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SYNTHESIS OF SILVER NANOPARTICLES FROM ALKANNA TINCTORIA AND THEIR FUNCTIONAL ACTIVITIES

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Abstract

Since prehistoric times, mankind have used medicinal plants to alleviate illnesses and relieve physical suffering. People are becoming increasingly interested in science, particularly biotechnology and biomedical procedures, because of nanotechnology. The term "nanoparticles" (NPs) describes nuclear or molecular structures that have at least one spatial dimension up to 100 nanometers. The manufacture of nanoparticles using plant extracts has increased in relevance because of its simplicity, affordability, environmental sustainability, and particle size control. The aim of this study is to investigate, within the overall framework of natural product research, the antiviral and antibacterial properties of plant extracts obtained from natural sources. Fruit (R. canina), leaves (S. pallidum V bithynicum), and roots (A. tinctoria). Additionally, the novel production of nanoparticles using the root extract of A. tinctoria is included in this study. Identifying any potential bioactive substances in these plant extracts and investigating their prospective uses in the fields of biotechnology and medicine are the main objectives of the study. In the present study, ethanol was utilized as a solvent to extract the bioactive components from the relevant plant materials. The standard diffusion and broth microdilution testing techniques were used to evaluate the extracts' antibacterial activity against various bacterial strains. Using the DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging method, the sample's antioxidant capacity was evaluated. With variable levels of effectiveness against various microbial strains, the results demonstrated that the extracts from A. tinctoria root, S. pallidum, V. bithynicum leaves, and R. canina fruit had strong antibacterial activity. Additionally, the extracts demonstrated significant antioxidant capability, underscoring their ability to counteract oxidative stress and free radicals. The study examined the potential use of A. tinctoria root juice in the production of nanoparticles. The bioactive chemicals in the plant extract demonstrated characteristics that made it possible to use them as a stabilizing and reducing agent during the synthesis of nanoparticles, therefore supporting manufacturing procedures that are ecologically friendly. Scanning electron microscopy (SEM) and UV-V is spectroscopy were used to evaluate the nanoparticles in order to ascertain their structural characteristics and to peer into the method of production.

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INTRODUCTION

A plant is referred to as a "medicinal plant" if it has chemical components that can effectively cure infectious illnesses in both people and wildlife. Since prehistoric times, people have used medicinal plants to alleviate ailments and ease physical suffering. It is known that several of the compounds that plants generate are toxic to microbes. The bioactive components of both native and farmed botanical species have drawn more attention from researchers in recent years in an effort to examine their potential uses in the domains of nutrition and medicine (Redzic, 2010). People have used medicinal plants for centuries to treat illnesses and ease physical discomfort. The natural world has been a major source of medicinal qualities throughout human history, and a sizable amount of modern medical therapies are derived from organic materials, particularly botanical specimens (Ma *et al.,* 2010).

Individuals are getting becoming more interested in science, particularly biotechnology and biomedical procedures, thanks to nanotechnology. Everyone had access to food during the first green revolution, but agricultural progress has since stalled. We need the Second Green Revolution to meet the rapidly increasing demand for food from the burgeoning population. The objective of nanoscale science and nanomaterials is to fundamentally alter agricultural and food systems (Norman and Hongda, 2013). Since nanoparticles are tiny and have a large surface area, research on them is quite active. This implies that they have the ability to undergo physical and chemical changes that bulk materials cannot. The term "nanoparticles" (NPs) describes nuclear or molecular structures that have at least one spatial dimension up to 100 nanometers. Health and medicine have made substantial use of nano-biotechnology (*Singh et al.,* 2011).

Pseudomonas stutzeri AG259, the first bacterial strain used to produce Ag-NPs, was extracted from the silver mine. Ag+ ions are absorbed by fungal cells, which then use their enzymes to reduce Ag+. The primary advantages of plant-mediated Ag-NP synthesis over microbial synthesis are its availability, affordability, speed, safety in a variety of settings, and variety of metabolites involved in reduction. Phytochemicals are responsible for the reduction of silver ions in plant-mediated synthesis (Hasan, 2015). Flavones, terpenoids, ketone bodies, amides, carboxylic acid, and aldehydes are the main phytochemicals. Hydrophilic chemical compounds such as flavones, amines, and acidic substances quickly reduce silver ions, which helps to both immediately reduce and create Ag-NPs (Singh *et al.,* 2016). Because of its simplicity, affordability, environmental sustainability, and capacity to regulate particle size, the use of plant extracts for nanoparticle production has grown in significance. These friendly plants can produce stable nanoparticles, offer longer storage times, and stop aggregation (Ghoreishi *et al.,* 2011).

An alternate method of synthesizing nanoparticles is using plant extracts (Rao *et al.,* 2016). Numerous studies have been conducted on the biosynthesis of nanoparticles in plants. The *Enteromorphaflexuosa* (Wulfen) *J. Agardh* extracts (Yousefzadi *et al.,* 2014), *Piper nigrum* Wall (Ghoreishi *et al.,* 2011), *PalergoniumgraveolensL'Her* (Rao *et al.,* 2016), *OnosmadichroanthaBoiss* (Sankar et al., 2014), *Bacillus subtilis* (Khodadadi *et al.,* 2017), *Arbutus Unedo* (Kouvaris *et al.,* 2012). Since their invention, nanoparticles have been used to great advantage in a wide range of scientific domains. This section provides a thorough summary of the latest developments in the application of silver nanoparticles. Industrialization's advancement has always been intimately associated with the control and eradication of several dangerous compounds that pose a risk to human health and ecological systems. Many challenges arise from the natural breakdown of contaminants (Adeyemi *et al.,* 2022). Various industrial dyestuffs and synthetic textile dyes make up the bulk of chemicals produced worldwide. Water and the ecosystem become contaminated when dye wastewater is released into rivers, causing pollution. Aquatic life and the different biological processes that occur in river ecosystems are negatively impacted by contaminants. Thus, it is essential to remove dyes from industrial effluents to safeguard the environment and advance public health (Zulfiqar *et al.,* 2024).

