Green synthesis of Silver Nanoparticles from Red cabbage extract assisted Response Surface Methodology, physical characterization and its antibactericidal efficacy against urinary tract pathogen.

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Abstract:

Nanoparticles emerging materials that influence many application-oriented fields like medicine, biology, agriculture, environment, and space research. Synthesizing the silver nanoparticles (AgNPs) through a chemical approach is expensive and laborious. In the present study, a biological mode green synthesis of AgNPs from red cabbage extract and its physiochemical parameter validation through response surface methodology (RSM), Scanning Electron Microscopy (SEM), Fourier transform infrared spectroscopy (FT-IR), Transmission Electron Microscopy (TEM) and Dynamic light Scattering (DLS) was achieved. The red cabbage-mediated AgNPs showed a better RSM curve value of 2.7 with variables like pH and temperature-dependent manner which is more significant than the standard value. The SEM and TEM analysis revealed that the AgNPs are cylindrical with agglomerated morphology. The elemental composition analysis revealed the synthesized nanoparticle with predominant silver (Ag) followed by carbon and other organic matter. FTIR spectral results support the presence of silver and organic anthocyanin content from red cabbage present in the AgNPs. Particle analysis of AgNPs through DLS states that the average size of synthesized silver nanoparticles is good as per standard values. The antibacterial effect of AgNPs from red cabbage extracts against urinary tract infective (UTI) pathogenic *Staphylococcus saprophyticus* bacteria exerted significant antibactericidal effect at the concentrations of 0.5 µg/mL by damaging the cell membrane integrity after 12 hours and 24 hours treatment period which is observed through Field Emission Scanning Electron microscopy (FESM) analysis. As a plausible conclusion, the red cabbage-mediated AgNPs is a feasible mode of green synthesis and its application as an antibactericidal agent.

Keywords: Red cabbage, Silver nanoparticles, FTIR, SEM, TEM, DLS, *Staphylococcus saprophyticus.*

Introduction:

Nanotechnology is an emerging field that involves the synthesis of nanoparticles ranging from 1-100nm in size using different elements like Gold, silver, palladium other metallic substances. Due to silver nanoparticles' size, shape, high surface area to volume ratio, and optical, physical, and chemical properties, nanoparticles are put into a wide range of applications. Silver nanoparticles (AgNPs) are one such intriguing type due to their physical properties, feasibility, and biological uptake by living tissues in terms of drug delivery or carrier molecules. AgNPs are used in a wide range of applications such as biological product development, cancer drug delivery systems, combating cancer, antimicrobial activity, and water purification systems (Guilger-Casagrande et al., 2019). Although silver is toxic to mammals at a low concentration it has proven to be non-toxic to human cells (Zhang et al., 2016; Hamouda et al., 2019), hence it has numerous uses in in-vitro and in-vivo studies. Subsequently, research on the synthesis and characterization of AgNPs has increased extensively since high-yield nanoparticle synthesis and their size study have become a fundamental focus of demanding research, many chemical mediated methods AgNPs synthesis has been reported by many researchers, for example, spherical AgNPs synthesis (Abou El-Nour et al., 2010), and triangular AgNPs production (Demirbas et al., 2016). Fabrication of Ag-NPs is the process involves use of plant extracts/phyto-compounds (e.g.alkaloids, terpenoids, flavonoids, and phenolic compounds) to synthesize nanoparticles in more economical and feasible. Several findings concluded that in the field of medicine, Ag-NPs play a major role in pharmacotherapy (infection and cancer) (Mohanta et al., 2023).

Red cabbage has potent free radical scavenging effect and nutritional properties due to its promising rich anthocyanins, phenolic acid derivatives, flavonoids, vitamins, glucosinolates, and isothiocyanates (Kolonel et al., 2000; Veeranki et al., 2015). It is documented that the red cabbage has a prominent compounds such as 4-(methylsulfinyl) butyl ITC (4MSOB-ITC, sulforaphane) that has strong chemo preventive properties (Hanschen and Schreiner, 2017; Palliyaguru et al., 2018). Literatures have documented that there is wide range of polyphenols and anthocyanins in the red cabbage and the results showed that more than 30 anthocyanins were found in red cabbage, predominantly cyanidin-based anthocyanins in non-acylated or acylated by one or two acids from among cumaric acid, sinapic acid, and caffeic acid (Ahmadiani, Sigurdson, Robbins, Collins, & Giusti, 2019).

Urinary tract infections (UTI) is most common type of infections especially in elderly aged which causes 236,790 deaths worldwide annually (Ahmadi et al., 2022). UTI are very difficult to treat since UTI pathogens are multi drug resistant and the pathogenicity it cause is very severe (Yang et al., 2022). Among many pathogenic UTI bacteria's *Staphylococcus saprophyticus* is the more prevalent type of pathogens which accounts for major urinary tract complications (Tandogdu and Wagenlehner, 2016; Ozturk and Murt, 2020). Hence the search of new antimicrobials with less adverse side effects to the host physiology and effective toxicity to UTI pathogens is mandatory to treat UTI related complications. Antimicrobial capabilities of silver nanoparticles (Ag NPs) have been demonstrated against a wide spectrum of bacteria (Mehata, 2021; Quintero-Quiroz et al., 2019; Breig and Lutj, 2021). AgNPs are environmentally beneficial due to their applications in electronics, catalysis, medicines, and controlling microorganism proliferation in biological systems (Mehata, 2021; Quintero-Quiroz et al., 2019). Bacteria, fungi, yeast, actinomycetes, and plant extracts are all involved in the biogenic synthesis of AgNPs (Breig and Lutj, 2021). In addition to enzymes, a variety of plant parts such as flowers, leaves, and fruits have recently been employed in the creation of gold and silver nanoparticles (Sarabia and Ortiz, 2009).

There is plenty of literatures depicting the optimization strategies of AgNP and enhancing their maximum yield with greater stability. The present study was aimed at the biological production of silver nanoparticles from red cabbage extract and its antimicrobial potential against urinary tract infective (UTI) pathogenic bacteria *Staphylococcus saprophyticus* was validated. The physicochemical characterization of the synthesized AgNPs were optimized by using the statistical tool Response Surface Methodology (RSM) and physical characterization through field emission scanning electron microscopy (FESEM), Fourier transform infrared (FTIR) spectroscopy, particle size analyzer (DLS) were done.

Materials and Methods:

Chemicals and Reagents:

All the necessary chemicals and reagents were procured from Sigma Aldrich, and Hi-Media India. All the chemicals used in the present study are of Analytical grade (A.R) with at most 99% purity. The bacterial pathogen used for antimicrobial screening in the present study is of American type cell culture (ATCC) strain which were purchased from Hi-Media, Mumbai India.

