to its small crystal size as compared to red onion AgNPs.

Keywords: biosynthesis, silver nanoparticles, antimicrobial

Introduction:

The synthesis of silver nanoparticles or nanomaterial was a main point for the recent nanotechnology research. Because of broad used AgNPs were much needed, therefore produced better yields. Investigation had been conducted and the process had improved with regard to effects of physical and cultural conditions on AgNPs biosynthesis. The expiry date and production could be improved to ideal performance through optimization of different particular variables.(Soni and Prakash 2012).

In biological approaches aqueous extract could also be used as the reducer or protection agent to manufacture metal nanoparticles from microbes and plants. (Moghaddam 2010). This approach could not have used any of poisonous chemicals, high energy, high pressure, and high temperature rather it was environment friendly. The extracts of any plants were used to obtain nano-silver of various sizes.(Zuas, Hamim, and Sampora 2014).Synthesis of nanoparticles procedures make uses biomaterials such as microbes, fungus, and flora, environment friendly supportable methods as compared to conventional chemical approaches.(Navazi, Pazouki, and Halek 2010) Microorganisms or plants, they consume a little energy, produce minute harmful emissions, as well as function mostly under standard environment.(Nazeruddin *et al.* 2014). Plants have long been known because of their ability to biologically reduce metal ions and hyper- accumulate them. Due to exceptional properties, plants are used in ecofriendly biological process for manufacturing of metal nanoparticles, as well as beneficial for detoxifying purposes.(Weng *et al.* 2022). Extracts of plants have a mixture of bioactive components including proteins, sugars, salts and acids (phenolic acid). All of these have been shown to contribute significantly in first decreasing and afterwards maintain metal ions. (Nalawade, Mukherjee, and Kapoor 2012; Mosa *et al*. 2016). Phenolic and flavonoids levels had been present in significant amount among red onions compared to several colored varieties. (Shon *et al*. 2004). The red onions had been shown to have considerable quantity of anthocyanins.(Poulose *et al*. 2014) (Gorinstein *et al.* 2008).

Silver nanoparticles were most effective anti-bacterial competitors of other various metal nanoparticles. Silver nanoparticles had vast array of biological activities allowing it to be attractive objects fighting against pathogens. (Durán and Marcato 2013). Silver nanoparticles (AgNPs) allow antimicrobial activities to occur appropriately used for several domestic applications such as food storage, home appliances and medical purposes. (Jha and Prasad 2010). Flavonoids and proteins were the main elements for the formation of silver nanoparticles in ahuhu (*Tephrosia purpurea*) leaf extract. It was found that the size of silver nanoparticles was 16nm, and XRD results were worthy .(Ajitha, Reddy, and Reddy 2014).

Plant extracts were indeed useful for microbes given the ease of quantification, less bio-hazardous nature. Moreover, plant extract do not required any complex process for cell culture maintenance. (Ardelean 2015). The plant based metallic nanoparticles were having antimicrobial activity against different pathogenic bacteria. (Mie *et al.* 2014; Morones *et al*. 2005).

Yellow onions were noticed to have greater quantities of thiosulphinates about 0.35 mole-percent than red onions about 0.20 mole percent. Average diameter AgNPs were produced by constant mixing of silver nitrate solution with 50-60oC with extract of *Allium cepa* and demonstration anti-bacterial action against *Salmonella typhimurium* and *E.coli* at 50 μg/ml. (Saxena, Tripathi, and Singh 2010).

In this study silver nanoparticles were developed using red and yellow onions (Allium cepa L.) bulb extracts. The anti-microbial activity of red and yellow onions bulb extracts, silver nitrate and developed silver nanoparticles evaluated against pathogenic bacterial strains *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Klebsiella pneumoniae* and results were compared. This will be the first study to biosynthesized silver nanoparticles and antimicrobial activity from red and yellow onions (*Allium cepa L.*) bulb extracts.

Material and Methods

Materials

The plant materials used were yellow onion and red onion (*Allium cepa L.*) bulb. The onions were purchased from local market of Multan (Multan, Pakistan). Silver nitrate $AgNO_3$ (Duksan Korea or equivalent) was purchased from M/S A.K. Traders Multan (Multan, Pakistan), peptone (BioPLUS™), NaCl (SIGMA®), yeast extract powder (MC001), agar (OXOID Technical Agar No 2, UK) were purchased from M/S Linker Enterprises Lahore (Lahore, Pakistan). Distilled water was obtained from laboratory of Biochemistry and Biotechnology Women University Multan (Multan, Pakistan). All these chemicals were analytical grade and taken from the Laboratory of Department of Biochemistry and Biotechnology Women University, Multan (Multan, Pakistan).

The bacteria strains *Staphylococcus aureus* (NR-45003), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 13883), and *Pseudomonas aeruginosa* (ATCC 9027) were gifted from Agriculture University Faisalabad, Department of Microbiology (Faisalabad, Pakistan).

Onion extracts preparation

Red and yellow onions (*Allium cepa L.*) were washed in 500 ml beaker first washed two times with tap water and then washed five times with distilled water. The onions peels were removed and bulb outer parts were ground in the mortar and pestle. Both types red and yellow onions were weight in weight balance [SC4020 (OHAUS, USA)] about 250 grams of red onion and 250 grams of yellow onions. The course pieces of onions were taken in a 1000 ml beaker and add about 500 ml of deionized water. Both onions were taken in separate beakers. The beaker was placed on the Hot Plate with Magnetic stirrer [MS-H-S (Scilogex, USA)] and boiled for over 45 minutes between 75 ^{0}C. The mixtures were allowed to cool down at room temperature, filtered contents through Whatman filter paper 1(Figure.1 A).

Preparation of silver nitrate solution

Producing silver nanoparticles, fresh silver nitrate solution was used. Different concentrations of silver nitrate solutions were prepared (1M, 1N, and 1mM). For making 1 M silver nitrate solution 0.17grams dissolved in 100 ml distilled water. For 0.1N silver nitrate solution, 1.69 grams added in 1000 ml distilled water. For 1mM solution weighted about 0.051 grams and dissolved it in 300ml distilled water in a flask (two times weighed the silver nitrate for both types of onions). Silver nitrate was added gradually by constant stirring on a magnetic stirrer. The clear water turned milky white in color further stirring was stopped.

Silver nanoparticles green synthesis

Silver nanoparticles were synthesized by gradually adding red onion extract to the freshly prepared silver nitrate solution. The flasks were placed on magnetic stirrer. The same process was repeated for the second flask in which yellow onion extract was added. The clear silver nitrate solution started changing its color. When it became light yellow then stopped adding onion extract. A total of 90 ml of the extract was added to the silver nitrate solution. Stirrer both reaction mixtures for about 20 mints after that took solution off from stirrer. The reaction mixture contained silver nanoparticles. Cover reaction mixture flask with aluminum foil. The reaction mixture flask further incubated for 24 hours at room temperature. After 24 hours of incubation time the reaction mixture color changed from light yellow to dark brown. The change in color was an indication aimed at effective production of AgNPs (Figure 1 B).