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Materials and method

Plant Collection and Identification

The research was conducted at Abbottabad University of Science and Technology. The experimental plant material was identified using Google Lens, a plant identification app called Plant Net, and by comparing it with field guidelines. The initial step involved collecting plant samples from the mountainous region of Nathia Gali, specifically from Village Nagribala, Bagla Mohallah, in the Abbottabad district, after proper identification. The plants identified in this study were *A. tinctoria* (L.), *S. Pallidum V. bithynicum* and *R. canina*. The plants were chosen according to their local availability.

Pallidum V. bithynicum

Cacana fruit

A. tinctoria root

A. tinctoria plant

Preparation of Ethanolic Extracts of leaves, Fruits and Roots

Following a thorough examination for illness or damage, the plants were cleaned with tap water and then distilled water to get rid of any remaining dirt. The plants were shaded for two weeks to provide for the best possible drying conditions. After being dried, the plants' roots, fruit, and leaves were ground into a fine powder (Adeyemi *et al.,* 2022).

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Fine powder of *A. tinctoria*

Fine powder of *R. canana*

Preparation of Ethanolic Extracts

Twenty grams of *A. tinctoria* roots, five grams of *Sedum Pallidum V. bithynicum* leaves, and three grams of R. canina fruit were weighed, and each sample was soaked in 70% ethanol with a 1:5 ratio. The samples were then put in a shaker for about 14 days to help the bioactive chemical compound dissolve (Aisha *et al.*, 2014).

Filtration of Ethanolic Extracts

Following a 14-day interval, Whatman No. 1 filter paper was used to carefully filter the soaked plant material. To optimize the amount of active chemical components extracted from the plant, the procedure was carried out three times. Any remaining plant material is eliminated during the filtration process, leaving only a transparent extract (Arsene *et al.*, 2022).

Evaporation of Ethanolic Extract

To obtain the extract material, the filtered material is concentrated. As seen in figure 3.7, this was accomplished by evaporating the ethanol in a water bath set at 60°C. The ethanolic extract was what was left behind after the ethanol was totally evaporated. After being meticulously weighed, the condensed substance was put into falcon tubes (Arsene *et al.*, 2022).

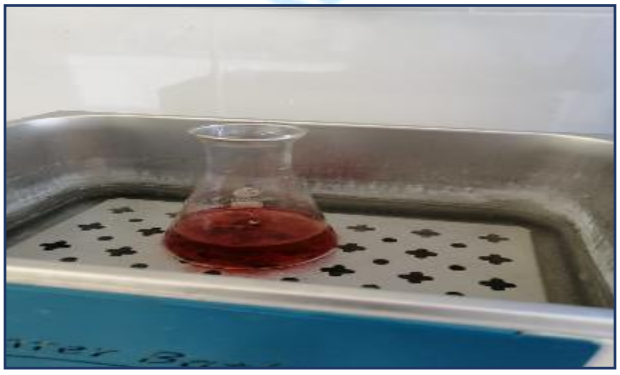

Figure 1. Shows extract in the water bath

Protocols for Antibacterial Activity

The well disc technique and the subsequent procedures were used to investigate the antibacterial activity of extracts from *Sedum Pallidum V. bithynicun* leaves, A. tinctoria roots, and *R. canina* fruit. (Klančnik *et al.*, 2010)

Subculture of Bacterial Stock Culture

A small amount of bacterial stock that had previously been successfully maintained in the lab was aseptically placed onto L.B. agar after the inoculation loop was sterile. After covering the plates, incubate at 37°C, an appropriate temperature (Kim *et al.*, 2017) .

Preparation of Mueller Hinton Agar Plates

In accordance with the manufacturer's instructions, easily accessible Mueller Hinton Agar (MHA) powder was dissolved in distilled water to produce a specific quantity of MHA. Cotton plugs were used to firmly seal the media-containing jar, which was then

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autoclaved for 30 minutes at 121°C. After being cleaned in a laminar flow hood, the MHA was gently transferred into a petri dish. The Petri dishes were allowed to dry out for around twenty minutes (Åhman *et al.*, 2022).

Quantification of Bacterial Stock Concentration Using (OD) Measurement

Pick two to three colonies from every fresh bacterial culture, then place them in five milliliters of autoclave water. Next, determine the optical density of the bacterial suspension at a particular wavelength of 600 nm using a spectrophotometer. We assessed each bacterial suspension's optical density (OD). After reducing the suspension once more, the OD value was examined if it was higher than 0.88 (Pan *et al.*, 2014).

Inoculation of MHA Agar Plates

A standardized suspension of microorganisms from their respective suspensions was obtained using autoclaved cotton buds. The solution was equally distributed over the outermost layer of MHA agar plates using the quadrant streaking technique. To reduce the excessive moisture, the contaminated plates were momentarily exposed to the air (Pan *et al.*, 2014).

Preparation of Plant Extract Wells

Using a sterile Cork borer, wells were made on the infected MHA agar plates, and each well is labeled. A micropipette was used to apply plant extracts to the wells. After that, the plates were given some time to dry.

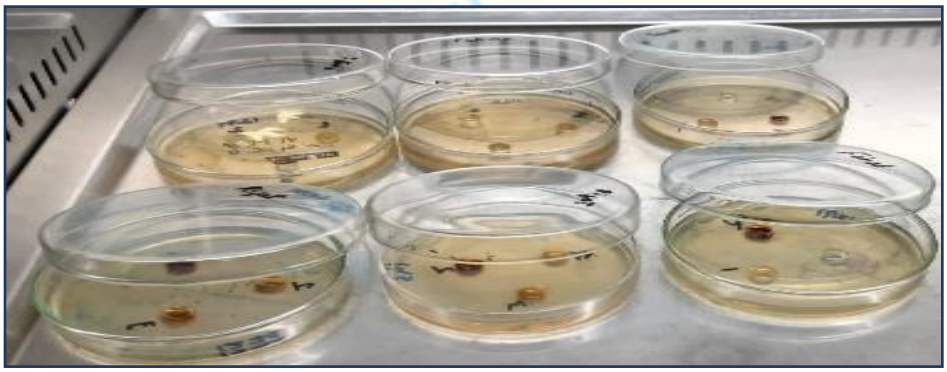

Figure 2: Shows inoculated MHA agar plates

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Incubation

The plant extract-inoculated MHA agar plates were incubated for 18 to 24 hours at 37°C, which is the right temperature for bacteria. To ensure ideal bacterial growth and precise assessment of antibiotic activity, the incubation conditions were carefully controlled.

