

## Lab 4

### Green synthesis of Nanoparticles

Nanotechnology evolved as the achievement of science in the 21st century. nanoparticle is a small particle that ranges between 1 to 100 nanometres in size. Undetectable by the human eye, nanoparticles can exhibit significantly different physical and chemical properties to their larger material counterparts. The material properties change as their size approaches the atomic scale. This is due to the surface area to volume ratio increasing, resulting in the material's surface atoms dominating the material performance. Owing to their very small size, nanoparticles have a very large surface area to volume ratio when compared to bulk material, such as powders, plate and sheet. This feature enables nanoparticles to possess unexpected optical, physical and chemical properties, as they are small enough to confine their electrons and produce quantum effects. The synthesis, management, and application of those materials with a size smaller than 100 nm. Nanoparticles have significant applications in different sectors such as the environment, agriculture, food, biotechnology, biomedical, medicines, etc. like; for treatment of waste water environment.

A variety of metallic nanoparticles have drawn a lot of interest because of their potential antimicrobial properties, including silver (Ag), gold (Au), Ag oxide (Ag<sub>2</sub>O), zinc oxide (ZnO), titanium dioxide (TiO<sub>2</sub>), calcium oxide (CaO), copper oxide (CuO), and magnesium oxide (MgO) .Even though, their antibacterial activities depend primarily on the size, shape, surface charge, pH, concentration, type of capping or stabilising agents, and bacterial gram-type.

**mechanisms of action:** metallic NPs have been shown to have antibacterial properties e some of the ways that metallic nanoparticles work: (i) attraction to bacterial cell walls due to opposite surface charges; (ii) membrane instability; (iii) production of reactive oxygen species (ROS); (iv) release of metal ions; and (v) modification of the signalling pathway.

## **Synthesis of Nanoparticles**

are three kinds of approaches for the production of nanoparticles There

1. Physical Methods
2. Chemical Methods
3. Biological Methods

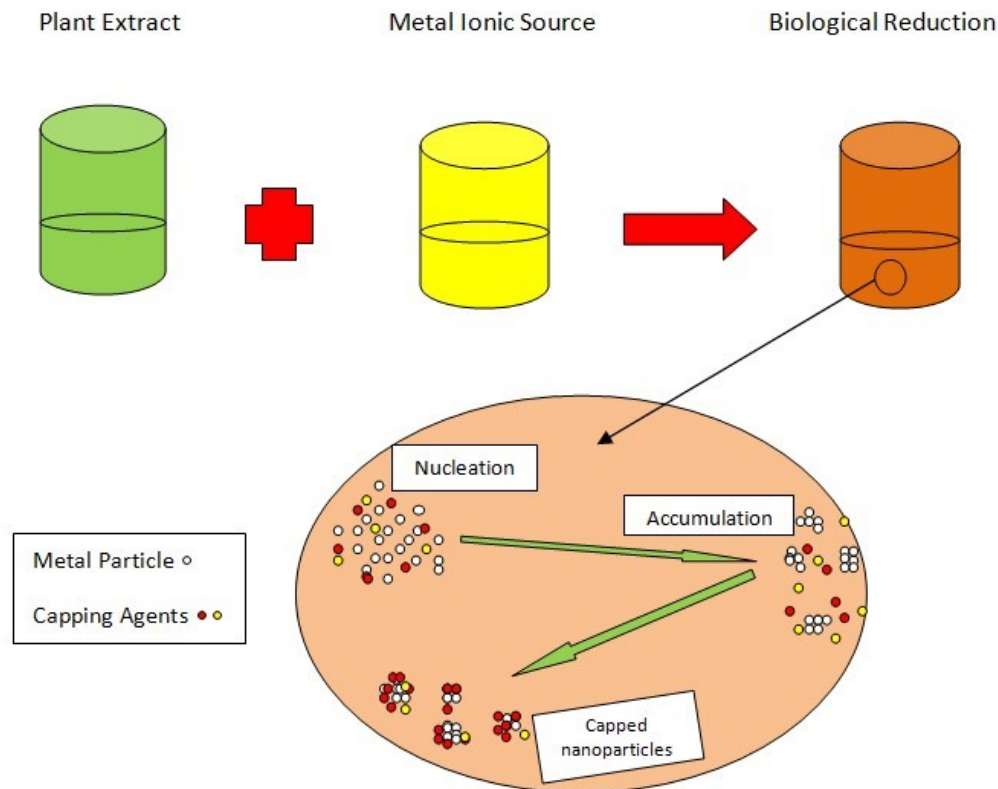
### **Biological methods**

#### **Synthesis using microorganisms**

synthesis of nanoparticles using microorganisms have gained more attention due to cost-effectiveness and eco-friendliness. There are two techniques by which nanoparticles can be synthesized from a microorganism, one is extracellular biosynthesis and another is intracellular biosynthesis

#### **Synthesis using plant extracts**

Plant extracts show a vital character in the biosynthesis of nanoparticles. This process is also recognized as green synthesis or a green process of manufacture of nanoparticles. because plant extracts can work as reducing and stabilizing agents. This opens up new possibilities for cost-effective, environmentally-friendly nanoparticle synthesis with enhanced size uniformity and stability. Moreover, bio-inspired nanoparticles derived from plants exhibit intriguing pharmacological properties, making them highly promising for use in medical applications due to their biocompatibility and nano-dimension.