Purification of silver nanoparticles

The reaction mixtures of red and yellow onions AgNPs were centrifuged at 13000 rpm in Micro centrifuge Machine [CF-10 (DAIHAN, USA)] for around 15 minutes to obtain purified pellet contained nanoparticles and the supernatant was discarded. Centrifuged processes (repeated twice) and each time pellets were collected. The pellets washed two times with distilled water. Pellets were dried in Memmert heating and drying universal U oven at 80 ^{0}C for 24 hours. The powder form pellets were stored at 4 ^{0}C for further testing (Figure 1 B)

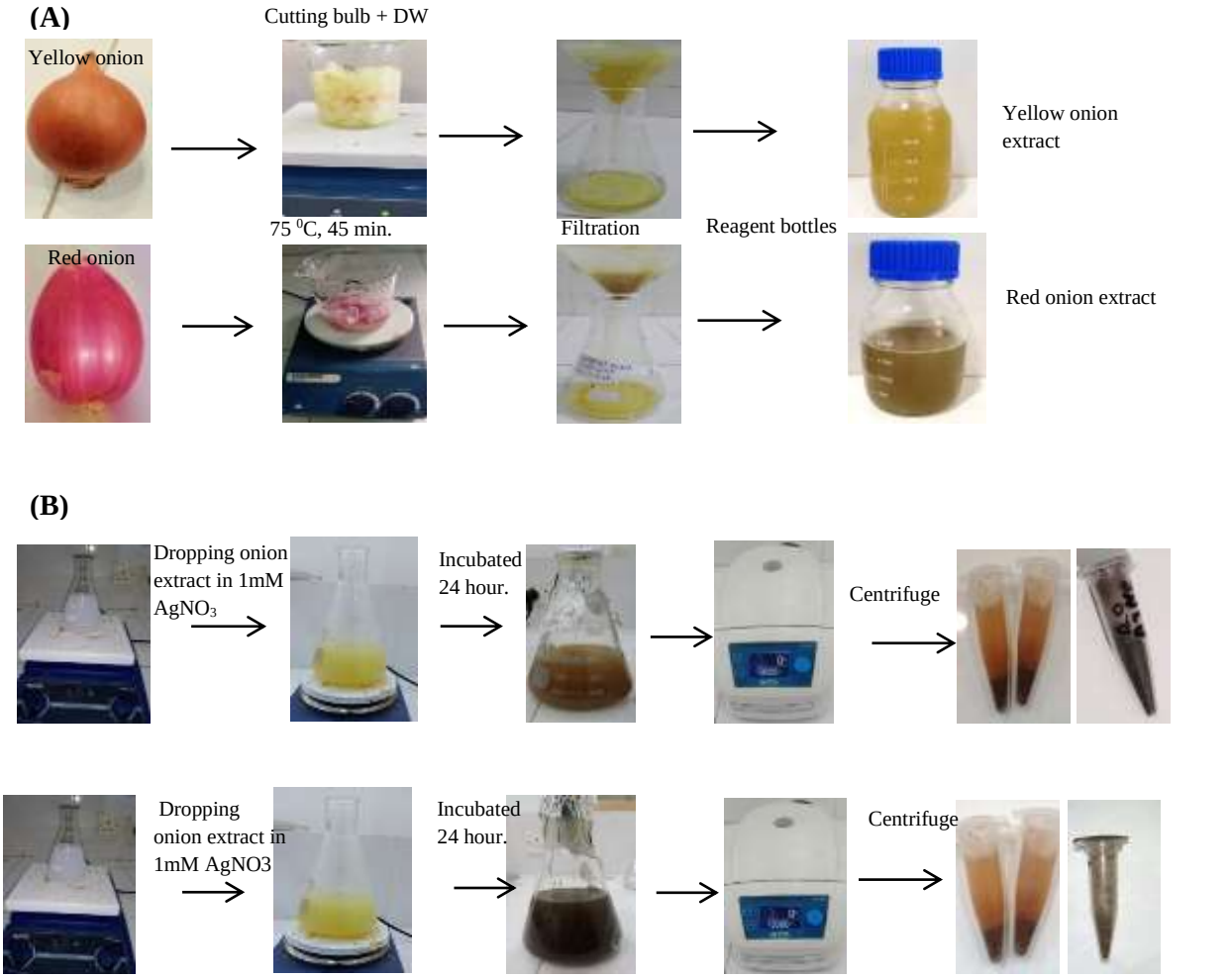


Figure 1 Stepwise representation of AgNPs development from red and yellow onions bulb extracts (A); Extraction procedure of aqueous red and yellow onions (*Allium cepa L.*) bulb extract (B); Biosynthesis procedure of silver nanoparticles using silver nitrate and red and yellow onions bulb extracts pellets taken and dried store at 4 ^{0}C.

Characterization of red and yellow onions AgNPs

Ultraviolet-visible spectroscopy analysis

The reductions of silver ions were examined by calculating peak in Double Beam UV-Vis spectrophotometer [C-7200S (PEAK Instruments Inc.USA)] using quartz cuvettes. UV-visible analysis of synthesize silver nanoparticles were executed at scanning speed of 300 nm to 900 nm and wavelength interval was 1 nm. The silver nanoparticles developments were confirmed by measuring the highest peaks of the reaction mixture, in the range of 300 to 400 nm that indicated formation of silver nanoparticles.

FT-IR analysis

The presences of functional groups on onion extracts and synthesized silver nanoparticles were analyzed using Bruker ALPHA FTIR spectrophotometer wavelength frequency ranging from 375 to 7500 cm^{-1} from department of Chemistry, The Women University Multan (Multan, Pakistan). FTIR spectroscopy demonstrated data regarding presence of functional groups. Red and yellow onions AgNPs and bulb extracts dried in hot air oven at 75 ^{0}C. The red and yellow onions extract and developed AgNPs were analyzed by placing small amount of dried AgNPs on the sample chamber after wiping it with ethanol. The baseline adjustment was done for each sample. Different peaks (bending, broadening)

on spectra were distinctive for specific functional group. The analysis was used to measure biomolecules responsible, which immediately work to reduced, help stabilized as well as capping agents. The regions between wavenumber 1500 to 800cm^{-1} (finger print region) reflected the biochemical molecules like carbohydrates, proteins, lipids and polyphenols in (*Allium cepa L.)* extract. Regions from 4,000 – 2000 cm^{-1} were showed functional groups.

SEM analysis

The morphological characterizations of the samples were analyzed by using SEM model Hitachi S2380 N, Japan and secondary electron beam mode is used for the analysis. The fresh samples were scatter on clean glass slide, dried it. The samples were then subsequently analyzed under SEM in different resolutions from 1 mm to 500 μm and magnifications powers from 40X – 3000X. The SEM data further characterized by using imageJ 1.53e and origin 2021 software. The SEM analysis was executed at Central Hi Tech Laboratory of University of Agriculture Faisalabad (Faisalabad, Pakistan)

X-ray diffraction analysis

X-ray diffraction (XRD) of AgNPs conducted to analyze the crystal nature of the developed nanoparticles. For XRD analysis, pellets of pure AgNPs were prepared. Each sample was analyzed using X-ray diffractometer (Panalytical, Germany) under array of Bragg angle at 2θ, and d spacing A^0 with angular speed 50/ minutes. XRD analysis performed in Central Research Laboratory, Lahore College for Women University, Lahore (Lahore, Pakistan). Patterns in the XRD recorded in origin 2021 software. The crystallite size and average crystallite size from XRD determined, by using Scherrer equation.

$$D=k\lambda/\beta COS\theta$$.(Langford and Wilson 1978).

Where,

D= grain dimension in nm.

k= sherrer constant (0.9),

$\lambda= 0.15406A^0$

β= FWHM in Radians,

θ=Bragg-angle

Antimicrobial activity of AgNPs

The antimicrobial activity of extract, silver nitrate and developed AgNPs were evaluated against four different strains of bacteria according to guidelines set by Clinical Laboratory standards Institute. Some bacterial strains were taken from agricultural university Faisalabad (Faisalabad, Pakistan). Bacterial strains that were used mentioned in the table 1.

Table 1 List of Bacterial Strains Used For Antimicrobial Activity

Pathogenic Bacteria	**Strain Type**	**Short form**
Staphylococcus aureus	Gram positive	*S.aureus*
Klebsiella pneumoniae	Gram negative	*K.pnemoniae*
Pseudomonas aeruginosa	Gram negative	*P.aeruginosa*
Escherichia coli	Gram negative	*E.coli*

Culturing media

Nutrient agar

This media was used for growth of bacteria. The media contained chemicals; yeast extracts 0.2 gram, peptone 0.5 gram, agar 1.5 gram and sodium chloride 0.5 gram. Total 2.7 gram of the medium was dissolved in one hundred milliliter of distilled water. The pH of medium was sustained on 7.2. Dissolved chemicals and sterilized by autoclave for 15 minutes at 121ºC. The nutrient agar media was cool down at room temperature and then poured it in petri plates. When nutrient agar plates solidified then using loop steaking of bacteria was done under UV illuminator.

