

## Green Nanotechnology: Application, And Characterization in Biological Synthesis of Herbal Nanoparticles

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### ABSTRACT:

Herbal therapy is the most complementary and alternative medical therapy without any serious side effects, The applications of nanotechnology can be very beneficial and have the potential to make a significant impact on society. To maximize patient compliance and prevent repetitive administration, Phyto therapeutics requires a systematic approach to administer the components over time. This might be obtained by making NDDSs for the herbal ingredients. NDDSs reduce the frequency of administration of dose to strive for noncompliance but also contribute to increasing therapeutic value by lowering the toxicity and boosting bioavailability. Hence, integration of the nanocarriers as an NDDS in the traditional medicine system is essential to conflict with more severe diseases like inflammation, pain, microbial infection, asthma, diabetes, cancer, and others.

**KEYWORDS:** Green Nanotechnology, Herbal Nanoparticles, Ecofriendly synthesis of Gold and Silver Nanoparticles.

### I. INTRODUCTION:

The variety in the plant species having reduction properties to produce nanoparticles makes them an optimal prospect for fabrication since the atmosphere present for synthesis determines the morphological properties of nanoparticles, and due to the differences in reducing capacities, capping properties, stabilizing properties among various species; plants provide an excellent platform for the control of this properties<sup>2</sup>. The use of biological components for the synthesis of nanoparticles has commonly called "green synthesis" and is considered to be more beneficial because it is eco-friendly, economical, and applicable for large-scale synthesis as it operates on less input of energy, low pressure, and low temperatures. The lack of poisonous byproducts and the consequent decrease in

degradation of the product made this technique preferable over physical and classical chemical methods<sup>1</sup>.

The novel carriers should ideally fulfill two prerequisites. First, it should deliver the drug at a rate directed by the needs of the body over the period of treatment. Second, it should channel the active entity of the herbal medicine to the site of action. Conventional dosage forms including prolonged-release dosage forms cannot meet any of these.

Nanotechnology has existed for thousands of years. In ancient times people used to stain their drinking glasses with nanoparticles. The disparity of nanotechnology within other fields of science and further discoveries have possessed a significant impact on biotechnology, medicine, pharmaceuticals, physics, chemistry, optics, etc. There is much evidence that metals are present in living systems in different forms, playing a significant role in various biochemical processes, growth metabolism, and healing. Blood contains the Zn, Mn, Cu, Hema protein, and other vital trace metals in the biological system<sup>2</sup>.

Nanotechnology mainly deals with the synthesis and fabrication of materials at the nanoscale level at 1–100 nm. It is the critical length scale at which certain novel size-related properties develop and the material starts to act differently than the molecules or bulk material<sup>3</sup>.

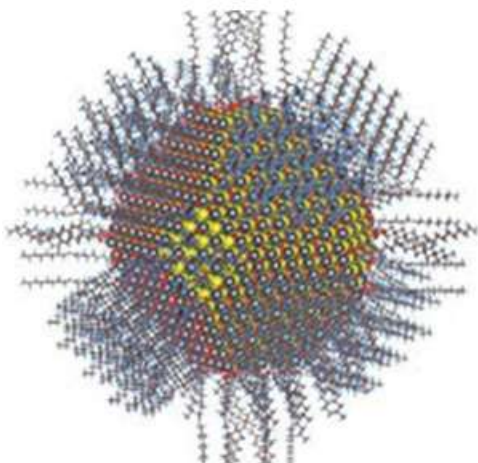


Figure: Nanoparticle

#### Role of Nanoparticles<sup>4</sup>:

- To distribute the medication in nanoscale level particles that help to improve the medicine's full surface area distribute it more quickly and remove it from the blood.
- The drug delivery method is target specific.
- The medication is permeable to epithelial and endothelial barriers.
- To distribute the medication to the site of action.
- Combining the two various techniques in treatment therapy drugs.