Antibacterial Activity for *A. tinctoria, R. canina* and *Sedum Pallidum V. bithynicum* Extract

The antibacterial activity of A. tinctoria was assessed against various bacterial strains, as presented in Table 1. Different bacterial strains were spread on the surface of MHA media in different plates by using a cotton buds. After spreading the bacterial strains on the MHA media in different plates. Wells of 6mm were punched by means of sterile Cork borer. 100µl of *A. tinctoria* extract were loaded in wells of each plate. The extract was dried in each hole of plates and subsequently set aside at 37 °C for 24 hours. The zones of restriction were measured after a 24-hour period (Terzioğlu and Coşkunçelebi, 2021).

3.5. Bacterial Strains Used for Evaluation of Antibacterial Potential of the Different Extracts

Table 1. Shows the bacterial strains used for antimicrobial activity

Name of species	Detail of the species used
Enterobacter cloacae	Obtain from department of Microbiology AUST
Staphylococcus aureus	Obtain from department of Microbiology AUST
Pseudomonas aeruginosa	Obtain from department of Microbiology AUST
Citrobacterfreundii	Obtain from department of Microbiology AUST
Klebsiellaaerogenes	Obtain from department of Microbiology AUST
Klebsiella pneumonia	Obtain from department of Microbiology AUST
Salmonella typhimurium	Obtain from department of Microbiology AUST

DPPH Assay

Using the DPPH test, the radical scavenging capacity of *A. tinctoria* and *R. canina* was assessed. A solution of 0.1 mM DPPH was made. Assemble the plant extract and create different concentrations of 1 ml by mixing 1 ml of the DPPH solution with a particular amount of plant extract in autoclave water. Each sample has a distinct concentration of plant extract. To enable the DPPH radical scavenging process, combine them in different test tubes and let the reaction mixtures sit at room temperature in a dark area for approximately half an hour. The reaction mixture shows a noticeable hue shift after half an hour. Measure each reaction mixture's absorbance at a wavelength of 517 nm using a UV visible spectrometer (Sharma and Bhat, 2009).

DPPH Assay for *A. tinctoria*

A 0.1 mM DPPH solution was made, and autoclave water was added to a test tube to create different quantities of 1 ml plant extract (as shown in table 2). Each sample is then mixed with a 1 ml solution of DPPH, which contains different amounts of plant extract. For half an hour, the 2 ml solution sample was incubated in a dark environment. All test samples were measured for absorbance at 517 nm using a UV-visible spectrophotometer. One milliliter of DPPH solution and one milliliter of distilled water were combined to create the control sample. This formula is used to quantify antioxidant activity (Sharma and Bhat, 2009).

$$\text{Antioxidant Activity (\%)} = \frac{(\text{Abs\_control} - \text{Abs\_sample})}{(Abs\ control)} \times 100$$

DPPH Assay for *R. canina*

In a test tube, a 0.1 mM DPPH solution was made. Table 2 shows the several concentrations of 1 ml R. canina extract that were made by adding autoclave water. Each sample, which contains different quantities of plant extract, is then mixed with a 1 ml solution of DPPH. For half an hour, the 2 ml

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solution sample was kept in a dark room. All test samples were measured for absorbance at 517 nm using the UV visible spectrometer. One milliliter of DPPH solution was dissolved in one milliliter of distilled water to create the control sample (Sharma and Bhat, 2009).

Table 2. Shows the total concentration of mixture used for DPPH assay

Sample concentration	Autoclave water	DPPH Concentration	Total Solution
50 µl	950µl	1ml	2ml
100 µl	900 µl	1ml	2ml
125 µl	875 µl	1ml	2ml
150 µl	850 µl	1ml	2ml
175 µl	825 µl	1ml	2ml
200 µl	800 µl	1ml	2ml

Biosynthesis of Silver Nanoparticles of *A. tinctoria* Root Extract

Silver nanoparticles were synthesized according to the following protocols

Washing the Glass Vessels

The glassware used for AgNPs synthesis was thoroughly washed with water that has been due to eliminate any metal impurities.

Preparation of Plant Extract

Twenty grams of *A. tinctoria* roots were weighed and submerged in 70% ethanol at a 1:5 ratios in order to biogenically create AgNPs. Falcon tubes that had been cleaned and dried were used to hold the roots. After that, the falcon tubes were sealed and shaken for a period of fifteen days. After 15 days, nanoparticles were created using a pure plant product that had been filtered and boiled down (Sirivibulkovit *et al.*, 2018).

Method of Biosynthesis

In a flask, 1 milliliter of the A. tinctoria root extract and 9 milliliters of AgNO3 solution were combined to create the AgNO3 solution. 17 milliliters of extract were used, and 153 milliliters of AgNO3 were added. Following a few intermittent stirs, the AgNO3 solution was added to the *A. tinctoria* extract at room temperature. After seeing the colour change of a reaction mixture, it was allowed to sit at room temperature for 48 hours. The combination is heated to 62°C at a certain temperature using a magnetic mixer two days later. People look at the jar containing the response combination. The reaction mixture's apparent hue shift suggests that nanoparticles are forming (Singh *et al.*, 2016).

Separation and Purification of Synthesized Nanoparticles

After this the mixture is centrifuge at 15000rpm for 20 minutes to separate the pallet from the supernatant and dry the pallet in an oven at a very

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low temperature to remove any remaining unreacted plant biomass (Singh *et al.*, 2016).

Characterization

UV visible and SEM methods were used to learn about the nanoparticles.