Red cabbage of extraction and Biosynthesis silver nanoparticles (AgNPs):

Red cabbages were purchased from an organic market and chopped in fine pieces approximately (1-2 cm). Weighed (100 g) chopped red cabbage in 100 mL of distilled water was ground using a mixer grinder. Adding a few drops of ethanol the aqueous extract was filtered by vacuum filter and stored at a cool temperature for further analysis and experiments (Lee and Jum, 2019). Biosynthesis of Silver nanoparticles (AgNPs) was done following the methodology of (Brewer, 2011; Chandrasekhar et al., 2012; Shumi et al., 2023; Kanipandian et al., 2014), with slight modifications. Silver nitrate (AgNO3) (8 mL of 5 mM) aqueous solution was mixed with 2 mL of red cabbage extract (10 % w/v) for the synthesis of AgNPs under vigorous stirring for 40 min. The reaction was stopped by centrifugation at 6000 rpm for 20 min. The precipitates (AgNPs) were dispersed in water and centrifuged again. The centrifugation process called the "washing step" was repeated at least three times to completely remove excess extract and non-reacted Ag^+. The total time of extraction to synthesize of AgNPs spanned for 48 hours. The final yield of 28 mg of AgNPs obtained from synthesis were redispersed in water and stored at 4˚C for further use.

Characterization of AgNps:

The silver nanoparticles were characterized by different characterization techniques (Kanipandian et al., 2014; Abdellatif et al., 2023) through Field Emission Scanning Electron microscope (FESEM) (JEOL 6610 LV SEM), Transmission electron microscope (JEOL 1400 TEM), Fourier Transform Infrared Spectroscopy (FTIR, Shimadzu IR Tracer-100). X-ray diffraction analysis (XRD-500, Anton Paar), Particle size Analysis (PSA-1190, Anton Paar).

Optimization parameters of AgNPS and statistical validation:

The synthesized AgNPs were optimized using response surface methodology (RSM) based on its high yield and design of experiment (DOE) (Ibrahim et al., 2021). The DOE was assessed statistically on a (DESIGN EXPERT Software -8.0) based on physicochemical parameters like pH, temperature, and concentration of the red cabbage extract. A three-level face-centered central composite design (FCCCD) was suggested at 20 experimental runs and the response is estimated at 200-600 nm wavelengths and the curve was plotted.

Antibacterial screening of AgNPS :

The red cabbage extract mediated AgNPS were subjected for antibacterial efficacy against selective urinary tract pathogen (UTI) of *Staphylococcus saprophyticus* ATCC 19701. Broth dilution method (Parvekar et al., 2020) was employed to assess the antibacterial effect of synthesized AgNPs and the minimum inhibitory concentration (MIC) was validated using 96 well microtitre plate. Red cabbage AgNPs was made into serial dilution up to two fold from the concentration series of 5 µg/mL to 0.1254 µg/mL and 10µL of the AgNPs suspension was added on to the bacterial inoculum (10^9 CFU/mL using McFarlands standard) in a brain heart infusion (BHI) broth medium. The negative control served as only the BHI broth without AgNPs. The bacterial inoculum was incubated for 24 hours at 37^{o}C and absorbance was recorded at 370 nm using ELISA reader (Agilent Technologies, USA). The lowest concentration of AgNPs where the bacterial growth inhibited was recorded as the minimal inhibitory concentration (MIC).

FESM analysis of microbial toxicity of AgNPs:

S. saprophyticus bacterial cells treated with AgNPs and the normal control cells were subjected for FESM (FESEM) (JEOL 6610 LV SEM) analysis for assessing the bacterial toxicity and cell membrane changes (Haddad et al., 2022). The cell membrane integrity between the control and AgNPs treated cells were scrutinized based on FESM observations.

Statistical analysis:

Statistical analysis was done using SPSS (version.19) software package. One way- ANOVA was performed to defer the level of significance along with and Tukey's post hoc test. p value < 0.05 was considered the high level of significance.

Results and Discussion:

AgNPs production and optimization through response surface methodology (RSM)

The study of the silver nanoparticles (AgNPs) synthesis based-green methods become more interesting recently due to their low-cost preparation, eco-friendly and non-toxic precursors (Sharifi-Rad et al., 2024). In the present study the most notable interaction between critical factors for high-yield AgNPs manufacturing was identified using a central composite design under Response Surface Methodology (RSM) and a system model containing their feasible interaction was developed in a minimum number of experimental runs (Carvalho et al., 2018). RSM is the result of combining several different techniques mathematical and statistical strategies for approximating and optimizing a system based on numerous variables (Kharissova et al., 2013). Responses and various types of experimental runs Table 1 illustrate the FCCCD of three factors, as well as their values. The reaction of AgNPs is given as (Area*) in response. The highest reaction was obtained at the center point (run 19), with a score of 2.7. At run 5, the lowest response of 1.4 was discovered.

To acquire all conceivable combinations of input variables that can optimize the response inside the 3-D space region, a three-level factorial design was used. The quadratic model was determined to be significant by analysis of variance (ANOVA)at a p-value less than 0.05. As a result, some of the numbers were not significant. The response surface approach was used to reduce the size of the model (RSM). Table 2 summarizes the results. The constructed model's appropriateness was demonstrated by Fisher's statistical analysis. The high yield of AgNPs synthesis was shown to be dependent on the concentrationof Red cabbage concentration, pH, and temperature during the reaction using a second-order regression equation. A second-order polynomial equation in terms of coded factors was used to establish the relationship between the selected parameters and each response:

Sqrt (AgNPs yield) = -9329+2.0579*A +0.16*B +1.69*C -0.005*A*B-0.043A*C -0.003

B*C -0.29 A2… .... Equation 1

The statistical Equation 1, where 'A' is Red cabbage concentration, 'B' is Temperature, and 'C' is pH, demonstrates that positive values have a synergistic influence on the reaction (AgNPs yield) and negative values have an antagonistic effect on the response (AgNPs yield). The coefficient of one element supplied the solution in this equation. The efficacy of this particular component to examine the generation of AgNPs using the coefficient values from the equation, it is evident that the silver nitrate concentration has a greater positive influence than the other variables. Values of "Prob > F" less than 0.05 indicated that the model terms were significant, with a lower probability value indicating more significance for the regression model, and a p-value of 0.0005 in this model.

The F-test value shows how themodel's mean square compares to the residuals' mean square. The model F-test score of 47.71 indicates that the model is significant, implying that the model has a 0.01 percent chance of being correct. Noise is expected to affect the F-value. A (Red cabbage concentration) and C (Temperature) are linear terms in this model. One of the interactions between AC and quadratic term C2 model terms was statistically significant. The coefficient of determination (R2) was employed to test the model's fitness. An R2 value near 1 indicates that the experimental and expected responses are moreclosely related. As a result, having a solid model R2 is crucial. The model's fit is measured on a scale of 0-1, with the closer it gets to 1, the better (Abdel-Aziz et al., 2014). In this scenario, the modified determination coefficient is in reasonable agreement with the correlation coefficient (R2) value of 0.9772. The (R2Adj) score of 0.9567 indicates that the model is highly significant. The signal is measured with sufficient precision. For the proposed model, a noise ratio of less than 4 is considered adequate. An algorithm has been created in the developed model 19.564 is an adequate accuracy value for AgNPs yield indicating the model can be used to navigate the design space.