#### Nanoparticles Advantages: -

- Because it is smaller in size than that liposomes and microspheres it can easily flow through the bone's sinusoidal gaps, bone marrow, and spleen versus other organ systems with long periods of circulation.
- Nanoparticles inhibit a drug or proteins from enzymatic breakdown.
- They provide a significant advancement above current therapy or practice.
- The administration routes of nanoparticles are mainly oral and intravenous (IV) in terms of efficacy and efficiency.
- It minimizes the liver's toxicity.

#### Nanoparticles Disadvantages: -

- Protracted and costly to expense.
- Significant immunogenicity.
- Potential for subpar targeting.

#### Need Of the Nanoparticle in Herbal Formulation<sup>4</sup>: -

- Does not require the involvement of any specific ligand moiety and exhibits passive targeting to the disease site of action.
- Due to their specific size and high-loading capacity, nanoparticles can deliver high concentrations of medication to the disease site.
- Targeting herbal medicines to specific organs with nanoparticles increase selectivity, medication delivery, safety, and efficacy.
- Giving the medication in nanoparticles enhances its overall surface area, and facilitates its absorption into circulation.
- Nanoparticles can be used to enhance the solubility of herbal drugs and to localize the medicine at a specific site, which improves efficacy.
- Demonstrates enhance penetration and retention effects, including greater permeation through the barriers due to the little size and retention due to low lymphatic drainage.
- Decrease negative effects.

#### Biological synthesis of herbal and medicinal plants mediated nanoparticles:

##### 1. Synthesis of Gold Nanoparticles:

1.1. In a typical experiment, AuNPs synthesis protocol was optimized by stirring a mixture of Aloe vera aqueous extract at three different concentrations with 1mM  $\text{HauCl}_4$  aqueous solution (1;1, 5;1, 10;1) at 200 rpm at room temperature for 1 hour. Within a particular time change in color was observed indicating nanoparticles synthesized<sup>5</sup>.

1.2. In the Erlenmeyer flask, 2.5 ml of appropriate extract, 2.5 ml of 0.001 M chloroauric acid, and 1 ml of appropriate pH (4, 7, and 9.2) buffer solution were added. This mixture was allowed to stir at 240 rpm using a magnetic stirrer. The reduction of  $\text{Au}^{+3}$  to  $\text{Au}^0$  was monitored by observing the change in color of the reaction mixture from light brown to dark brown. This procedure was extended with a higher concentration of chloroauric acid (0.01 M) to understand the effect of concentration<sup>6</sup>.

1.3. The plant extract for the reduction of  $\text{Au}^{3+}$  ions to  $\text{Au}^0$  was prepared by combining thoroughly washed Ennab leaves (10 g; leaves were collected in the month of June) in a 200

mL Erlenmeyer flask with sterile DD water (100 mL). The mixture was then boiled for 5 min. In a typical experiment, 5 mL of the plant extract was added to 1 mM aqueous HAuCl<sub>4</sub> solution (45 mL). Reduction of AuCl<sub>4</sub><sup>-</sup> was monitored by recording the UV-vis absorption spectrum as a function of time<sup>7</sup>.

1.4. In a conical flask, 20 mL of the peel extract was reacted with 10 mM of tetrachloroaurate at room temperature under static conditions. The color change of the reaction was observed and the time taken for the changes was noted. The solution color changes immediately from pale brownish to a purple color indicating the formation of [Au/G. mangostana]. The Au-NPs nanoparticle emulsion obtained was kept at 4°C<sup>8</sup>.

1.5. In a typical synthesis of Au NPs, 10 mL of the aqueous extract of *Mentha Longifolia* leaf was added dropwise to 50 mL of well-mixed 0.001 M aqueous solution of HAuCl<sub>4</sub> with constant stirring at room temperature. After 20 min, the light-yellow colored mixture changed to wine red, evidence for the preparation of Au NPs. Then the solution containing nanoparticles was centrifuged at 4000 rpm for 20 min and the upper transparent layer was decanted off. The residues obtained were washed several times with deionized water and finally dried in an oven at 50°C<sup>9</sup>.