Nutrient broth

This media was used for multiplication of bacterial strains. The medium contained yeast extract 0.2 gram, peptone 0.5 gm., NaCl 0.5 gm. Total 1.2 gram of medium was dissolved in distilled water and mixed it well. The dissolved medium was decontaminated by autoclave at 121ºC for 15 minutes. Media pH sustained at 7.2. After that the bacteria colony from nutrient agar plate was taken and mixed it in the nutrient broth media. The flasks containing bacteria were placed on electrical shaker for 24 hours so that the bacteria multiply in number. After that the bacteria culture was used for spreading on nutrient agar plates and checked for antimicrobial activity.

Agar well diffusion method

Antimicrobial activity was performed by using agar well diffusion test. The Nutrient broth was inoculated with single bacterial colonies and bacterial cultural were incubated for 24 hours at 37 degree with shaking at 450 rpm. Fresh nutrient agar prepared and poured in the petri plates. The petri plates were placed in UV illuminator for 20-25 minutes for solidification of nutrient agar. About 20µl fresh overnight prepared bacterial cultures in nutrient broth were spread evenly on each petri plates by using sterile glass spreader. The six wells were created in each petri plates using sterile blue tips of micropipette diameter 9 mm. First wells were sealed by pouring one or two drops of agar. The samples were placed in each well with sterile pipette along with control silver nitrate solution and plant extract. The sample plates were then kept warm at 37 degree for 24 hours in the incubator. The zone of inhibition diameter calculated in mm by measuring scale. The comparisons of both types of developed silver nanoparticles were described in forms of graph and tables.

Statistical Analysis

Antimicrobial activity performed in triplicate form. The obtained results of all investigation were handled in Microsoft Excel® 2010 (Microsoft Corporation, Redmond, USA). All the experiments were performed three time and data were presented. The data were presented in mean ± Standard Deviation (SD) and one way ANOVA. Significance level checked according to p values (<0.001, <0.05, <0.01) considered level of significance. Statistical analyses were performed by using Instat 3 software.

Results and Discussion

Visual analysis of biosynthesized AgNPs

Formulations made with different concentration of silver nitrate and plant extract were prepared under different temperature and show different color changes (MI, M2, N1, N2, S1, S2). The silver nanoparticles formation was observed under different temperature and incubation time periods. The formulation of M1 and M2 prepared in 1M of silver nitrate solution (0.17gram/100ml) about 2 ml of extract were inserted in 50 ml $AgNO_3$ solution. Three time conduct reaction mixtures for M1 and M2 gave same results. After 24 hours of incubation at room temperature slight color changes were observed for M1 and M2 formulation. The formulation N1 and N2 prepared in 0.1N (1.69grams in 1000ml distilled water). Take about 100 ml of silver nitrate solution from prepared stock solution and 20 ml of prepared extract added. The reaction mixtures were observed after 24 hours of incubation at room temperature for color changes. Two time conduct reaction and same results were seen as for formulation N1 it was medium brown and for N2 it was light brown color changes. The formulation (S1, S2) exhibited finest silver nanoparticle development from red and yellow onions (*Allium cepa L.*) bulb extract. The formulation S1 of red onions showed silver nanoparticles development in 1mM (0.017 gram/100ml) and 20 ml of extract color changed dark brown after

incubation time of 24 hours at room temperature. The formulation S2 of yellow onions showed silver nanoparticles development in 1 mM (0.017 gram/100ml) and 20 ml extract, dark brown color indicate confirmation of silver nanoparticles development. The silver nanoparticles development occurs, when the onion extract reacted with the silver nitrate Ag+ changed to Ag^0. A study was reported by Saxena, he stated that using onion extract for silver nanoparticle synthesis UV-Vis spectra close to 413 nm. The changed in range of peaks of silver nanoparticles were due to the changed in size and shape. (Arya *et al*. 2018)

(a) 1 M AgNO3 (b) 0.1 N AgNO3 (c) 1mM AgNO3

Figure 2 visual analyses of different formulations for development of AgNPs using different concentration of red and yellow onion extract and silver nitrate solutions

UV –visible spectrophotometry

UV-visible analysis of synthesize silver nanoparticles were executed at scanning speed of 300nm to 900 nm and wavelength interval was 1 nm. All formulations analyzed by using double beam UV-Vis spectroscopy. The formulation M1 of red onions (*Allium cepa L.*) developed AgNPs showed absorption peak at 299 nm and M2 of yellow onions AgNPs at 300 nm. The formulation N1 of red onions AgNPs showed maximum peak at 270 nm and N2 of yellow onions AgNPs at 313 nm. The UV-vis spectrum of red onions (*Allium cepa L.)* bulb extract formulation S1 showed maximum peak at 418 nm that certainly confirmed the development of silver nanoparticles. The yellow onion (*Allium cepa L.)* bulb extracts formulation S2 silver nanoparticles showed maximum peak at 399 nm. When the color changed, it's mainly due to the surface plasmon resonance that was present on the surface of AgNPs. The λ maxima of red onions (*Allium cepa L.)* silver nanoparticles showed it exhibited maximum capacity to developed silver nanoparticles as compared to yellow onions (*Allium cepa L.)* bulb extracts.