A 3D surface response plot is a graphical representation of the regression equation produced from the constructed model, which is used to investigate parameter interactions and determine the best condition of each parameter for maximum AgNPs yield production. In addition, the plot is based on the function of two variables while the third variable is at its best; the fourth variable is at its worst. When a contour plot is used to specify the level of interaction relevance, an elliptical or saddle plot is produced (Pandey and Rizvi, 2009). The 3D plot of AgNPs is shown in (Figures 1, 2, and 3) using the interactions of all three variables of temperature, pH, and red cabbage through which the results are generated. The influence of $AgNO_3$ concentration on AgNPs yield was optimized, and the effect of stirring duration on yield production was nearly constant. The surface map shows that the best condition for high-yield AgNPs is determined by red cabbage, temperature, and pH, and this was further illustrated in the perturbation plot.

Table 1. The FCCD optimization of three variables with AgNPs yields the response

		Factor 1	Factor 2	Factor 3	Response 1
Std	Run	A: Red cabbage	B. Temperature	C: pH	R1
12	1	2.7	71.93137	6	1.2
7	2	1.4	60	8	1.3
2	3	4	25	4	0.8
20	4	2.7	42.5	6	2.1
1	5	1.4	25	4	0.5
5	6	1.4	25	8	1.1
14	7	2.7	42.5	9.363586	1.3
6	8	4	25	8	1.2
19	9	2.7	42.5	6	2.4
8	10	4	60	8	0.7
10	11	4.886331	42.5	6	0.9
9	12	0.513669	42.5	6	0.8
17	13	2.7	42.5	6	2.3
3	14	1.4	60	4	0.8

11	15	2.7	13.06863	6	0.7
13	16	2.7	42.5	2.636414	0.6
16	17	2.7	42.5	6	2.2
18	18	2.7	42.5	6	2.4
15	19	2.7	42.5	6	2.4
4	20	4	60	4	0.9

Table 2. One-way ANOVA analysis on FCCCD quadratic model.

Source	Sum of Squares	df	Mean Square	F-value	p-value Prob>F
Model	8.83621	9	0.981801	47.70878*	<0.0001
A-Red cabbage	0.00034	1	0.00034	0.01654	0.9002
B-Temperature	0.064824	1	0.064824	3.14998	0.1063
C-pH	0.449356	1	0.449356	21.83562	0.0009
AB	0.10125	1	0.10125	4.920054	0.0509
AC	0.10125	1	0.10125	4.920054	0.0509
BC	0.06125	1	0.06125	2.976329	0.1152
A^2	3.542892	1	3.542892	172.1602*	<0.0001
B^2	3.055646	1	3.055646	148.4834*	<0.0001
C^2	3.055646	1	3.055646	148.4834*	<0.0001
Residual	0.20579	10	0.020579		
Lack of Fit	0.12579	5	0.025158	1.57238	0.3158
Pure Error	0.08	5	0.016		
Cor Total	9.042	19			
R-Squared	0.977241				
Adj R-Squared	0.956757				
Pred R-Squared	0.87626				
Adeq Precision	19.56423				
C.V%	10.78601				

* Indicates the level of significance at p <0.0001

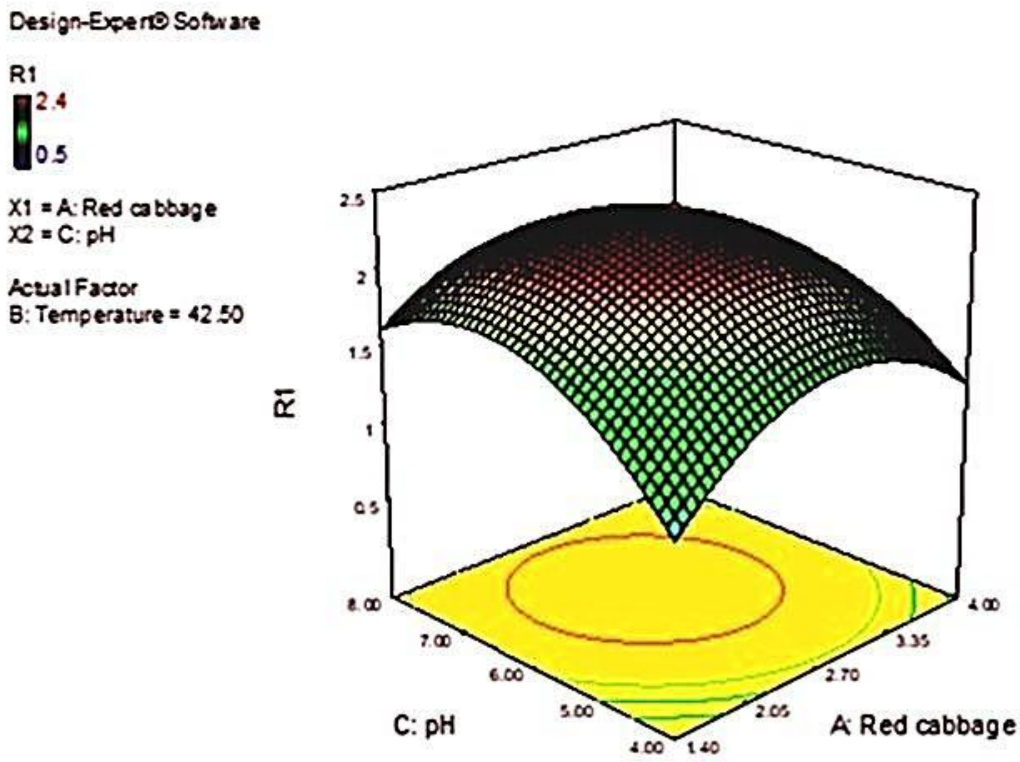


Figure 1: 3D interaction plot and RSM analysis of AgNPs yield, the interaction with Red cabbage concentration, and pH.

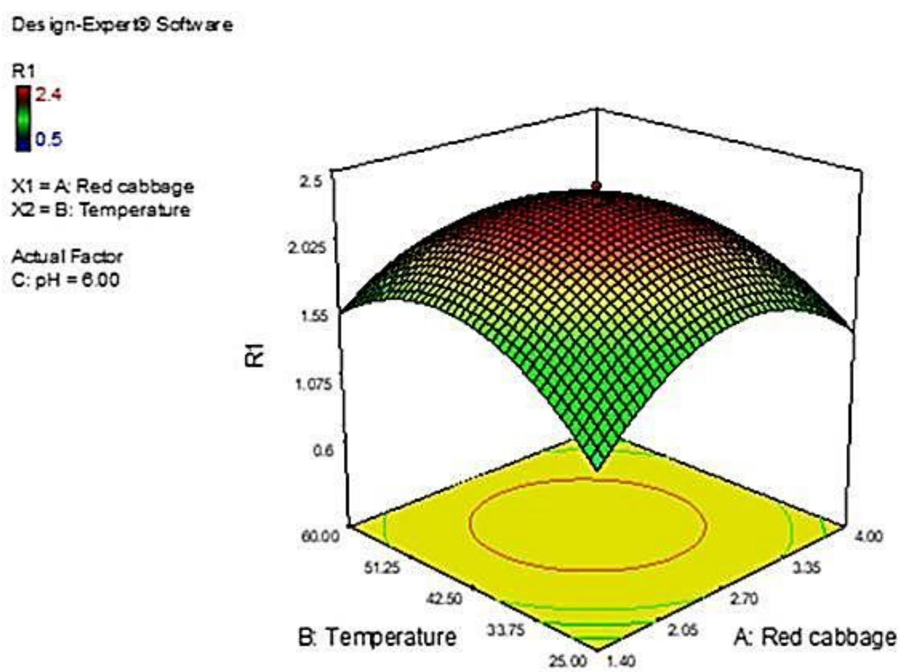


Figure 2: 3D interaction plot and RSM analysis of AgNPs yield, the interaction with Red cabbageconcentration and temperature.