## 2. Synthesis of Silver Nanoparticles:

2.1. Thirty grams of buchu ethanolic extract were dissolved in 100 mL of water and filtered to create a stock solution. From this stock solution, 10 mL was taken and mixed with 10 mL of 0.1 M AgNO<sub>3</sub>. This mixture was diluted to 50 mL with distilled water and heated to 40 °C under continuous stirring. Aliquots were taken at different reaction times to monitor the growth of Ag-NPs. The experiment was repeated at different temperatures of 60 °C and 75 °C<sup>10</sup>.

2.2. The samples were collected by using a randomized sampling method. The plant extract was purchased readymade to hasten the study. 0.5g of Piper longum extract was added to 100ml of distilled water and was boiled for 5 minutes at 50 degrees Celsius. The solution was filtered 1.16g of AgNO<sub>3</sub> was added to the extract and was kept in the shaker at 750rpm

for half a day. After the complete dissolution of silver. A sample of extracts with different readings in the spectrophotometer were taken in different time intervals. A spectrophotometer is an optical device that can determine the concentration of a compound or particles in a solution or suspension<sup>11</sup>.

2.3. For the green synthesis of silver nanoparticles, we used the aqueous leaf extract of *C. prophetarum* prepared in the previous step. For this, 10 mL of leaf extract was added to 90 mL of 1 mM aqueous silver nitrate solution, followed by heating at 80 °C for 3 h with constant stirring. The formation of the AgNPs was preliminarily detected by the change in color from yellow to dark brown. The green-synthesized nanoparticles were separated using centrifugation at 15,000 × g for 20 min. This process was repeated thrice to get rid of free silver associated with Cp-AgNPs. The final green-synthesized silver nanoparticles were denoted as Cp-AgNPs, which were freeze-dried and then stored at 4 °C until further use<sup>12</sup>.

2.4. The fresh leaf of *C. roseus* broth solution was prepared by taking 10 g of thoroughly washed and finely cut leaves in a 300 mL Erlenmeyer flask along with 100 mL of sterilized double distilled water and then boiling the mixture for 5 min before finally decanting it. The extract was filtered through Whatman filter paper no 1 and stored at -15°C and could be used within 1 week. The filtrate was treated with aqueous 1 mM AgNO<sub>3</sub> solution in an Erlenmeyer flask and incubated at room temperature. As a result, a brown-yellow solution was formed, indicating the formation of silver nanoparticles. It showed that aqueous silver ions could be reduced by aqueous extract of plant parts to generate highly stable silver nanoparticles in water<sup>13</sup>.

2.5. For the preparation of AgNPs, 150 mL of 1 mM silver nitrate aqueous solution was transferred to the Erlenmeyer flask holding 150 mL of extract of *Alysicarpus monilifer* leaves. After 10 min, the color of the extract turned brown indicating the preparations of AgNPs. The brownish color becomes denser, and after 1 h, no significant colour change was observed. The colour change is due to the coherent excitation of the SPR in the metallic

nanoparticles. Afterward, the AgNPs were rinsed and centrifuged with deionized water and then lyophilized. The lyophilized AgNPs were stored in an amber-colored bottle to be used for further characterization and potential application<sup>14</sup>.

### Characterization of nanoparticles:

#### 1. UV-visible Spectroscopy

The presence of Au-NPs is confirmed by UV-vis spectrain. The results showed that there is no obvious peak for *G. mangostan* peel extract. However, after the addition of tetrachloroaurate, a sharp peak appears at the range of 540–550 nm. It is further confirmed by other characterizations that this peak indicates the formation of monodispersed spherical shape Au-NPs. The reaction takes place within 3 minutes with obvious colour change<sup>15</sup>.

#### 2. X-Ray Diffraction Analysis

AuNPs synthesized from precursors at different concentrations such as 0.001 M and 0.01 M are shown in Fig. 2. Peaks at 38°, 44°, 64° and 77° are assigned to the face centered cubic (fcc) units of Au, which are represented by Bragg diffraction planes of (111), (200), (220) and (311) respectively [JCPDS file No. 01-089-3697]. Apart from aforementioned peaks, additional peaks are also observed, which are indicated by asterisk. These may be due to the bio-inorganic compounds and protein matters present in the extract. The average grain sizes calculated using Scherrer formula are 15 nm (with 0.001 M) and 20 nm (with 0.01 M) chloroauric acid<sup>16</sup>.