UV Visible Spectroscopy

The colour shift was seen when the reaction mixture including the metal ion solution and root extract was allowed to settle for some time. Record the absorbance range of the nanoparticle sample using a UV-Vis spectrophotometer. Graph 1 displays the UV and visible areas of the spectrometer, which measures the amount of light that the extract nanoparticles receive at various wavelengths (Mohammadian *et al.*, 2018).

Scanning Electron Microscopy

Sample Preparation

A small quantity of AgNPs powder was deposited in distilled water onto a carbon-coated copper grid to create the sample. After that, hot air at 50–60°C was used to dry the AgNPs layer on the SEM grid for five minutes. The dehydrated samples lacked moisture and remained stable.

Sample Mounting

After drying, the A. tinctoria nanoparticles were adhered to a sample holder using a conductive glue. This ensures that the sample won't move during imaging and remains consistent (Mohammadian *et al.*, 2018).

Sputter Coating

The specimen was then put through a sputter coater for 120 seconds at 30mA.

Instrument Calibration

The SEM apparatus was calibrated correctly before the imaging process began. To maximize the image quality, this entails modifying parameters including focus, working distance, and electron beam intensity.

Imaging

The imaging procedure was started by introducing dried-out nanoparticles into the SEM chamber after the device had been calibrated. When electrons are moved across the surface of the sample, their interactions with it produce observable signals that may be used for imaging.

Image Analysis

After acquiring SEM images, a specialized software was to analyze the images. This involve measuring particle sizes, assessing the distribution of nanoparticles, and examining surface features.

Interpretation

The SEM images depict the dried *A. tentoria* nanoparticles, providing information about their size, design, which is and surface properties.

Results

Antimicrobial activity of *A. tinctoria* root extract against *C. freundii*

Using the well diffusion technique, the antibacterial potential of A. tinctoria collected was evaluated. The growth of C. freundii was found to have decreased. A 27.5 ± 0.43 mm zone of inhibition was shown by the plant extract. This suggests that the extract could prevent the growth.

Figure 3: Shows ZOI against *C. freundii*

Antibacterial activity of *A. tinctoria* root extract against *E. cloacae*

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E. cloacae, which exhibits resistance to 36 antibiotics, was tested against an extract from A. tinctoria. Figure 4 shows the extract's zone of inhibition, which measured 25± 0.55 mm. To test the activity against *E. cloacae*, 200µl of the highest concentration of root extract was utilized.

Figure 4: Shows ZOI against *E. cloacae*

Antibacterial activity of *A. tinctoria* root extract against *P. aeruginosa*

In the experiment, 150µl of root extract was used to combat *Pseudomonas aeruginosa*. A zone of inhibition of 21 ± 0.5 mm was seen in *Pseudomonas aeruginosa*, indicating great sensitivity to the root extract of A. *tinctoria* (Figure 5). The results showed that *P. aeruginosa* is susceptible to A. *tinctoria's* ethanolic root extract.

Figure 5: Shows ZOI against *P. auriginosa*

Antimicrobial activity of *R. canina* fruit extract against *P. aeruginosa*

A 100µl extract made from ethanol of R. canina was tested for its antibacterial activity towards *P. aeruginosa*. The extract exhibited a zone of inhibition measuring 15 ± 0.4mm, as depicted in Figure 6. The study found that the ethanolic extract effectively inhibited the development of *P. aeruginosa*.

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Figure 6: Shows ZOI against *P. aeruginosa*

Antibacterial activity of *A. tinctoria* Root extract against *K. aerogenes, S. typhimurium, K. pneumonia, S. aureus*

In order to test the extract's antibacterial activity against each of the single bacteria *K. aerogenes*, *K. pneumonia*, *S. typhimurium*, and *S. aureus*. 100µl of *A. tinctoria* root extract was applied to each well of separate plates. Against each of these bacterial strains, the extract exhibited no zone of inhibition.

Figure 7 shows no ZOI against *K. aerogenes*

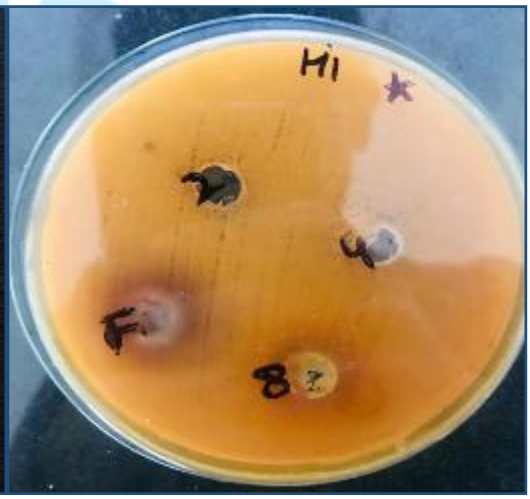

Figure 8 shows no ZOI against S.typhimurium

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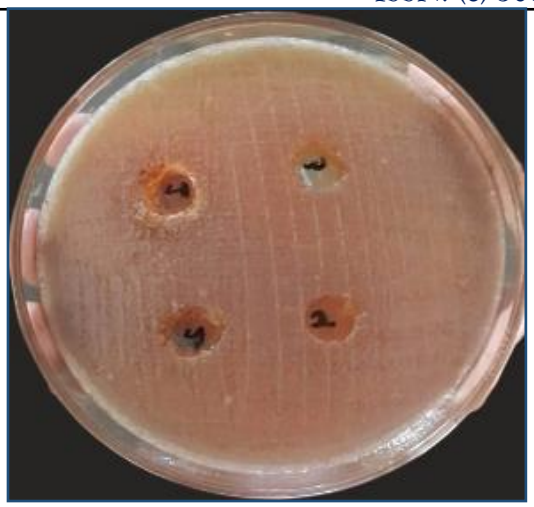

Figure 9 Shows no ZOI against *K. pneumonia*

Figure 10. Shows no ZOI against *S. aureus*

Antimicrobial activity of *R. canina* fruit extract against *E. cloacae, S. aureus, K. aerogenes, K. pneumonia, C. freundii, S. typhimurium*