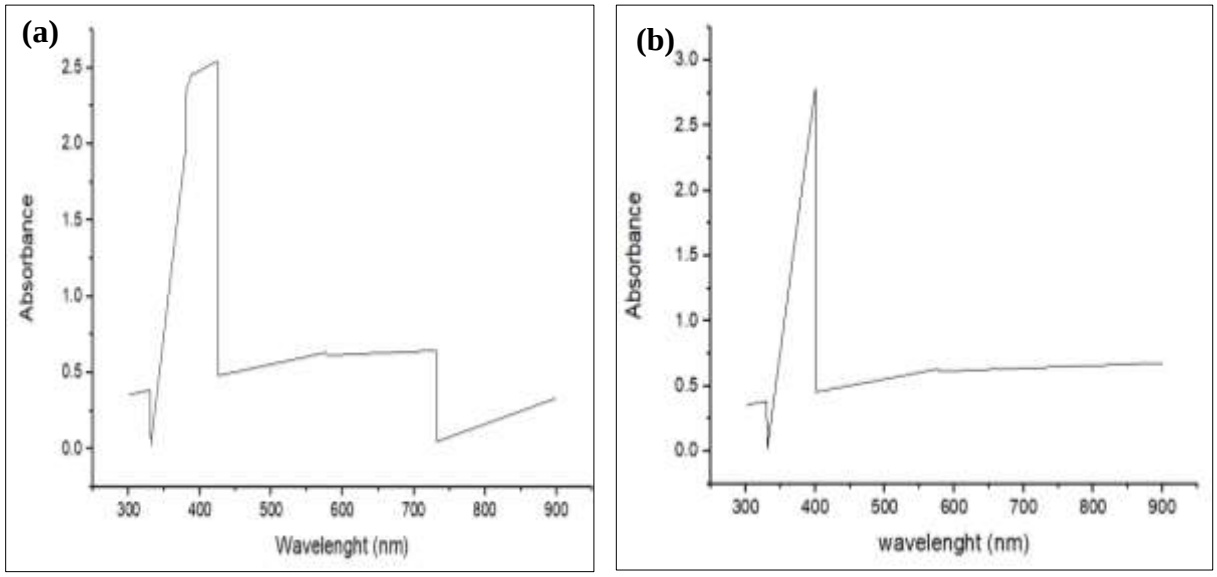


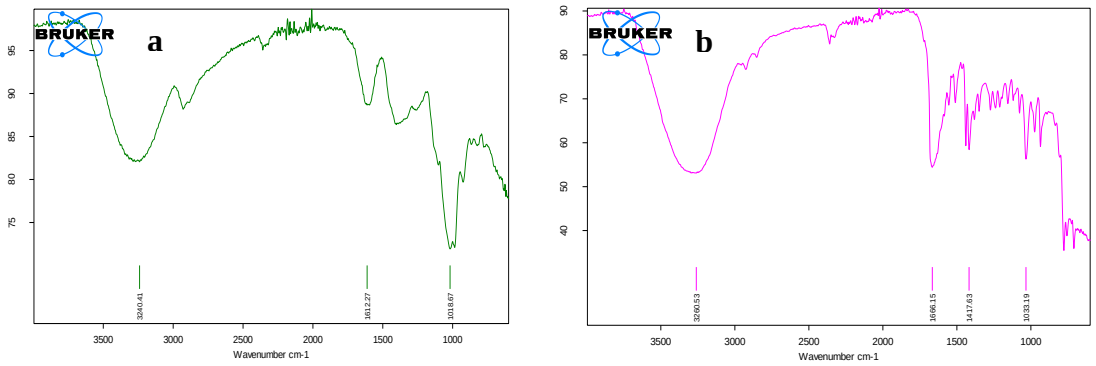
Figure 3 UV- Vis graphs of formulation S1 and S2 (a) The absorption peak of 418 nm was appeared in red onions AgNPs (b) The absorption peak 399 nm was appeared in yellow onions AgNPs indicated presence of silver nanoparticles.

Fourier-transform infrared spectroscopy (FTIR)

Red onions (*Allium cepa L.*) extract showed in figure 17 peaks at 3240, 1612 and 1018 cm^{-1}. Spectra showed strong and wide band at 3240 cm^{-1} allocated to C-H group stretching. The band 1612 cm^{-1} showed C=C medium extending. Peak 1018 cm^{-1} showed C-N stretching vibration aliphatic amines. Yellow onions (*Allium cepa L.*) bulb extract figure 18 showed peaks form 3260, 1666, 1417 and 1033 cm^{-1}. Peak 3260 cm^{-1} exhibited N-H stretch medium absorption contains functional group 1&2 amine, amide. Peak at 1666 cm^{-1} showed medium stretching between C=C (Alkene hydrocarbon). The peak at 1417 cm^{-1} presented C-H medium widening indicate presence of alkane. The peak at 1033 cm^{-1} wavenumber showed strong C-O stretching band of esters, carboxylic acids and alcohols residues.

The AgNPs prepared from red onions (*Allium cepa L.*) showed in figure 19 peaks at 3267 and 1004 cm^{-1}. Peak 3267 cm-1 displayed C-H group of strong and narrow stretching of alkyne. The peak at 1004 cm-1 C-N in variable region presence of aliphatic amines. As Awwad proposed that AgNPs synthesized from onion extract were bounded by proteins and having functional group like aldehyde, ketones, alcohols (Awwad, Salem, and Abdeen 2013). Spectra of AgNPs yellow onions showed in figure 20 peaks at 3260 and 1013 cm-1. Peak at 3260 cm-1 single narrow strong peak showed C-H group. The band at wavenumber 1013 cm-1 was from C-N stretch. The existence of amines remnants had stronger capability to bind with silver nanoparticles showed that it stabilizes AgNPs in aqueous form .(Sathyavathi et al. 2010). (Figure 4)

By comparing FTIR spectra of both types AgNPs and onions (*Allium cepa L.*), it was noticed that several bands formed by both types of extract have been repeated in FTIR spectra of AgNPs with slight variations in point of absorption peaks. As compared to onion extract peaks, AgNPs of red onions (*Allium cepa L.*), absorption peak at 3267 cm-1 shifted to higher frequency and 1004 cm-1 narrower down to low frequency. The peaks changes directed that stabilization and reduction of AgNPs proceed through these groups. The peaks found at 3267 and 1004 cm-1 of AgNPs spectrum showed that amines(C-N) and hydrocarbons (C-H) of onions interact with AgNPs. The peaks of AgNPs compared with yellow onions extract showed that peaks form between 1600-1000 cm-1 were involved in AgNPs stabilization. Band at 1033 cm-1 (C-O) alcohols reduced to 1013 cm-1 spectrum (C-N) aliphatic amines clearly depicted narrowing down and sharp stretching help in AgNPs development. This evidences proposed that AgNPs produced from yellow and red onions (*Allium cepa L.*) extract were bounded by either free amines or hydrocarbon residues. The same type of stretching also reported by Gomaa, in his study AgNPs synthesized from onion extract had C-N stretching at peak 1387 cm-1 showed presence of amine group and bands at 1061 – 971 cm-1 showed C-N stretching of carboxylic acids, esters and ethers. (Gomaa 2017).



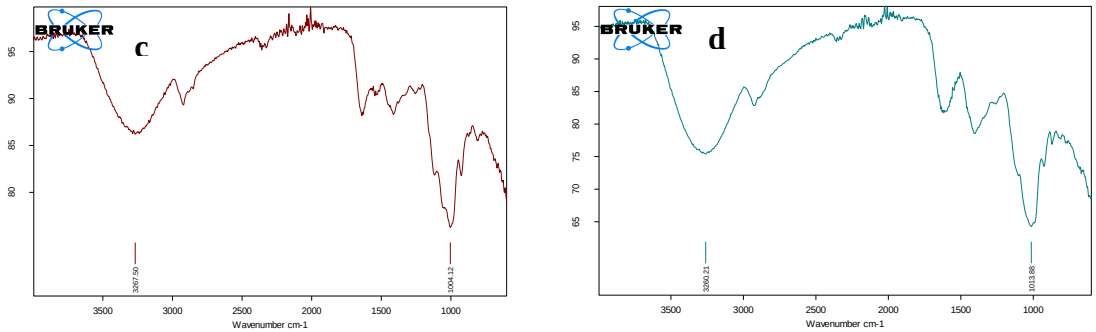


Figure 4 FTIR spectra of (a) red onion bulb extract (b) yellow onion bulb extract (c) red onions AgNPs (d) yellow onions AgNPs showed the presence of amine and hydrocarbons functional groups.

Table 2: Comparison FTIR Spectra of red and yellow onions and their synthesized AgNPs

Red onions				Yellow Onions			
Peak position in extract (cm^{-1})	**Peak position in AgNPs (cm^{-1})**	**Change in peak position (cm^{-1})**	**Type functional group**	**Peak position in extract (cm^{-1})**	**Peak position in AgNPs (cm^{-1})**	**Change in peak position (cm^{-1})**	**Type functional group**
3240.41	3267.50	+27.09	C-H	3260.53	3260.21	-0.32	N-H
1612.27	-	-	C=C	1666.15	-	-	C=C
1018.67	1004.12	-14.55	C-N	1417.63	-	-	C-H
-	-	-	-	1033.19	-	-	C-O
-	-	-	-	-	1013.88	-	C-N

Note: (-) mean no peak value in FTIR spectra.

X-ray diffraction (XRD)

XRD patterns were analyzed by determining the intense peak position, full width half maxima (FWHM), and determined the average silver nanoparticles crystal size by using Scherer equation mention in detailed material and method chapter. The XRD pattern indicates that developed silver nanoparticles from both types of onions were contain assorted appearance (spherical to cubical) structures of AgNPs. Red onions AgNPs more crystal in nature as compared to Yellow onions AgNPs.

Red onions AgNPs

The XRD of red onion silver nanoparticles (S1) showed four intense peaks at different points. The peaks form at 10.44 at 2θ , 256 counts, 27.25 at 2θ, 127 counts, 32.09 at 2θ, 187 counts 46.07 2θ, 128 counts. The average size to silver nanoparticle using Scherrer equation $D=k\lambda/\beta COS\theta$ was 9.65 nm determined by using origin software 2021.