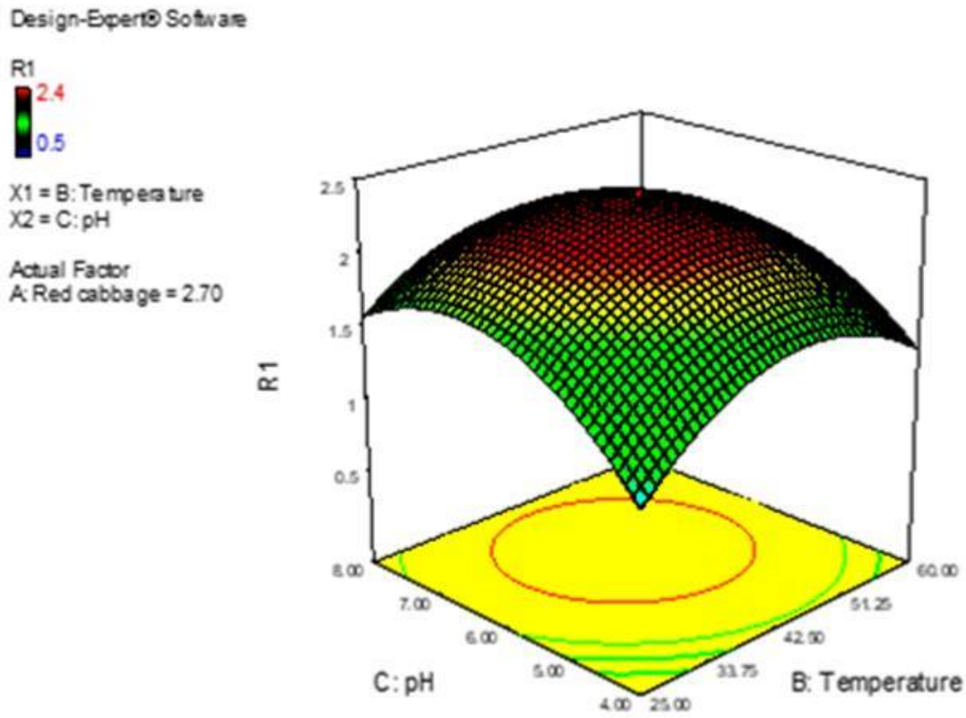


Figure 3 : 3D interaction plot and RSM analysis of AgNPs yield, the interaction with Red cabbageconcentration of pH andTemperature.

Additionally, the perturbation plot in Figure 4 (which came up by default using thedesign expert program and perturbation theory employing mathematical methods for finding an optimum condition to solve the problem) clearly shows interactions between the variables. In Figures 5 and 6, the probability plot between the residual and predicted is represented by the interaction of line which is the reference point where X = 0.00, and the side point's actual circumstances TSC concentration (A) and stirring time (B) have a linear relationship relation. The optimum manufacturing condition for AgNPs for a certain application was determined using statistical and perturbation plot analysis. The generated model has a concentration of A = 3.14 mL, a temperature of 48.465 Celsius, and a pH of 5.995 with 47.71 percent of 5-50 nm AgNPs production yield The concentration of $AgNO_3$ is the most prominentfrom equation 1.

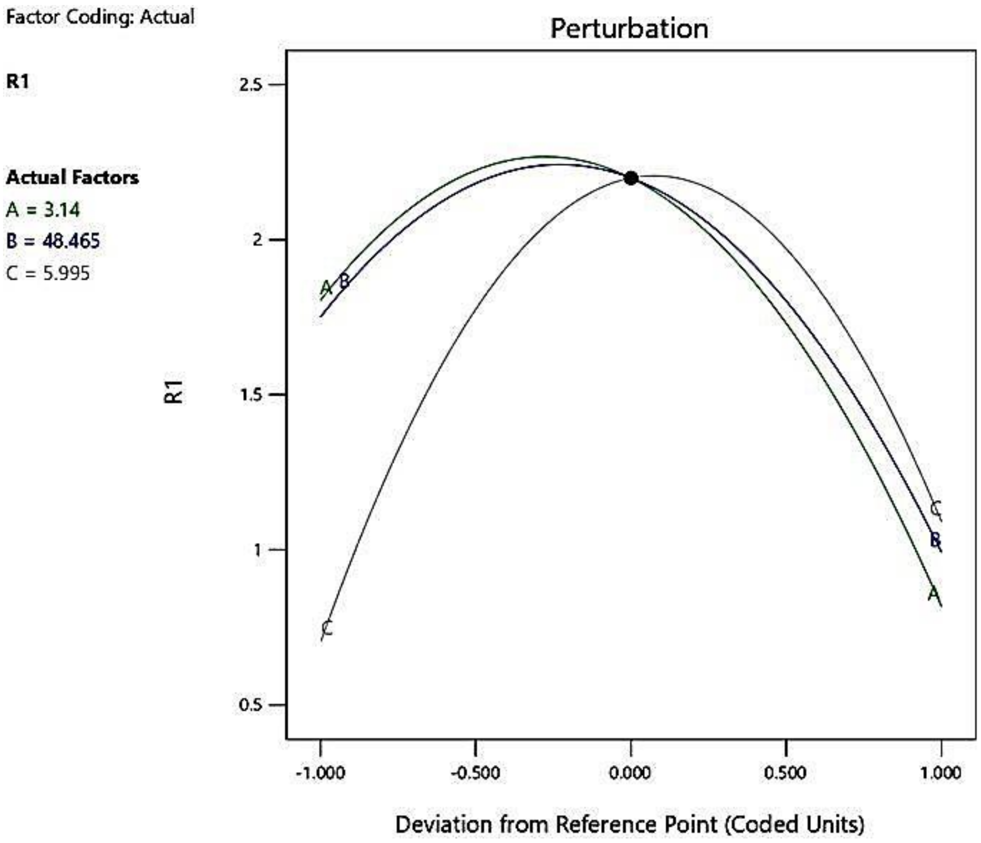


Figure 4: Perturbation plot of AgNPs yield and its optimization.