The study employed the well diffusion method to assess the antimicrobial activity of the ethanol fruit extract of R. canina. Each bacterium was inoculated onto the surface of a separate Petri plate containing Mueller-Hinton agar (MHA). Different bacteria were put on different plates, and 100µl of extract was put in each well. As shown in well over half of the pictures, no zone of inhibition was seen against any of the bacteria

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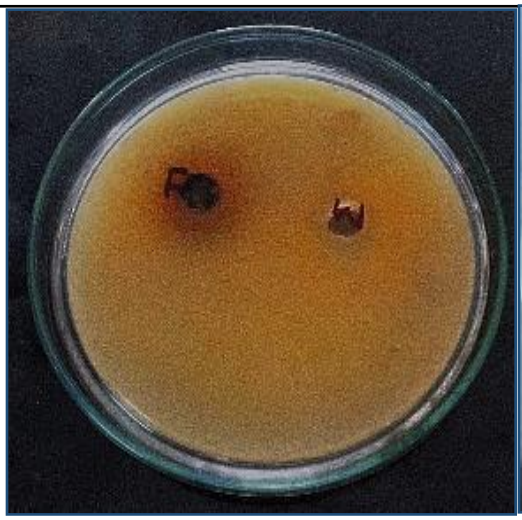

Figure 12 shows no ZOI against *K. Pneumonia*

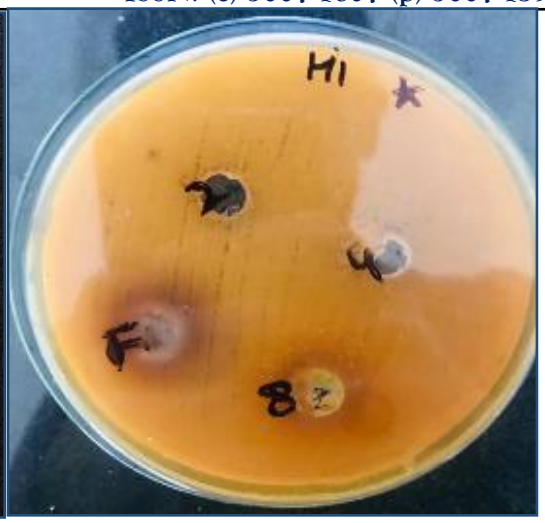

Figure 11 shows no ZOI against *K.aerogenes*

Antimicrobial activity of *S. pallidum V. bithynicum* leaves extract against *E. cloacae, S. aureus, K. aerogenes, S. aureus, C. freundii*

The antibacterial qualities of an ethanolic extract of *S. Pallidum V. bithynicum* leaves were evaluated using the well diffusion technique. Mueller-Hinton agar (MHA) plates were streaked with different microorganisms. After making wells, 100 µl of extract was put into each well on a different plate. No zone of inhibition against any bacteria was found.

4.8. Antioxidant Activity of *A. tinctoria* Root extract

Using DPPH, the extract's capacity to scavenge free radicals was assessed. Figures (4.8) and (4.8.1) illustrate the colour changes that were seen after 30 minutes. A UV visible spectrometer was used to detect each test sample's absorbance at 517 nm. The extract's results indicated high scavenging activity (39.80%) at a concentration of 150 µl/ml, whereas graph 1 showed the lowest activity (6.60 concentration of 200 µl/ml. These findings imply that the root extract of *A. tinctoria* has a concentration-dependent activity and an ideal range for efficacy.

Before colour change

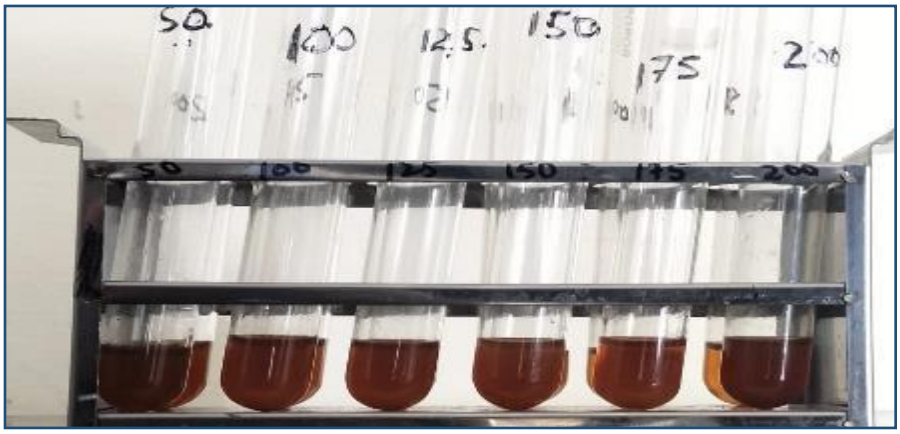

Figure 13. Shows before colour change DPPH solution

After colour change

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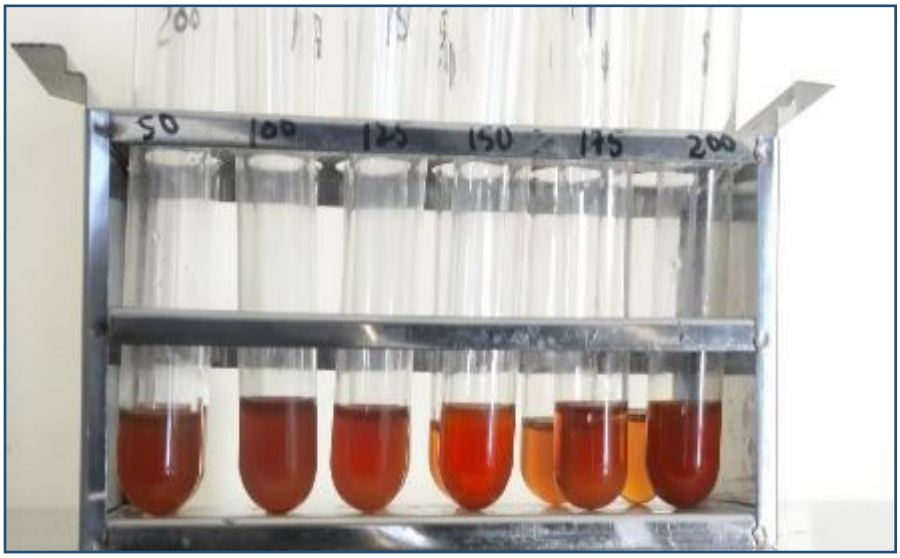