### Synthesis of silver NPs Green

Biologically synthesised silver NPs could have numerous applications in regions, there are three major sources of synthesising silver nanoparticles: bacteria, fungi and plant extracts. This biosynthesis of silver nanoparticles mostly involves oxidation reduction reactions. In plants, the real point of utilising plant concentrates for silver nanoparticle combination is that they are safe and nontoxic much of the time and that they have an expansive mixed bag of metabolites that can help in the reduction of silver ions. Compared with bacteria, fungi have the capacity to produce larger amounts of nanoparticles because they are regarded as the organisms that produce nanoparticles extracellularly due to extensive secretory components, which is included in the reduction and capping of nanoparticles. Silver nanoparticles have received much attention as antimicrobial agents and have been shown to be an effective antimicrobial agent.

## **Characterization silver nanoparticle**

- 1-UV-Vis spectrophotometry is used to assess their optical characteristics
- 2- FTIR spectroscopy assists the identification of the functional groups present on the nanoparticle surface. For a detailed analysis of their size, shape, and structure
- 3- Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) measurements are utilized to determine the size and surface charge of the nanoparticles. These methods provide valuable insights into the properties and behaviour of the synthesized nanoparticles.

## **Material and methods**

**Preparation of silver nitrate solution with 1mM** :1 mM of silver nitrate solution was prepared by adding 0.0849 g of AgNO<sub>3</sub> to 500 ml of steriledistilled water and mixing the solution thoroughly by the stirrer to dissolve the material. the final solution was kept in fuscous coloured bottle to prevent the auto oxidation of silver nitrate solution

**.Preparation of plant extracts**:the plant's leaves were used for fabrication Ag NPs which were collected from local markets and washed several times with tap water to remove the dusts and other pollutants and cut it into small pieces. Plant extracts of *Petroselinum crispum* , *Beta vulgais* was prepared by adding 20g of fresh green leaves of each plant separately into clean Erlenmeyer conical flask containing 100 ml of sterile distilled water then were incubated in water bath at two temperature (60-100)C° for 20 minutes to facilitate the formation of aqueous plant extracts. The plant extracts were filtrated by using filter paper NO.1 to remove insoluble fractions and macromolecules Finally ,plant extracts were centrifuged at 10000 rpm for 10 minutes .and was stored at 4 C° in refrigerator for further experiment .

**Preparation of Ag NPs** from plant extract was achieved by adding 5 ml of plant extract to dark bottle containing 45ml of 1mM AgNO<sub>3</sub> solution to avoid photo activation of silver. The reaction mixture was monitored in water path at two temperature (60-100)C° separately for 30 minutes ,the

colour change of reaction mixture is indication proving the formation of silver nanoparticles. Finally, the mixture was centrifuged at 5000 rpm for 10 minutes for getting the pellet which is used for checking antimicrobial activity

### Antibacterial activity of silver nanoparticles

The antibacterial, antifungal activity of biosynthesized Ag NPs was done by agar well diffusion method against two bacteria *Staphylococcus aureus* example for gram positive and *E. coli* example gram negative bacteria. grown in nutrient broth at 37°C for 24 hr. density of bacteria was fixed at  $1.5 \times 10^8$  by comparison with McFarland No.(0.5). 20 ml of Muller Hinton agar was poured in sterilized Petridishes and allowed to solidify. Then, wells in the agar plates were made by using sterile cork borer with diameter of 6.0 mm, the bacterial strains used in the current research were swabbed uniformly onto the surface of plates by sterile cotton swabs. The inoculated plates were left for moments to dry. Subsequently, 20 µL for each precipitate and supernatant of synthesized Ag NPs which were obtained after centrifuging of reaction mixture that was incubated at two temperatures were added separately into wells of inoculated Mueller-Hinton Agar plates by using micropipette while 1m AgNO<sub>3</sub> solution was used as standard sample. The treated plates were left for one hour for allowing the diffusion to occur. Then, these treated plates were incubated overnight at 37°C. After that, clear inhibition zones were observed around the well and measured. The diameter of inhibition zone was calculated using a meter ruler, and the values for each type of bacteria were recorded