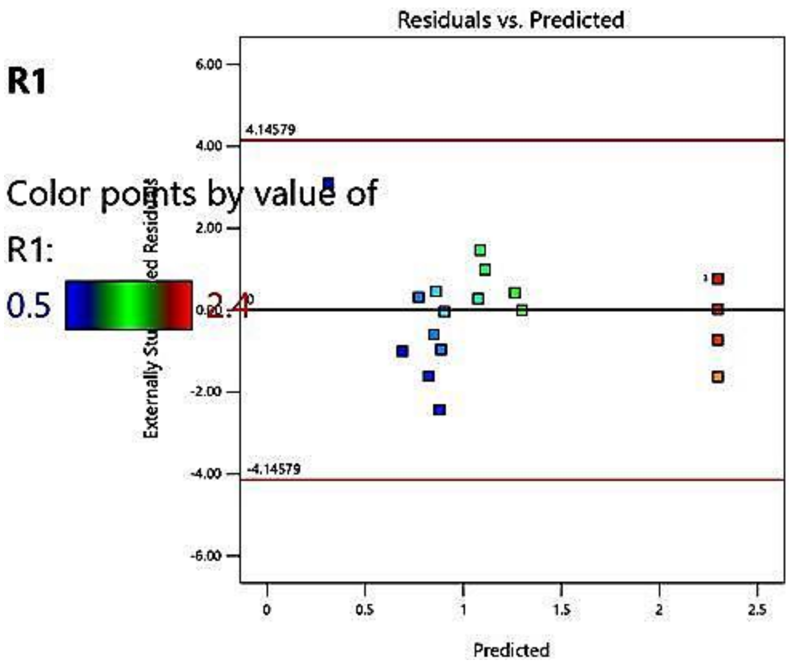


Figure 5: Normal probability plot of AgNPs residuals vs predicted.

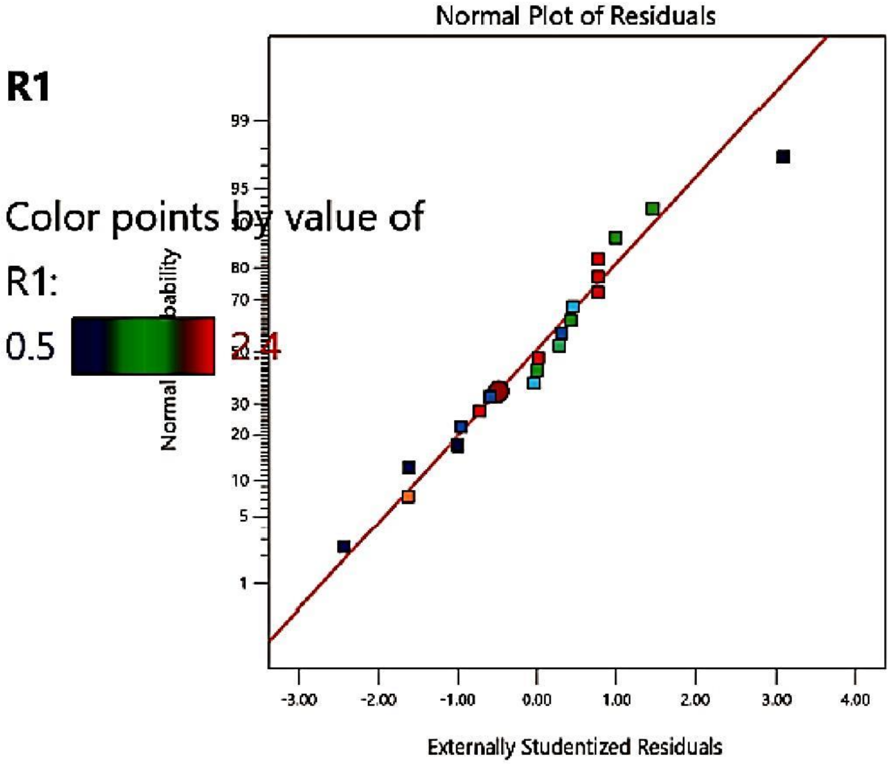


Figure 6: Normal plot of AgNPs residuals.

Characterization of silver nanoparticles

UV-visible spectroscopy is a method for measuring how much light is absorbed and scattered by a sample (the extinction, which is defined as the total of absorbed and scattered light). The wavelength-dependent extinction spectrum of the sample was quantified by comparing these data at each wavelength (Rautela et al., 2019; Carvalho et al., 2019). In the present study, the AgNP synthesized from red cabbage extract showed a broad band with maximum absorption at 420 nm nanometers wavelength when compared to the normal silver nanoparticle with a narrow band peak at 404 nm (Figure 7). This indicates that the strong absorption band recorded at 430 nm is due to the interaction of $AgNO_3$ with red cabbage extract particles like anthocyanin pigments (Baudot et al., 2010). The absorption spectra of cylindrical silver nanoparticles were reported to have a high peak at 420 nm wavelength and dark brown as seen in Figure 7.

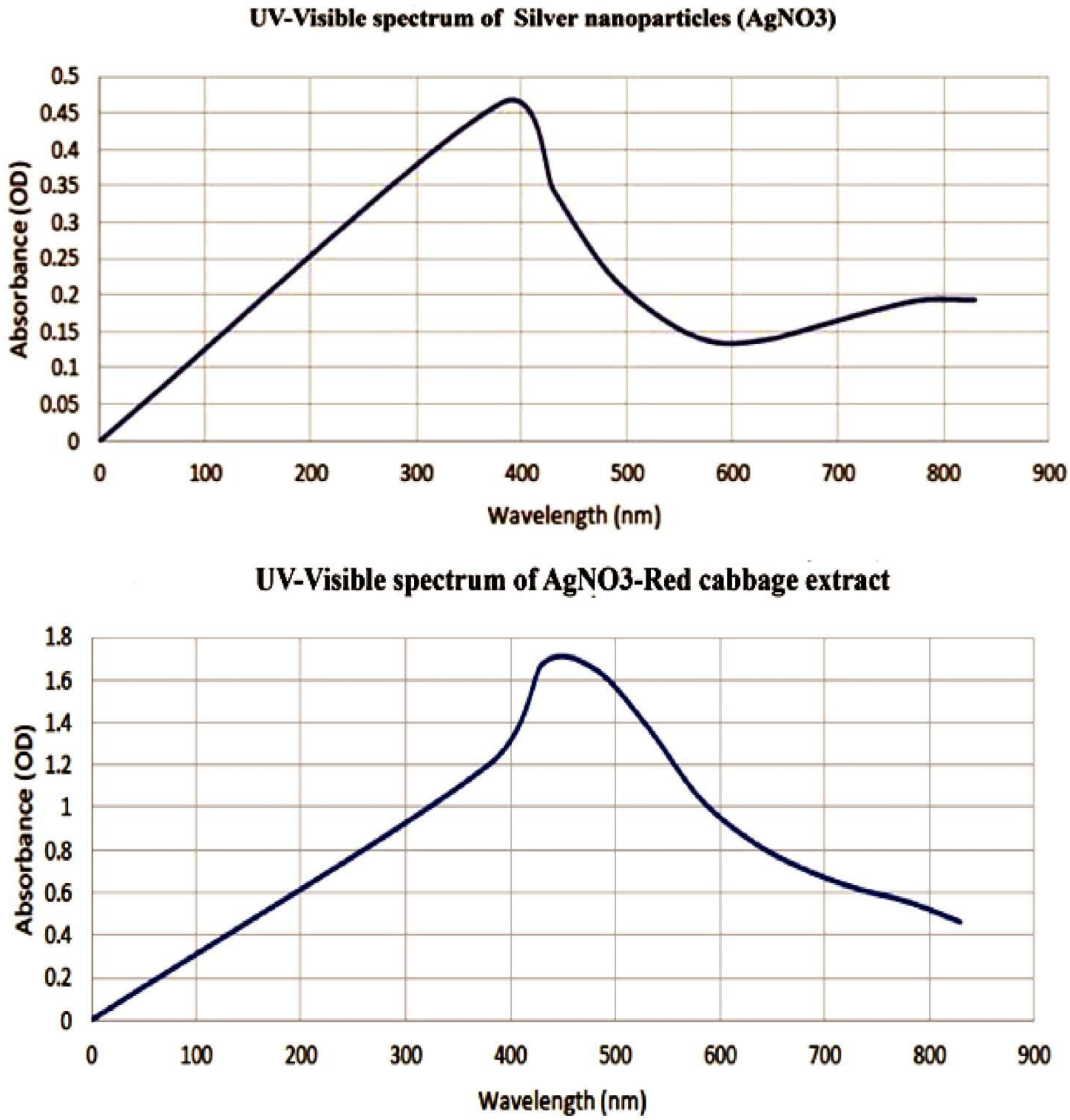


Figure 7: UV-Visible spectrum of $AgNO_3$ and UV-visible spectrum of $AgNO_3$-red cabbage extract.