Yellow onions AgNPs

The XRD of yellow onions silver nanoparticles (S2) showed maximum peaks at point 8.06 2 θ and 214 counts per second, 11.46 2θ, 150 counts, 29.03 2θ, 80 counts, 46.28 2θ, 55 counts. The average grain size calculated from

Scherrer equation was 7.48 nm determined using origin software. The XRD result showed that the red onions (*Allium cepa L.)* AgNPs have slightly larger grain size as compared to yellow onion (*Allium cepa L.)* AgNPs. The calculated crystal size of present study exhibited similar results with the previously synthesized silver nanoparticles (Khalilzadeh and Borzoo 2016) from onions extract. There were intense nanoparticles peaks, which were indexed as a facial, crystalline silver coupling phase (Figure 5).

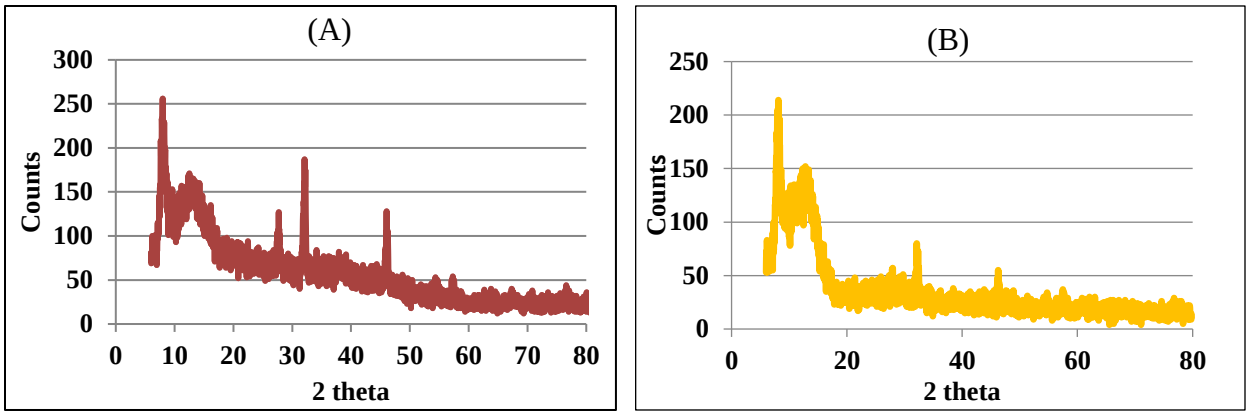
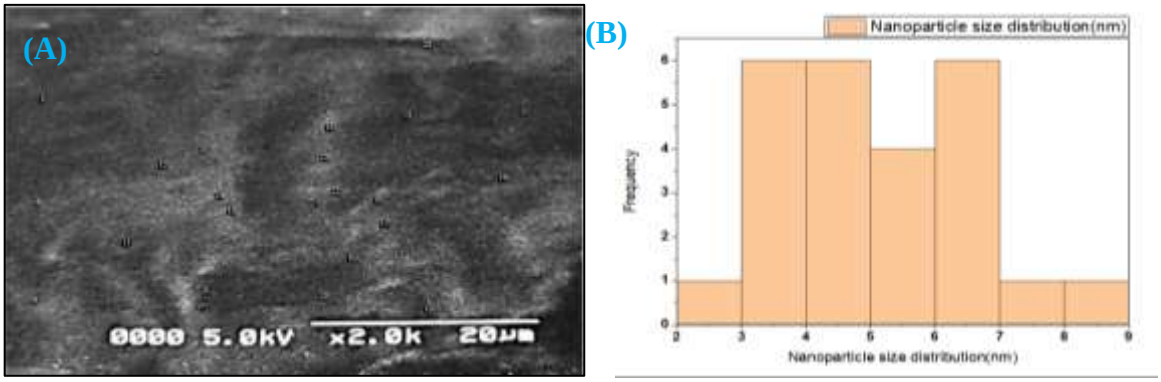


Figure 5 XRD spectra of (A) red onions AgNPs showed various intense peaks indicated crystal nature (B) yellow onions AgNPs showed few intense peaks that confirm crystal nature

SEM analysis

The morphological structures of biologically synthesized silver nanoparticles of formulation of S1 and S2 using red and yellow onion (*Allium cepa L.)* bulb extract confirmed through SEM. The SEM result showed that silver nanoparticles exhibit surface morphology spherical to cubical and highly dispersed particles. These results were quite similar with the results of previously synthesized nanoparticles from onion extract.(Al-Kalifawi et al. 2015) .Results showing that developments of silver nanostructures under different magnification and resolution powers. Lankoff also synthesized AgNPs from onion extract. He also reported that AgNPs had spherical shape with minor agglomeration.(Lankoff et al. 2012) The figure showed particle size of synthesized AgNPs from red onions (*Allium cepa L.*) bulb extract. After analyzing the data of SEM image, graphical demonstration of average particle size distribution were range of 2-9nm. The average diameter of AgNPs was 4.96nm. Similarly, figure showed particle size of synthesized AgNPs from yellow onions (*Allium cepa L.)* bulb extract. The graphical representation of SEM data showed that average particle size distribution were ranged of 3-8nm. The average diameter of AgNPs was 4.27nm.



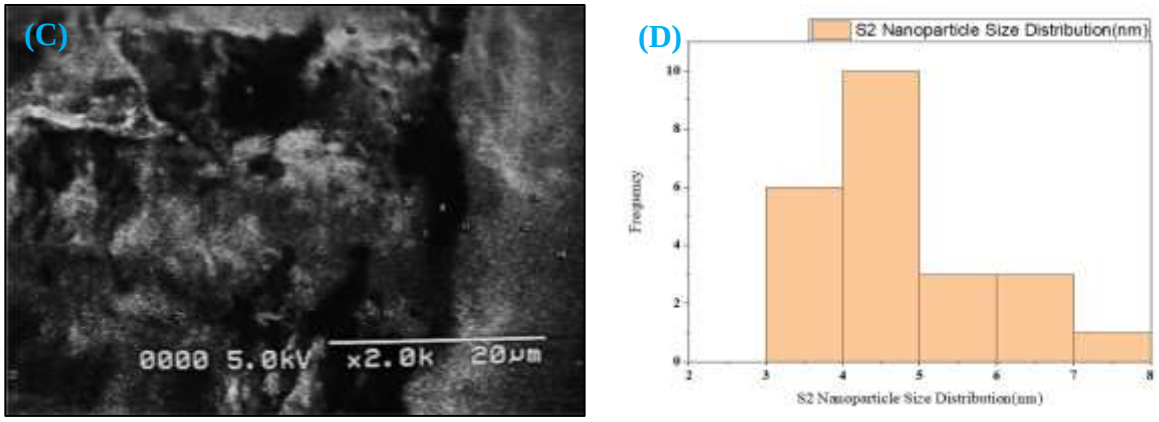


Figure 6 Particle size distribution from SEM micrograph (A) exhibited that particle were appeared spherical to cubical in shape and most of particles were agglomerated (B) the particle size distribution graph of red onions AgNPs dimensions were ranged from 2-9 nm (C) showed that yellow onions AgNPs were cubic to spherical in shape and mostly particles were clustered (D) particle size distribution graph of yellow onions AgNPs dimensions were ranged from 3-8 nm

Anti-microbial assay of red and yellow onions AgNPs

Agar well diffusion method used for determining antimicrobial action of prepared silver nanoparticles from red and yellow onions (*Allium cepa L.*). Antimicrobial assay performed by using five different pathogenic one gram positive and three gram positive strains of bacteria *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Klebsiella pneumonia*. The bacterial cultures were spread on agar plates and activity of red and yellow onions AgNPs and extract calculated easily. The zones of inhibition around the well were calculated after 24 hours incubation at 37 degree. The zone were calculated in diameter (mm) from one side of well to the start point of clear zone to other clear zone side together with the well width. The measured zone of inhibitions of silver nanoparticles prepared from red and yellow onions (*Allium cepa L.*) S1 and S2 displayed in (Table 2). About 20µl of bacterial culture was taken for each petri plates. The both types of synthesize silver nanoparticles used in different concentrations like 5 µl, 10 µl, 15 µl and 20 µl, onions (*Allum cepa L.)* bulb extract 30µl and silver nitrate solution 30 µl (1mM) used as control. The resulted diameter values were summarized in table 3, 4.