#### 3. Dynamic Light Scattering (DLS) and Zeta Potential ( $\zeta$ )

DLS analysis of the generated AuNPs showed an average hydrodynamic diameter of 51.8 ± 0.8 nm. The polydispersity index of the AuNPs was 0.340%, which is consistent with a 'medium monodisperse' distribution. Medium monodispersity may arise from the size or shape heterogeneity. TEM images confirmed the presence of various geometries in the samples that are dominated by spheres. Zeta potential values are often used as a hallmark indication of the stability of colloidal particles. The absolute values replicate the net electrical charge on the particles' external surface that arises from the surface functional groups. Nanoparticles are considered to exist as stable colloids if their zeta potential is more than 25 mV or less than -25 mV. The zeta potential of the AuNPs was -40.4 ± 0.2 mV; the suspension of

AuNPs in a buffer formed a stable colloid (well-dispersed) with no visible aggregation over 6 months<sup>17,18</sup>.

#### 4. Particle Size Analysis:

Laser diffraction particle size analyzer provides the detail about the particle nature, such as monodispersed, didispersed and polydispersed. Our investigation revealed that nanoparticles show polydispersity at 0.419 indexing and various sizes of nanoparticles ranging with effective diameter around 441<sup>19</sup>.

#### 5. FESEM analysis:

The surface morphology of AgNPs was investigated using FESEM. The micrographs showed that synthesized AgNPs have rough surfaces and spherical and uneven irregular beads of different sizes. The result indicates the reduction process is being held at the surface. The rough surface may be advantageous for the immobilization of enzymes. As we can see from the micrograph, the nanoparticles were not in direct contact even within the aggregates, indicating the stabilization of the nanoparticles by a capping agent<sup>1</sup>.

#### 6. Dynamic Light Scattering (DLS):

DLS is one of the most popular techniques which is used to determine the size of particles. Shining a monochromatic light beam, such as laser, onto a solution with spherical particles in Brownian motion causes a Doppler shift when the light hits the moving particle, changing the wavelength of incoming light. This change is related to the size of the particle. Using DLS, it is possible to compute the sphere size distribution and give a description of the particle's motion in the medium measuring the diffusion coefficient of the particle by using autocorrelation function<sup>20</sup>.

#### 7. Energy-dispersive X-ray spectroscopy (EDX):

EDX is an important technique for the analysis of the elemental composition of a sample and its application to nanotechnology has been documented. All elements have different atomic structures producing a unique set of peaks in the X-ray spectrum and these can be used to study the elemental composition of any nanoparticle<sup>21</sup>.

#### 8. Transmission electron microscopy (TEM)

TEM is a very useful technique for the characterization of nanoparticles, which provides

information on the site and morphology of nanoparticles. TEM has a 1,000-fold higher resolution compared with SEM and its images give more exact information related to size, shape and crystallography of the nanoparticles<sup>22,23,24</sup>.

#### 9. Auger electron spectroscopy (AES)

AES is a surface-sensitive analytical technique that derives from the interaction of an electron beam and atoms in residence at the surface of a sample and is an outstanding analytical method for nanotechnology. The oxidation state of silver as a component of a hybrid substance can be probed by AES<sup>25,26,27</sup>.

## II. CONCLUSION:

A number of plants and their extracts have been used for synthesis of silver and gold nanoparticles extracellularly. Some modern analytical techniques and antimicrobial assays have been applied to characterize the nanoparticle morphology. The use of such eco-friendly and economical nanoparticles in various diseased medications such as bactericidal, wound healing and other medical applications make biological point of view potentially exciting for bulk synthesis of other nanoparticles. From a technological point of view, the silver and gold nanoparticles have potential applications in biomedical fields, agriculture, foods and herbal research among several others. The biological procedures have considerable advantages such as cost-effectiveness, compatibility for medical and pharmaceutical applications as well as large scale commercial production. In future, it would be significant to understand the clear mechanism of biosynthesis and to technologically engineer the nanoparticles in order to achieve better control over size, shape, and Absolute uniform particles size which would further increase the use of nanoparticles in related fields.

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