Scanning electron microscopy (SEM), TEM and EDX:

SEM is a surface imaging technology that can resolve various particle sizes, size distributions, nanomaterial forms, and surface morphology of manufactured particlesat the micro and nanoscales (Taha et al., 2019). By manually measuring and counting the particles or using appropriate software, we can explore the morphology of particles using SEM and obtain a histogram from the images. In the present study, the red cabbage extract coupled silver nanoparticles are subjected to SEM and EDX analysis. The morphological features of the synthesized AgNPs seem to be cylindrical rod-shaped like cocci bacteria clumped together at 100μm thickness resolution (Figure 8). The elemental composition analysis of the AgNPs through EDX dispersive analysis revealed that the AgNPs nanoparticle consists of predominantly Carbon elements at 48.4% followed by Silver (Ag) at 25.2%, Oxygen (O) at 16.4% and Sulfur (S) and Copper (Cu) as 1% and 1.0% (Figure 9). A TEM image confirms the homogeneous AgNPs from red cabbage extract (Figure 10). The particle size average is AgNPs were found to have a diameter 5–500nm size range, with only a few particles agglomerated as spherical.

Figure 8: SEM characterization of silver nanoparticles.

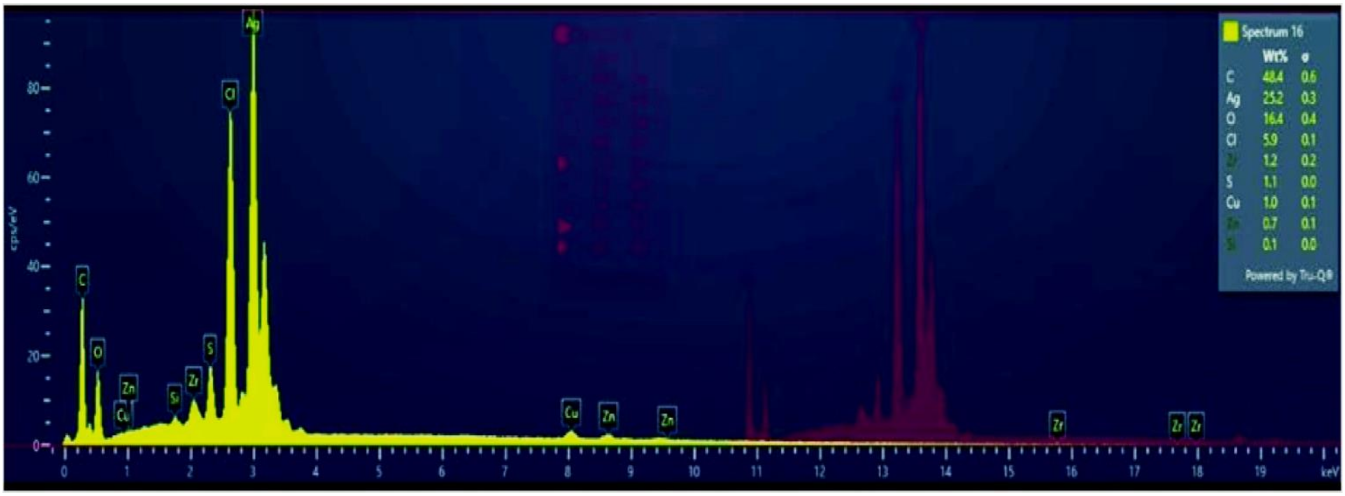
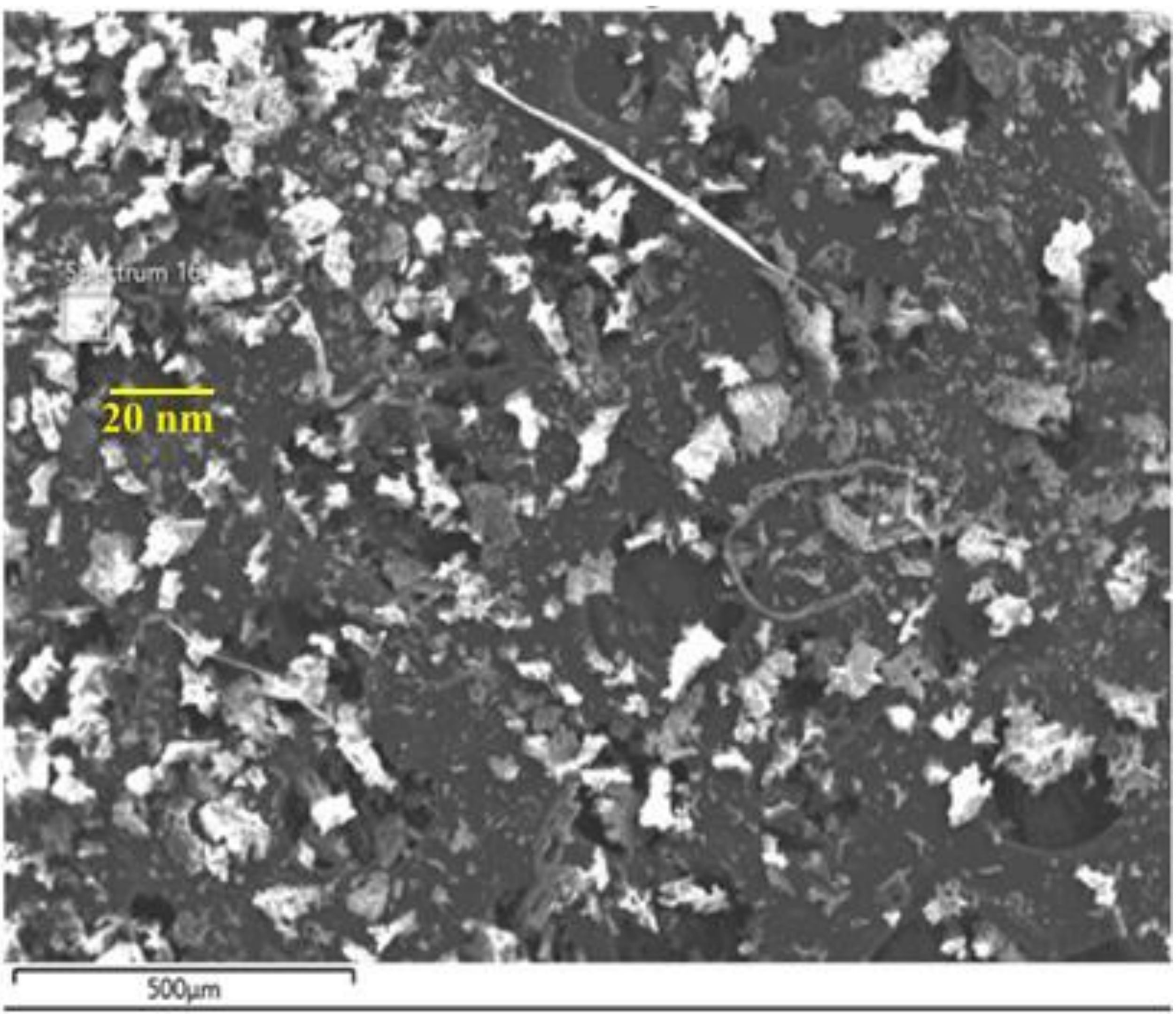