After performing each experiment thrice results showed that developed silver nanoparticles from red onions (*Allium cepa L.)* S1 formulation showed zones of *E.coli* at concentration 5 µl 0.0 mm, 0.0 mm.0 mm; at 10 µl AgNPs diameter 12.0, 12.4, 12.7 mm; at 15µl AgNPs 13.0, 13.2, 13.6 mm respectively at 20 µl AgNPs diameter were 16.0, 16.4, 16.9 mm.The measured zone diameter of *Klebsiella pneumonia* were at 5 µl AgNPs 13.0, 13.5, 13.7 mm; at 10 µl AgNPs concentration 14.0, 14.3, 14.8 mm; at 15 µl concentration 17.0, 17.2, 17.5 mm and at 20µl AgNPs concentration diameter were 11.0, 11.5, 11.8 mm. The clear zones diameters of Staphylococcus aureus were about 13.0, 13.4, 13.8 mm at 5µl AgNPs concentration; at 10 µl AgNPs 12.0, 12.5, 12.8 mm; at 15 µl AgNPs 15.0, 15.2, 15.5 mm respectively at 20 µl 16.0, 16.3, 16.7 mm. The measured zones against *Pseudomonas aeruginosa* at 14.0, 14.2, 14.8 mm at 5 µl AgNPs; at 10 µl concentration of AgNPs diameter were13.0, 13.4, 13.6 mm; at 15 µl AgNPs 17.0, 17.3, 17.9 mm; respectively at 20 µl diameter were 12.0, 12.1, 12.7 mm. The absence of antibacterial activity experimental in small concentration of AgNPs for *E.coli* bacteria was due to several reasons one of which was size of AgNPs. Results showed that at concentration 15 µl zone of inhibition found to be larger 17 mm against *Psedomonas aeruginosa*, and 17 mm for *Klebsiella pneumonia* then for other bacterial strains as shown in figure 7. The antimicrobial test carried out using red onions AgNPs in triplicate and standard deviation and mean values showed p-values <0.001 that indicated that results were significant.

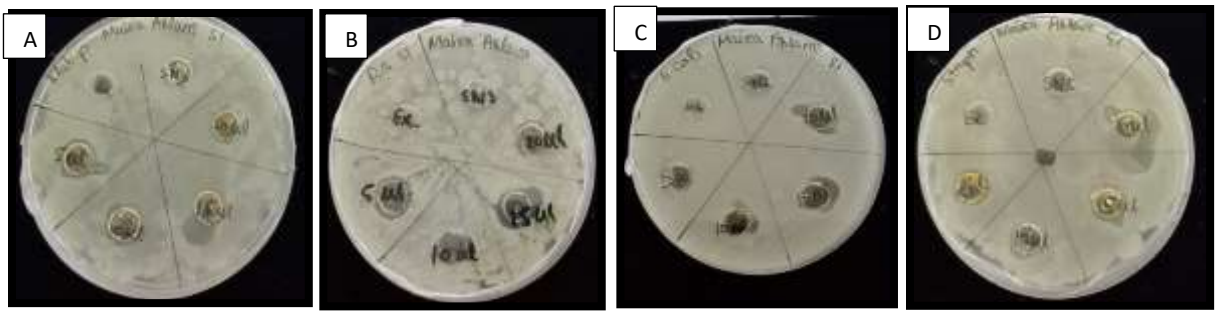


Figure 7 Diagramatic representation of antimicrobial activity of red onions AgNPs against (A*) Klebsiella pneumonia* (B) *Psedomonas aeruginosa* (C) *E.coli* (D) *Staphylococcus aureus* and controal silver nitrate 1mM solution and red onion bulb extracts

Table 3 Zone of inhibition of red onions AgNPs against different bacterial strains

Bioactive compound	Concentration µl/ml	Zone of inhibition (mm) Mean ± Standard Deviation				P-Value
		Klebsiella pneumonia	*Pseudomonas aeruginosa*	*E. coli*	*Staphylococcus aureus*	
AgNPs	5µl	13.4±0.36	14.33±0.41	0±0.00	13.4±0.40	<0.0001
	10µl	14.36±0.40	13.33±0.30	12.36±0.35	12.43±0.40	
	15µl	17.23±0.25	17.4±0.45	13.26±0.30	15.23±0.25	
	20µl	11.43±0.40	12.26±0.37	16.43±0.45	16.33±0.35	
AgNO3 Control	30µl	Nil	Nil	Nil	Nil	
Onion extract	30µl	Nil	Nil	Nil	Nil	

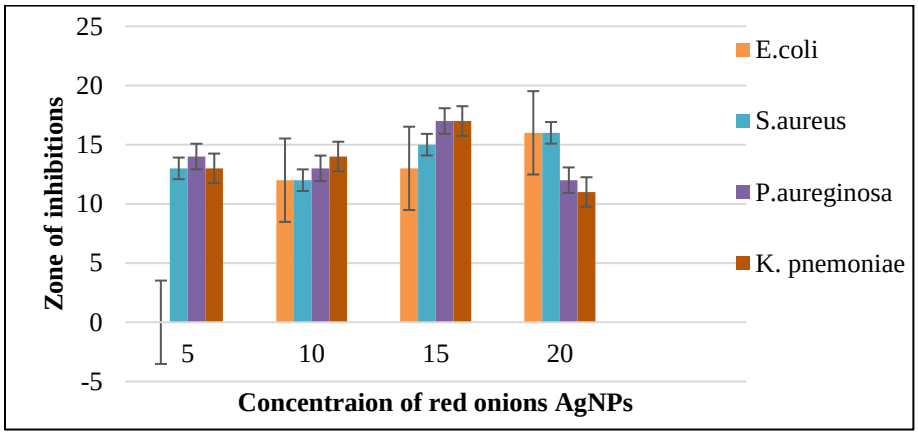


Figure 8 Graph showing zone of inhibition form by red onions AgNPs against different bacterial strains

The results for yellow onions (*Allium cepa L.*) S2 formulation showed antibacterial activity against pathogenic bacterial strains clear zones measured at different concentration of AgNPs 5 µl, 10µl, 15µl and 20µl. The measured

clear zones against *Pseudomonas aureus* diameter were at 5µl 19.0, 19.3, 19.5 mm; at 10 µl 16.0, 16.3, 16.9 mm; at 15 µl AgNPs concentration 17.0, 17.3, 17.4 mm and at 20 µl AgNPs 14.0, 14.6, 14.8 mm.The measure zone for *E.coli* 17.0, 17.1, 17.2 mm; at 10 µl AgNPs 15.0, 15.2, 15.3 mm; at 15 µl concentration 13.0, 13.5, 13.7 mm and at 20 µl 17.0, 17.1, 17.6 mm. The clear zones against *Staphylococcus aureus* were at 5 µl 13.0, 13.2, 13.4 mm; at 10µl 17.0, 17.5, 17.8 mm; at 15 µl 13.5, 13.2, 13.7 mm and at 20 µl diameter were 15.0, 15.3, 15.7 mm. The *Klebsiella pneumonia* was 12.0, 12.3, 12.7 mm; at 10 µl 13.0, 13.6 13.9 mm, at 15 µl AgNPs zone diameter were 14.0, 14.2, 14.6 mm, and at 20 µl 14.0, 14.3, 14.5 mm respectively. Results indicated that the *Pseudomonas aureus* at 5µl concentration have larger clear zone 19 mm and E.coli 17 mm clear zone, depicted that they displayed higher antibacterial activity than for other bacterial strains. The antimicrobial test carried out using yellow onions AgNPs in triplicate and standard deviation and mean values showed p-values of obtained result were in ranged in <0.001.