Figure 14: Shows after colour change of DPPH solution

Antioxidant Activity of the *R. canina* fruit extract

The extract's ability to eliminate free radicals was assessed using DPPH. The observed colour change over a 30-minute period is shown in Figure 4.9.1. All of the samples under study had their absorbance measured at a wavelength of 517 nm using a UV-visible spectrometer. Graph 2 shows that the extract had the highest scavenging activity (32.70%) at a concentration of 50 µl/ml and the lowest activity (3.17%) at a concentration of 200 µl/ml. The findings imply that the concentration of *R. canina* fruit extract affects its efficacy, suggesting that there is a range of ideal activity.

Before colour change

Figure 15: Shows before colour change of DPPH solution

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After colour change

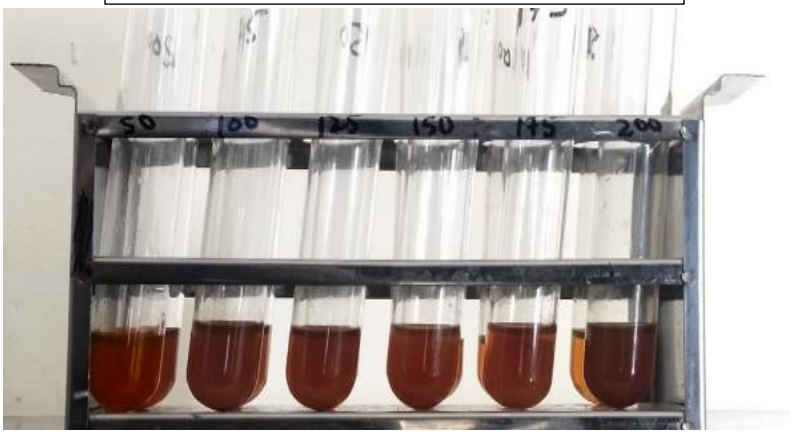
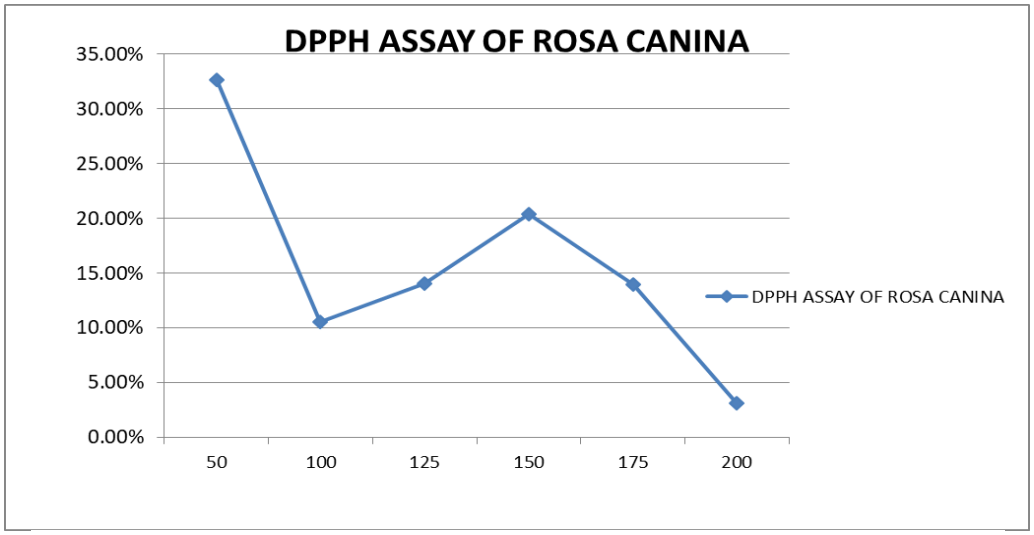


Figure 16: Shows after colour change of DPPH solution



Graph: 2 Shows DPPH assay of *R. canina*

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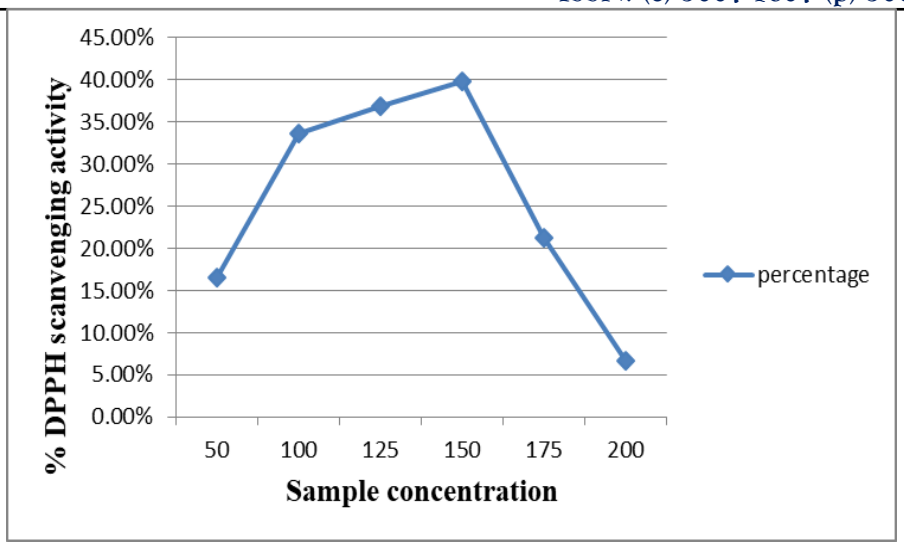
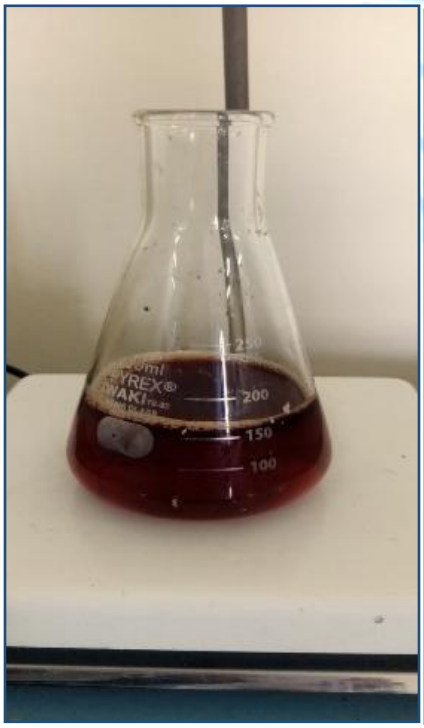