Figure 9: EDX composition analysis of AgNPs



Cues

Figure 10: TEM characterization of silver nanoparticles

FTIR –analysis:

FTIR analysis of AgNPs from red cabbage extract revealed the presence of carbonyl group (COO-) in the range of 2942.7 cm^{-1} and 3244.6 cm^{-1} alongside $-NH_2$ and COOH in the spectral range of 1638.2 cm^{-1} and 1561.8 cm^{-1}. This indicates the presence of anthocyanin a flavanoid pigment a predominant source of red cabbage extract that gives the red coloration (Huang et al., 2003). In addition to this, the FTIR spectral analysis indicates the presence of –CH=CH ethylene stretch at 1407.1 cm^{-1} (Figure 11). The FTIR spectra showed strong absorption peaks at 1239.3 cm^{-1}, 1030.6 cm^{-1}, 898.3 cm^{-1},780 cm^{-1} which assigns to C-N, C-H, (NH) C=O, and CH_2 groups from the silver nanoparticles (Sagadevan and Koteeswari, 2015).

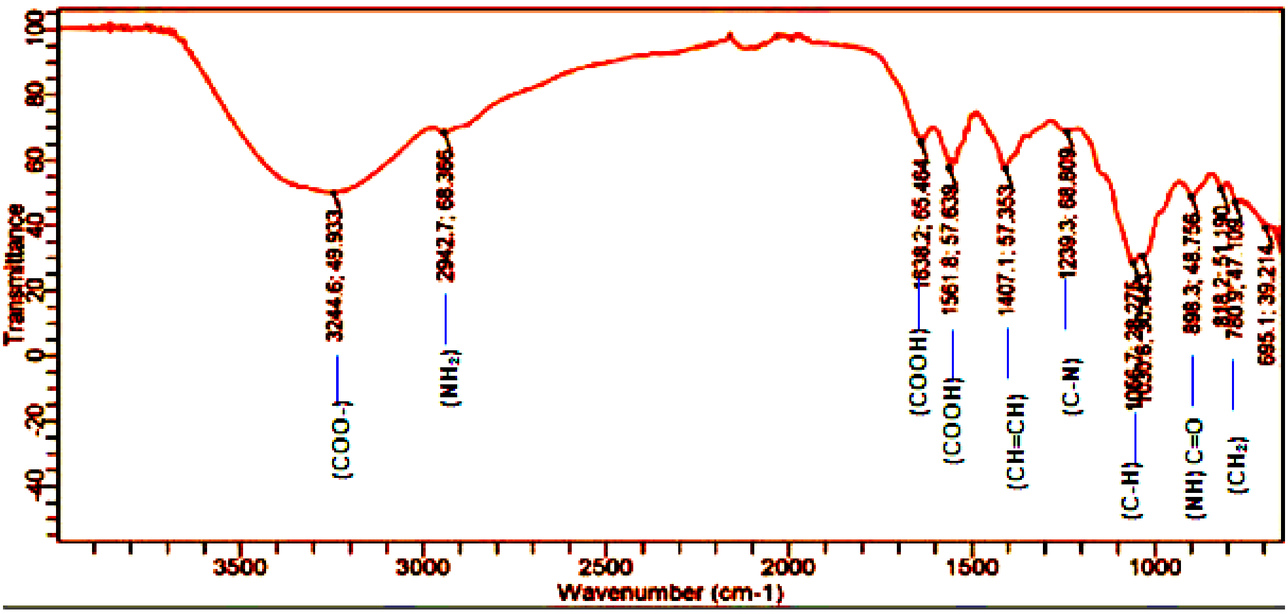


Figure 11: FTIR spectral analysis of Red cabbage-AgNPs

Particle size analysis:

The physicochemical characterization of produced Nanomaterials is critical for employing radiation scattering techniques to analyze biological activities (Taha et al., 2019; Huang et al., 2003; Sagadevan and Koteeswari, 2015; Jemal et al., 2017). Dynamic light scattering (DLS) can measure the size distribution of tiny particles in solution or suspension on a scale ranging from submicron to one nanometer (Feng et al., 2023). In the present study, the red cabbage synthesized silver nanoparticles were analyzed and the size distribution of the AgNPs measures from 90-100 nm. There is a strong peak at 100 nm (Figure 12A) that denotes that the Z-average size of the silver nanoparticle from red cabbage extract is strong in dynamic properties (Siddiqi et al., 2018). The method of dynamic light scattering is based on the interaction of light with particles. The light scattering effect of AgNPs reveals the scattering effect at 1000 µ seconds (Figure 12B) states the intensity of AgNPs in light scattering and its average nanoparticle size is good.

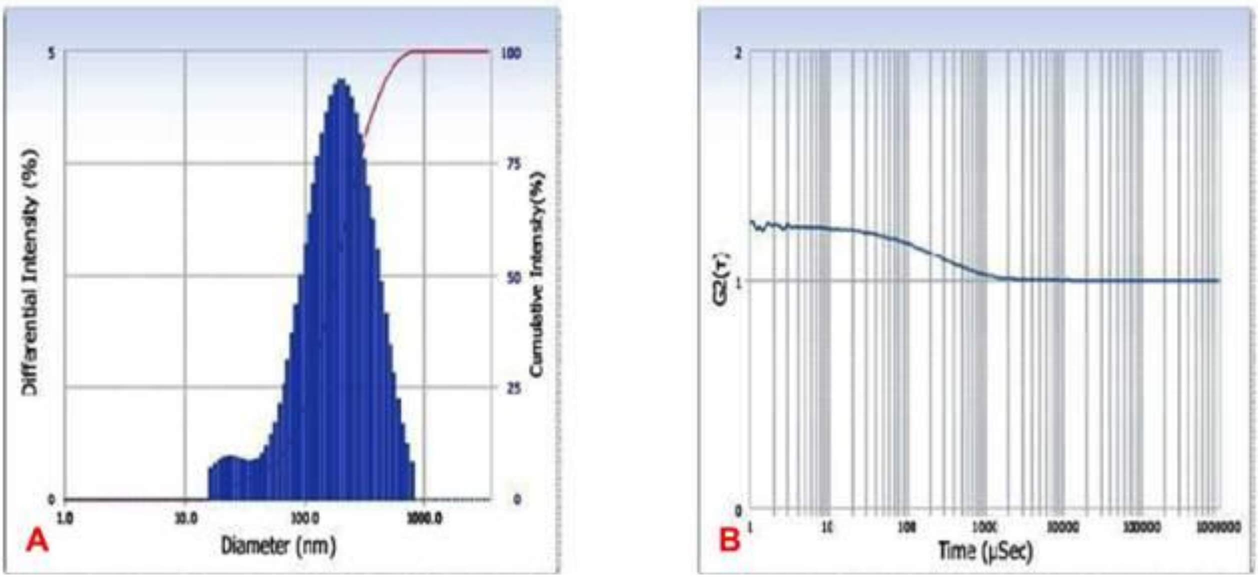


Figure 12A: Particle size DLS analysis of silver nanoparticles: **B**. Light Scattering effect of AgNP