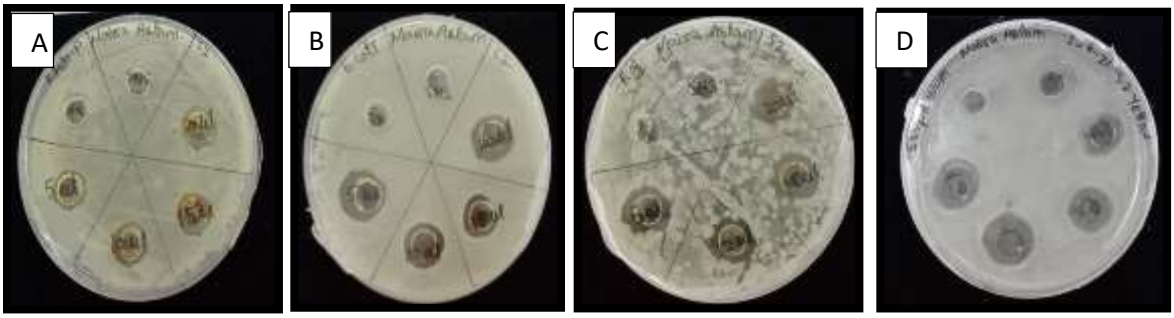


Figure 9 Diagrammatic representation of antimicrobial activity of yellow onions AgNPs against (A) *Klebsiella pneumonia* (B) *Psedomonas aeruginosa* (C) *E.coli* (D) *Staphylococcus aureus;* silver nitrate and yellow onion extract as control not form any zones

Table 4 Zone of inhibition of yellow onions AgNPs against different bacterial strains

Bioactive compound	**Concentration µl/ml**	**Zone of inhibition Diameter(mm) Mean ± Standard Deviation**				**P-Value**
AgNPs		*Klebsiella pneumonia*	*Pseudomonas aeruginosa*	*E. coli*	*Staphylococcus aureus*	<0.0001
	5µl	12.33±0.35	19.26±0.25	17.1±0.10	13.2±0.20	
	10µl	13.5±0.45	16.4±0.45	15.16±0.15	17.43±0.40	
	15µl	14.26±0.30	17.23±0.20	13.4±0.36	13.46±0.25	
	20µl	14.26±0.25	14.46±0.41	17.23±0.32	15.33±0.35	
AgNO3 Control	**30µl**	Nil	Nil	Nil	Nil	
Extract Control	**30µl**	Nil	Nil	Nil	Nil	

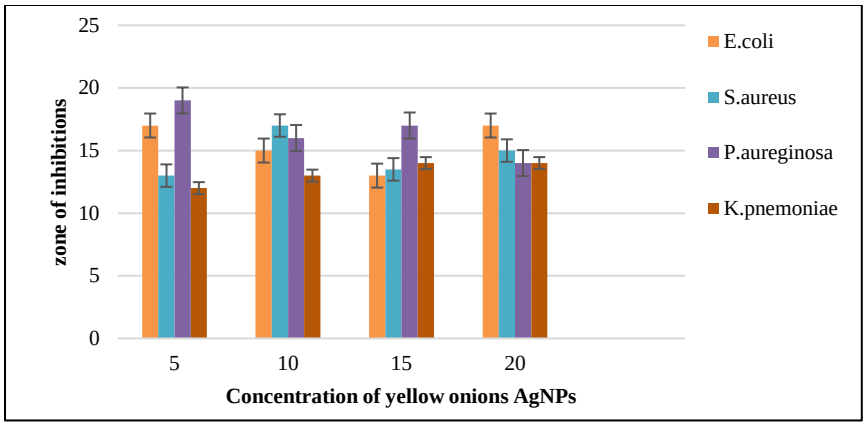


Figure 10 Graph showing zone of inhibition form by yellow onions (*Allium cepa L.*)

AgNPs activity against different bacterial strains

Comparison of anti-microbial activity of synthesized AgNPs from red and yellow onions (*Allium cepa L.)*

The comparative evaluation of anti-bacterial activity of synthesized silver nanoparticles from red and yellow onions (*Allium cepa L.*) bulb extract from formulation S1 and S2 showed in figure 35. The S1 formulation AgNPs at concentration 15 µl, the bacteria *Klebsiella pneumonia* and *Pseudomonas aeruginosa* showed higher zone of inhibitions about 17 mm which shows that red onions (*Allium cepa L.)* AgNPs exhibit maximum antibacterial activity at 15 µl. The developed AgNPs has no effect on E.coli bacteria when use in low concentration (no zone of inhibition), but when the AgNPs concentration increases it exhibit antibacterial activity at 20 µl concentration larger zone about 16 mm developed. Other AgNPs concentrations such as 5 µl and 10 µl have low zone of inhibitions. The present study indicated that developed nanoparticles have antibacterial activity. Sondi and Salopek identified AgNPs antimicrobial activity on gram negative bacteria *E.coli* was depends on concentration of AgNPs. As formation of pits occur in cell wall of bacteria and AgNPs mount up in bacterial membrane that cause cell death.(Moradi *et al.* 2021) (Sondi and Salopek-Sondi 2004).

The S2 formulation of AgNPs at concentration 5 µl form clear larger zone against *Psedomonas aeruginosa* bacteria 19 mm, but *Klebsiella pneumonia* show low zone of inhibition 12 mm at this concentration. At concentration 5 µl and 20 µl *E.coli* showed same inhibition zone size about 17 mm. The *Staphylococcus aureus* show maximum zone about 17 mm at 10 µl concentration.

The present study result clearly showed that developed AgNPs from both onions (*Allium cepa L.*) types have antimicrobial potential as they show different zone of inhibitions at different concentrations. The yellow onions (*Allium cepa L.)* S2 formulation showed maximum antibacterial potential even used in low concentration. The increased in antimicrobial activities were described as nano-sized AgNPs resulting in exterior region help cellular membranes binds immediately, enhanced the penetration potential of silver nanoparticles. In a related study silver nanoparticles synthesize from onion (*Allium cepa L.*) extract that exhibited maximum antimicrobial activity against *E.coli* at high concentration of AgNPs about 50µl .(Saxena, Tripathi, and Singh 2010).

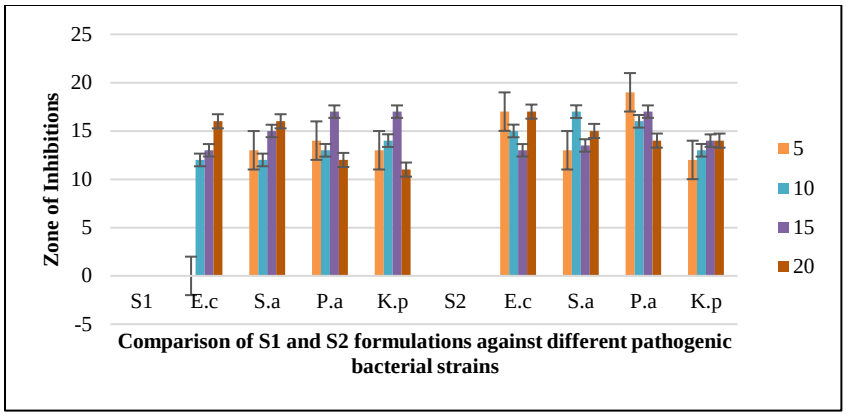


Figure 11 Bar chart of zone of inhibition comparison of red and yellow onions AgNPs

Conclusion

Conclusion drown from recent study were silver nanoparticles were successfully synthesized from both types red and yellow onions (*Allium cepa L.*) bulb extract. The onion extract used as capping and reducing agent of silver ion. The AgNPs were further characterized by UV-vis spectroscopy, SEM, XRD, FTIR. The SEM analysis showed that average particles size distribution of red onions AgNPs were ranged 2-9 nm, yellow onion AgNPs were in range 3-8 nm. The averaged AgNPs size of red onion XRD were 9.65 nm and yellow onions AgNPs were 7.48 nm. The average size from SEM and XRD showed that the biosynthesized red onions AgNPs were larger as compared to yellow onions AgNPs. The synthesis method of silver nanoparticles using onion extract (Phytonanotechnology) had maximum yield at very low expanse. The potential of antimicrobial activity were tested against four different pathogenic bacteria like *E.coli, Pseudomonas aeruginosa, Klebsieila pneumonia* and *Staphylococcus aureus*. The tests were performed by agar well diffusion assay method. From the study, both types of biosynthesized AgNPs red and yellow onions (*Allium cepa L.*) were observed to showed antimicrobial potential. However, yellow onions AgNPs exhibited maximum antimicrobial activity due to its small crystal size as compared to red onion AgNPs.