Graph: 1 Shows DPPH assay of *A. tinctoria*

Colour changes of AgNPs

AgNPs were made from the root extract of A. tinctoria, and when AgNO3 was added to the extract, colour changes occurred. After two days, the extract's colour changed from yellow to dark brown, as seen in Figs. 4.19 and 4.20. Figure (4.21) illustrates the formation of nanoparticles after the supernatant was separated from the pallet and dried at a very low concentration.



Before Colour Change

Figure 18: shows dark brownish colour of silver nanoparticles of solution

After Colour Change

Figure 17: shows yellowish colour of silver nanoparticles of solution

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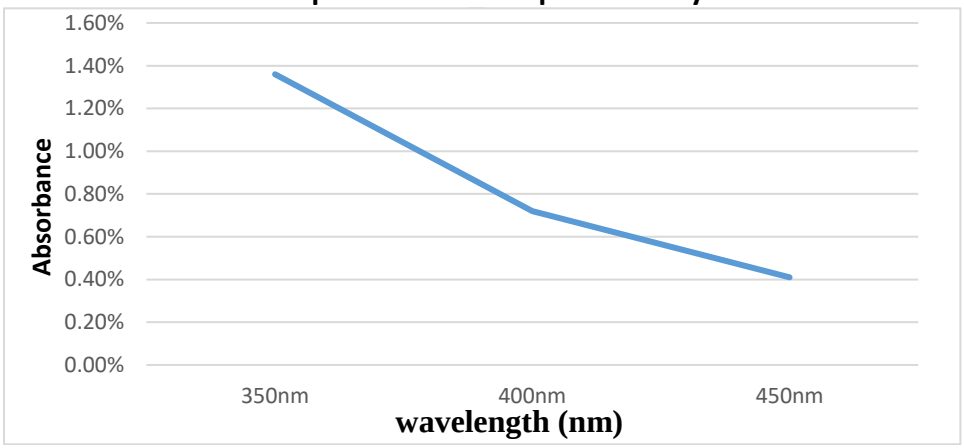
Figure 19: Shows silver nanoparticles of *A.tinctoria* root

Characterization of *A. tinctoria* extract

Root extract of *A. tinctoria* was characterized by using UV-visible spectrometry and SEM.

UV- visible spectrometry

Graph 3 UV- visible spectrometry



The UV-visible spectrometry technique was used to evaluate the silver nanoparticle of the ethanol extract of A. tinctoria. The absorption spectrum of the nanoparticle was measured at various wavelengths, and the highest peak was observed at 350nm, as depicted in graph 3.

SEM analysis results

Following the completion of sample preparation, a minimal quantity of silver nanoparticles (AgNPs) was affixed onto a sample holder. The sample underwent sputter coating. Nanoparticles were introduced into the scanning electron microscope (SEM) chamber. The findings indicate that the nanoparticles had a size of 50nm and exhibited complex gathers with circular and cubic shapes, as depicted in figures (20) and (21).

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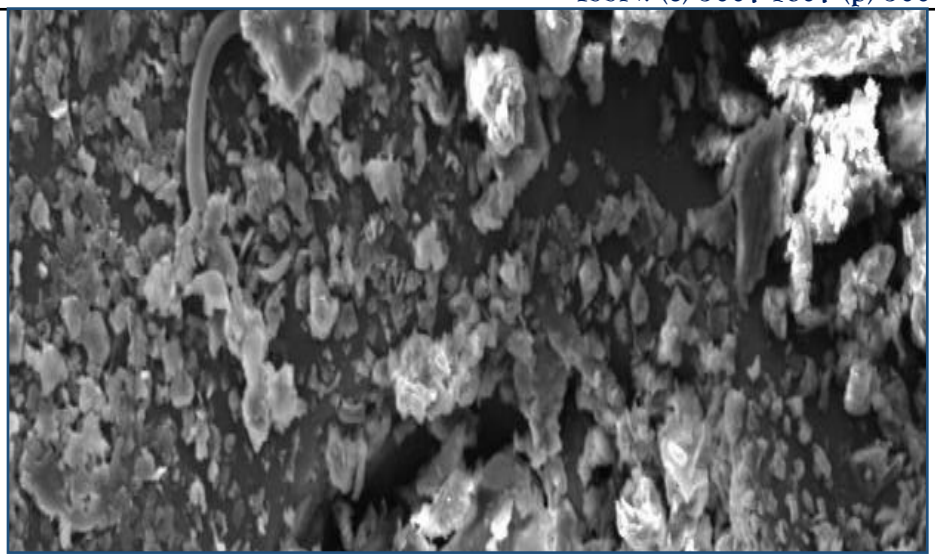