Minimum Inhibitory concentration of Red cabbage- AgNPs and its antibacterial effiacy:

Biogenic silver nanoparticles from biological source have immense antibacterial properties. A biogenic AgNPs synthesized from *Morinda citrifolia* exerted cytotoxic effect against *S.saprophyticus* bacteria at the MIC range of 0.4µg/mL (Rajivgandhi et al., 2020). In the present study the MIC of AgNPs synthesized from red cabbage extract treated bacterial strain *Staphylococcus saprophyticus* ATCC 19701 was assessed after 12 and 24 hours of AgNPS treatment. The results revealed that there is no bacterial growth was observed from the concentrations starting from 5.0 µg/mL (Table 3). A confirmatory test of spread plating the inoculum added as 5.0 µg/mL, of AgNPs on BHI agar plate inferred no bacterial growth in the agar plates and hence it confirms that the red cabbage AgNPs was bactericidal against the tested UTI bacterial strain *Staphylococcus saprophyticus.*

Table 3: MIC effect of Red cabbage AgNps against *S. saprophyticus*

AgNps concentrations	12 hours (OD value)	24 hours (OD value)
0.1 µg/mL	86.41±0.4	39.87±0.12
0.5 µg/mL*	32.17±0.1	12.39±0.67
1.0 µg/mL	19.22±0.5	2.04±0.11
3.0 µg/mL	8.01±0.25	0.84±0.1.9
5.0 µg/mL	2.36±0.41	0.021±0.11

***MIC - µg/mL**

FESM analysis of AgNPS bacterial toxicity

The morphological changes occurred in *Staphylococcus saprophyticus* ATCC 19701 after the treatment with red cabbge derived AgNPs was observed through FESEM. The results revealed that the control bacterial cells have normal cell membrane and its shape remains unique in the culture whereas AgNPs treated bacterial cells exhibited shrinkage of cell membrane as well rupture in terms of cytotoxic at 12 hours treatment at 5.0 µg/mL (Figure 13A-B). The effect of cell membrane rupture increases over the point of time treatment with AgNPs and maximum bacterial cell damage was observed during 24 hours of treatment with AgNPs (Figure 13C). The results revealed that red cabbage mediated AgNPs are highly cytotoxic to *S. saprophyticus* bacterial cells. A similar experimental findings was documented with *Syzigium cumini* mediated copper nanoparticles treatment with *S. saprophyticus* and other UTI bacterias like *Proteus mirablis*, *Streptococcus pyogenes* and *Pesudomonas aeruginosa* exerted massive cell membrane damage and cytotoxicity (Chauhan et al., 2018). The phytofabricated AgNPs of *Otostegia persica* leaf extract also exhibited significant antibacterial activity against both Gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*, and *Streptococcus pyogenes*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella typhi*) bacteria (Sharifi-Rad et al., 2021).

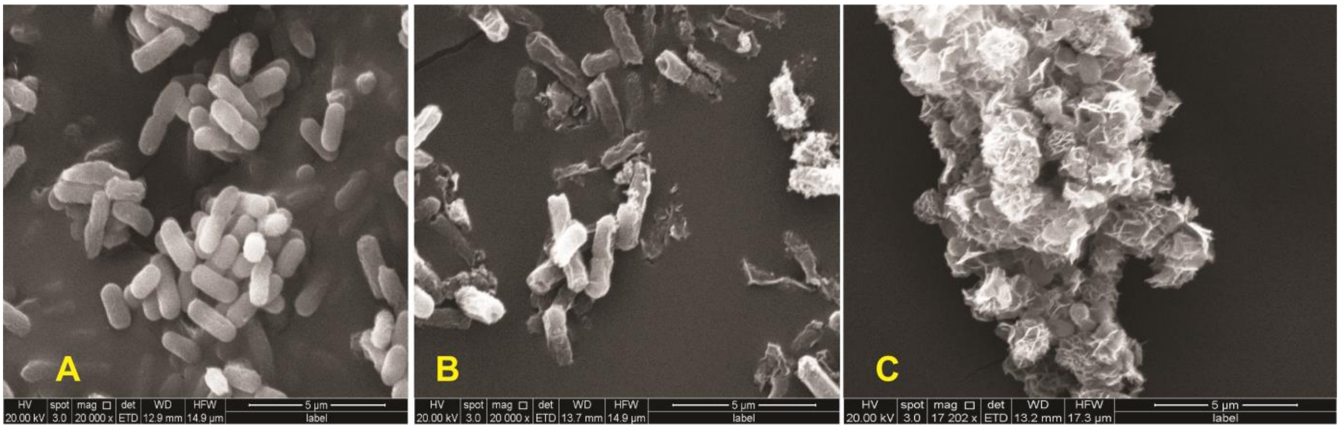


Figure 13 A: Control group of *S. saprophyticus* without AgNPs treatment. **B:** 12 hours treatment of *S. saprophyticus* with AgNPs. **C:** 24 hours treatment of *S. saprophyticus* with AgNPs at 0.5µg/mL.

Conclusion

In the present a green synthesize approach of AgNPs from red cabbage extract was physico-chemically characterized by FE-SEM, FTIR, UV and biological validation through antibacterial effect. The physico-chemical properties of Red cabbage mediated AgNPs reveals that it is stable enough to go through further lab and industrial scale production. The antibactericidal effect of AgNPs against the tested UTI pathogenic bacteria *S. saprophyticus* proven to be effective in terms of bacterial cytotoxic and brings considerable bacterial cell wall and structural damage. The biological approach to silver nanoparticle synthesis appears to be cost-effective and it's the best alternative to traditional physical and chemical approaches which would be suited for establishing a biological process for large-scale manufacturing. Further molecular level insight testing of this AgNPS in bacterial cell and in-vivo experiments will lead to candidate drug development in Nano drug delivery. These silver nanoparticles which are of biologically synthesized can be utilized in effectively for the treatment to reduce UTI disease risk and its microbial burden, as well as its futuristic biomedical applications.

Data availability statement: The raw datas associated with the present study is with corresponding author and made available based on request.

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Author's contribution: Conceptualization, data collection and investigation: writing – original draft **Kumaravel Kaliaperumal, Kumaran Subramanian and Abinaya Gayathri**.

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