CHAPTER-II

A Short Review on Synthesis of Gold Nanoparticles and its Biomedical Applications
2.1. Review of Literature on Green Synthesis of Gold Nanoparticles

Nanoparticles from various materials can be prepared by relatively simple methods. During the past few decades, a variety of different methods have been reported and reviewed for synthesizing gold colloids of monodisperse and uniform sizes particles (Turkevich, J. et al.; Frens, G. et al.; Hayat, M. A.; Schmid, G.; Dung N. et al.). Currently, there are green approaches generally carried out to prepare nanoparticles (Kharissova, O. V. et al.).

Research in the past few years has demonstrated that biological synthesis of gold nanoparticles can be equally important due to the environmental hazards caused by chemical synthesis methods. Many biological systems exert exquisite control over formation of the metal nanoclusters through concerted mechanisms. It is quite surprising that from simple microbes and bacteria up to more evolved plants it is possible to get nanoparticles (Oxana, V. et al.; Mittal, A. K. et al.; Roy, N. et al.). The production of nanoparticles is environmentally friendly because this involves natural phenomena that take place in the biological systems. Moreover, the biologically fabricated nanostructures offer substantially different properties: good adhesion, tribologically good properties, optical and electrical properties of high interest in optoelectronics. Up today, several procedures have been used for gold nanoparticle production and applications (Schröfel, A. et al.). In the last decades numerous enrichment and pure cultures isolated from a variety of environments have been shown to enzimatically reduce many dissolved heavy metals, metalloids and radionuclides to insoluble nano and microparticles. The potential application of biological manufacture gold nanoparticles has been pointed out in a few studies. In this study, it proves that how plants are able to give rise of nanomaterials of interest in science and technology.

The plant extract contains different biomolecules such as proteins, sugars, amino acids, enzymes and other traces of metals. These metabolites are strongly involved in the bioreduction process. The proposed reaction was Au⁺ ions reduction into metallic AuO nanoparticles in the presence of metabolites and redox enzymes (Thakkar, K. N. et al.). The reaction is given below.

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\text{HAu}^+\text{Cl}_4, 4\text{H}_2\text{O}^+ \text{ Plant Extracts} \rightarrow \text{Au}^{0}\text{NPs} + \text{Byproducts}
\]
Recently, the plant mediated nanomaterial has drawn more attention due to its vast application in various fields due to their physico-chemical properties. The gold nanoparticles were synthesized from natural resources and have been studied exclusively. The different parts of plant such as stem, root, fruit, seed, callus, peel, leaves and flower are used to synthesize metallic nanoparticles in various shapes and sizes by biological approaches. Biosynthesis reaction can be altered by wide range of metal concentration and amount of plant extract in the reaction medium, it may transformed the shapes and size of the nanoparticles (Muthuvel, A. et. al.\textsuperscript{117} ; Chidambaram, J. et. al.\textsuperscript{35}; Zayed, M. F. and Eisa, W. H.\textsuperscript{182}).

2.1.1. Stem as Source for Nanoparticles Synthesis
Daisy P (2012)\textsuperscript{40} Cassia fistula aqueous extract used for synthesis of gold nanoparticles and formed Au\textsuperscript{0}. The plant extract contains aldehyde group and it’s mainly involved in the reduction of gold ions into metallic Au nanoparticles. The different functional group –C=0, C=N indicates amide I, polypeptides which are the responsible compounds in the capping of ionic substances into metallic nanoparticles. The stem part of plant extract shows the different functional groups, particularly the carboxyl, amine and phenolic compounds which are involved in the reduction of gold ion. Thus, synthesized gold nanoparticles have capable in the treatment of hyperglycemia.

2.1.2. Fruits Mediated Synthesis of Metallic Nanoparticles
Mohanan (2013)\textsuperscript{113} used plant fruit bodies Citrus limon, Citrus reticulata and Citrus sinensis extract with addition of different molar concentrations of auric acid solution in order to synthesize eco-friendly AuNPs with specific morphological features. The extract contains active phytochemical compounds which are liable for the single step reduction reaction. The spherical shapes of gold nanoparticles were produced by the fruit extract. There is similar report on using of polyphenol from pear and Emblica Officinalis to synthesize and characterization gold nanoparticles (Ghodake, et. al.\textsuperscript{62}; Ankamwar, B. et. al.\textsuperscript{2}.)

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2.1.3. Seeds as Source for Nanoparticles Synthesis

The fenugreek seed extract contains high flavonoids and other natural bioactive products such as lignin, saponin and vitamins. The reduction of chloro-auric acid was carried out by using the powerful reducing agents *Abelmoschus esculentus* seed extract act as a better surfactant. The COO-group (carboxylic) C=N and C=C functional groups are present in the seed extract. The functional group of metabolites act as a surfactant of gold nanoparticles and the phenol group can stabilize the electrostatic stabilization of AuNPs (Chidambaram, J. et al.)35. The aqueous extract of *Abelmoschus esculentus* enhanced the reduction rate of gold ions. Thus, synthesized gold nanoparticles showed more activity against *Puccinia graminis* pathogenic fungus. Therefore, the biosynthesized metal nanoparticles were act as good antifungal agents.