Figure 20 shows SEM representation of *A. tintoria* root AgNPs

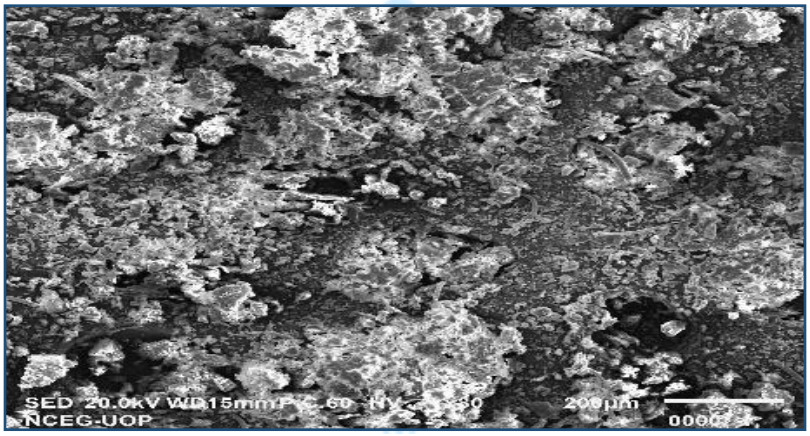

Figure 21: shows SEM representation of *A. tintoria* root AgNPs

DISCUSSION

Ethanolic extracts from the root of A. tinctoria, the leaves of Sedum pallidum, V. bithynicum, and the fruit of R. canina were evaluated for their antibacterial and antioxidant properties. Furthermore, A. tinctoria extract was used to synthesize silver nanoparticles, which were then characterized using a UV visible spectrometer and a SEM. In this investigation, ethanolic extracts from these plants were prepared (Alwahibi and Perveen, 2017, Dahpour *et al.*, 2012, Pehlivan *et al.*, 2018) prepared the ethanolic extracts of the *A. tinctoria*, *sedum pallidum V. bithynicum* and *R. canina* while (Mohammed et *al.*, 2016, Al-Qudah *et al.*, 2012, Fattahi *et al.*, 2012). *A. tinctoria*, *S. pallidum*, *V. bithynicum*, and *R. canina* were all extracted in methanol. Different bacterial strains were used to test the antibacterial activity of various plants. This study (Jaloob, 2018) shows that the zone of inhibition of *A. tinctoria* for *P. aeruginosa* was 22.61± 0.26mm to 24.85± 0.13mm, which is nearly identical to our findings of 21.0± 0.5mm. The zone of inhibition for Klebsiella and *S. aureus* was 28.87± 0.35 to 24.88± 0.09mm, while the ZOI for S. aureus in our study was 23.45± 0.35mm, which is comparable even though Klebsiella does not form any ZOI. A. tinctoria did not exhibit ZOI against Salmonella in our investigation while (Papageorgiou, 1978) revealed effective *A. tinctoria* against Salmonella.

In our study *S. pallidum V. bithynicum* has no activity against *P. aeruginosa*, *Klebsiella*, *E. cloacae* and Citrobacter which is similar to (Karadağ and Tosun,

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2019, Yaylı *et al.*, 2010) where it show weak activity. According to study in Slovakia (RovnÃ *et al.*, 2015) R. canina show strong activity against pathogenic Klebsiella and *P. aeruginosa* in our data *P. aeruginosa* show activity but not Klebsiella .(RovnÃ et al., 2015) reported that *R. canina* show less activity against *E.aerogenes* and *S. typhimurium* although our analysis *R. canina* show no ZOI against *E. cloacae* and *Salmonella typhimurium*. A spectrophotometer set at 517 nm was used to measure the antioxidant activity of A. tinctoria. The extract that shown the highest antioxidant activity was the one that was concentrated at 150µl/ml, whereas the extract that was concentrated at 200µl/ml had the lowest activity (Assimopoulou and Papageorgiou, 2005) employed a spectrophotometer set at 517 mm to gauge the root of A. tinctoria's antioxidant capacity. They noted that a high concentration reduced the extract's activity and that the extract's activity is concentration dependant (Sabih Ozer *et al.*, 2010) also shown that the medium-concentration extract in DPPH has more activity than the methanolic extract at higher concentrations (Sabih Ozer *et al.*, 2010) shown that the chemicals shikonin, alkannin, and esters found in A. tinctoria are quite effective in scavenging free radicals (Zannou and Koca, 2020). Because of its strong antioxidant qualities, the researchers showed in their study that *A. tinctoria* is used in cosmetic compositions.

At a wavelength of 517 nm, *R. canina's* antioxidant capacity was evaluated. The maximum antioxidant activity was shown by the extract with the lowest concentration in the sequence (50µl/ml). Additionally, R. canina exhibited the least amount of activity at high extract concentrations (200µl/ml) (Fattahi *et al.*, 2012). According to the study, R. canina's methanolic extract exhibited the most radical scavenging efficacy (Jemaa *et al.*, 2017) shown that *R. canina* hip has greater activity and a low EC50 value. By measuring absorbance at different wavelengths, UV-Vis spectroscopy was used to characterize the silver nanoparticles. The wavelength at which the greatest peak was seen was 350 nm (Roman *et al.*, 2013) also reported the nanoparticle characterization of A. tinctoria and peaks was formed between 300-350nm. UV spectra of nanoparticle residual solutions was also analyzed by (Shabbir and Mohammad, 2018). At a wavelength of 435 nm, a peak was seen. The shape and size properties of the nanoparticles were evaluated using scanning electron microscopy (SEM). The nanoparticles were 200µl in size and showed complex aggregates with oval and cubic forms. A. tinctoria-treated wool fabric samples' silver nanoparticle morphology was examined (Shabbir and Mohammad, 2018).

CONCLUSION

Ethanolic Extract of different plants were used to check the antibacterial and antioxidant activity. The growth of various bacterial strains was suppressed by these extracts. The root of *A. tinctoria* showed the most notable effects against bacterial strains of any extract. When large concentrations of extract were employed, all of the extracts' effectiveness against bacterial strains improved. At 121°C, A. Tinctoria showed considerable thermal stability. Significant antioxidant activity was shown by all extracts. AgNPs of *A. tinctoria* were characterized by their cubic shape and low frequency peaks.

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