References

Ajitha, B, Y Ashok Kumar Reddy, and P Sreedhara Reddy. 2014. 'Biogenic nano-scale silver particles by Tephrosia purpurea leaf extract and their inborn antimicrobial activity', *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, **121**: 164-72.

Al-Kalifawi, Esam J, Tagreed M Al-Saadi, Sanaa A Al-Dulaimi, and Entisar E Al-Obodi. 2015. 'Biosynthesis of silver nanoparticles by using onion (Allium cepa) extract and study antibacterial activity', *Journal of Genetic and Environmental Resources Conservation*, **3**: 1-9.

Ardelean, Ioan I. 2015. 'Metallic nanoparticle synthesis by cyanobacteria: fundamentals and applications.' in, *The Algae World* (Springer).

Arya, Anju, Khushbu Gupta, Tejpal Singh Chundawat, and Dipti Vaya. 2018. 'Biogenic synthesis of copper and silver nanoparticles using green alga Botryococcus braunii and its antimicrobial activity', *Bioinorganic Chemistry and Applications*, 2018.

Awwad, Akl M, Nidá M Salem, and Amany O Abdeen. 2013. 'Green synthesis of silver nanoparticles using carob leaf extract and its antibacterial activity', *International journal of Industrial chemistry*, **4**: 1-6.

Durán, Nelson, and Priscyla D Marcato. 2013. 'Nanobiotechnology perspectives. Role of nanotechnology in the food industry: a review', *International Journal of Food Science & Technology*, **48**: 1127-34.

Gomaa, Eman Zakaria. 2017. 'Antimicrobial, antioxidant and antitumor activities of silver nanoparticles synthesized by Allium cepa extract: a green approach', *Journal of Genetic Engineering and Biotechnology*, **15**: 49-57.

Gorinstein, Shela, Hanna Leontowicz, Maria Leontowicz, Jacek Namiesnik, Kasia Najman, Jerzy Drzewiecki, Milena Cvikrová, Olga Martincová, Elena Katrich, and Simon Trakhtenberg. 2008. 'Comparison of the main bioactive compounds and antioxidant activities in garlic and white and red onions after treatment protocols', *Journal of Agricultural and Food Chemistry*, **56**: 4418-26.

Jha, Anal K, and K Prasad. 2010. 'Green synthesis of silver nanoparticles using Cycas leaf', *International Journal of Green Nanotechnology: Physics and Chemistry*, **1**: P110-P17.

Khalilzadeh, Mohammad A, and Mina Borzoo. 2016. 'Green synthesis of silver nanoparticles using onion extract and their application for the preparation of a modified electrode for determination of ascorbic acid', *journal of food and drug analysis*, **24**: 796-803.

Langford, J Il, and AJC Wilson. 1978. 'Scherrer after sixty years: a survey and some new results in the determination of crystallite size', *Journal of applied crystallography*, **11**: 102-13.

Lankoff, Anna, Wiggo J Sandberg, Aneta Wegierek-Ciuk, Halina Lisowska, Magne Refsnes, Bożena Sartowska, Per E Schwarze, Sylwia Meczynska-Wielgosz, Maria Wojewodzka, and Marcin Kruszewski. 2012. 'The effect of agglomeration state of silver and titanium dioxide nanoparticles on cellular response of HepG2, A549 and THP-1 cells', *Toxicology letters*, **208**: 197-213.

Mie, Ropisah, Mohd Wahid Samsudin, Laily B Din, Azizan Ahmad, Nazlina Ibrahim, and Siti Noor Adnalizawati Adnan. 2014. 'Synthesis of silver nanoparticles with antibacterial activity using the lichen Parmotrema praesorediosum', *International Journal of Nanomedicine*, **9**: 121.

Moghaddam, K. 2010. 'An introduction to microbial metal nanoparticle preparation method', *Journal of Young Investigators*, 19.

Moradi, Fatemeh, Sajjad Sedaghat, Omid Moradi, and Samira Arab Salmanabadi. 2021. 'Review on green nano-biosynthesis of silver nanoparticles and their biological activities: With an emphasis on medicinal plants', *Inorganic and Nano-Metal Chemistry*, **51**: 133-42.

Morones, Jose Ruben, Jose Luis Elechiguerra, Alejandra Camacho, Katherine Holt, Juan B Kouri, Jose Tapia Ramírez, and Miguel Jose Yacaman. 2005. 'The bactericidal effect of silver nanoparticles', *Nanotechnology*, **16**: 2346.

Mosa, Kareem A, Ismail Saadoun, Kundan Kumar, Mohamed Helmy, and Om Parkash Dhankher. 2016. 'Potential biotechnological strategies for the cleanup of heavy metals and metalloids', *Frontiers in plant science*, **7**: 303.

Nalawade, Pradnya, Tulsi Mukherjee, and Sudhir Kapoor. 2012. 'High-yield synthesis of multispiked gold nanoparticles: Characterization and catalytic reactions', *Colloids and surfaces A: Physicochemical and engineering aspects*, **396**: 336-40.

Navazi, Zahra Ranjbar, Mohammad Pazouki, and Farah Sadat Halek. 2010. 'Investigation of culture conditions for biosynthesis of silver nanoparticles using Aspergillus fumigates', *Iran J Biotechnol*, **8**: 61.

Nazeruddin, GM, NR Prasad, SR Waghmare, KM Garadkar, and IS Mulla. 2014. 'Extracellular biosynthesis of silver nanoparticle using Azadirachta indica leaf extract and its anti-microbial activity', *Journal of Alloys and Compounds*, **583**: 272-77.

Poulose, Subin, Tapobrata Panda, Praseetha P Nair, and Thomas Theodore. 2014. 'Biosynthesis of silver nanoparticles', *Journal of nanoscience and nanotechnology*, **14**: 2038-49.

Sathyavathi, R, M Balamurali Krishna, S Venugopal Rao, R Saritha, and D Narayana Rao. 2010. 'Biosynthesis of silver nanoparticles using Coriandrum sativum leaf extract and their application in nonlinear optics', *Advanced science letters*, **3**: 138-43.

Saxena, Antariksh, RM Tripathi, and RP Singh. 2010. 'Biological synthesis of silver nanoparticles by using onion (Allium cepa) extract and their antibacterial activity', *Dig J Nanomater Bios*, **5**: 427-32.

Shon, Mi-Yae, Sang-Do Choi, Goon-Gjung Kahng, Sang-Hae Nam, and Nak-Ju Sung. 2004. 'Antimutagenic, antioxidant and free radical scavenging activity of ethyl acetate extracts from white, yellow and red onions', *Food and chemical toxicology*, **42**: 659-66.

Sondi, Ivan, and Branka Salopek-Sondi. 2004. 'Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria', *Journal of colloid and interface science*, **275**: 177-82.

Soni, Namita, and Soam Prakash. 2012. 'Efficacy of fungus mediated silver and gold nanoparticles against Aedes aegypti larvae', *Parasitology research*, **110**: 175-84.

Weng, Xiulan, Keran Yang, Gary Owens, and Zuliang Chen. 2022. 'Biosynthesis of silver nanoparticles using three different fruit extracts: Characterization, formation mechanism and estrogen removal', *Journal of Environmental Management*, **316**: 115224.

Zuas, Oman, Nuryatini Hamim, and Yulianti Sampora. 2014. 'Bio-synthesis of silver nanoparticles using water extract of Myrmecodia pendan (Sarang Semut plant)', *Materials Letters*, **123**: 156-59.