2.1.4. Leaves Mediated Synthesis of Nanoparticles

Plant leaves extract used as a mediator to synthesis of nanoparticles was reported. Leaves of *Coleus amboinicus*, *Cassia auriculata*, *Sorbus aucuparia*, *Cinnamomum zeylanicum* and many plants leaves extract have been studied. Recently, plant leaves were stated to contain an important bioactive compound which is involved in the nanoparticles synthesis by eco-friendly method. The AuNPs have effective drug in cancer medicine to cure various oncologies and dreadful diseases. It acts as a capping agent for the formation of gold nanoparticles and may enhance the cytotoxic effects of the tumor cells (Narayanan, K. B. et al.121; Kumar, V. G. et al.91; Dubey, S. P. et al.45; Smith, S. L. et al.165).

2.1.5. Flowers as source for Synthesis of Nanoparticles

Eco-friendly method of synthesis of gold nanoparticles by using ethanol and aqueous flower extract of the plant were studied (Das, R. K. et al.42; Vankar, P. S. et al.173). The extract medium contains abundant phenol group. These functional compounds are the main sources for reduction of tetrachloro aurete salt into bulk AuNPs. Likewise, *Catharanthus roseus* and *Clitoria ternatea* diverse group of flowers are used for the metallic nanoparticles synthesis with desired sizes and shapes. The plant synthesized nanoparticles have been effectively control harmful pathogenic bacteria and similarly the medicinal usable *Nyctanthes arbor-tristis* flowers of gold nanoparticles extract is synthezed via green chemistry method (Das, R. K. et al.)42.
The aqueous extract of *Mirabilis jalapa* flowers acts as a reducing agent and produced gold nanoparticles with eco-friendly method (Vankar, P. S. and Bajpai, D.)\(^{173}\). The plant metabolites which represent the bioreduction reaction to synthesis of metallic nanoparticles and its pharmacological applications.

### 2.1.6. Secondary Metabolites effect in Bio-reduction Reaction

Several secondary metabolites and enzymes have relatively promoted the formation of metallic nanoparticles from the corresponding ionic compounds. The reduction reaction mainly involved plant biomolecules (secondary metabolites) such as sugars (polysaccharides), proteins, organic compounds, pigments and plant resins etc. Plant natural products are involved in the reduction reaction to synthesize green nanoparticles. Plants are particularly participating in defence mechanisms to produce various chemical compounds such as polyphenols, saponins, antioxidant enzymes, terpenoids and alkaloids. These secondary metabolites are known as key sources for controlling the various acute diseases (Park, Y. et. al.)\(^{134}\). The proposed reduction reaction proved that the secondary metabolites are the main factors for the biosynthesis of metallic nanoparticles. The plant extracts contain numerous functional groups such as C=C (Alkenyl), C=N (amide), O=H (phenolic and alcohol), N-H (amine), C-H and COO- (carboxylic group). It is mainly symbolized as plant secondary metabolites and might be micro and macro biomolecules (Panda, T. and Deepa, K.)\(^{133}\). These chemical substances are fully participating for the nanoparticle production. For instances *Rumex hymenosepalus* plant extract promotes the nanoparticles syntheses at room temperature with fast reaction kinetics. Therefore, the solvent extract of *Rumex hymenosepalus* is rich in polyphenols such as catechines and stilbenes molecules that act as a reducing and stabilizing agents for gold nanoparticles production (Masumeh, N. et. al.)\(^{107}\). The plants derived secondary metabolites are phenolics (Kumar, K. M et. al.)\(^{89}\), proteins (Ren, F. et. al.)\(^{148}\) and polysaccharides (Haizhen, H. and Xiurong, Y.)\(^{70}\) etc. which was synthesized nanoparticles in eco-friendly method.

### 2.1.7. Importance of Gold Nanoparticles

- It is used for purification and quality management of air, biosensing, imaging, drug delivery system.
• Biologically synthesized gold nanoparticles have many applications like coatings for solar energy absorption and intercalation material for electrical batteries, as optical receptors, as catalysts in chemical reactions, for biolabelling, and as antimicrobials.

• Though gold nanoparticles are cytotoxic but they have tremendous applications in the field of high sensitivity bimolecular detection and diagnostics, antimicrobials and therapeutics, catalysis and micro-electronics.

• It has some potential application like diagnostic biomedical optical imaging; biological implants (like heart valves) and medical application like wound dressings, contraceptive devices, surgical instruments and bone prostheses.

2.2. Earlier works and the lead points for taking the present work
In literature review, biosynthesis of gold nanoparticles using plant derivatives is extremely studied in the last two decades. The plant metabolites are induces the production of gold nanoparticles in eco-friendly manner. As a prospect, the eco-friendly synthesis of nanoparticles using plant crude extracts and purified metabolites are novel substrates for the large scale production. The plant mediated nanoparticles have the potential to be used in various fields such as pharmaceuticals, therapeutics, sustainable and renewable energy and other commercial products. The leading points of the present works are as follows:

• Biological methods of nanoparticles synthesis using plant extract have been suggested as possible eco-friendly alternatives to chemical and physical methods.

• Using plant for nanoparticles synthesis can be advantageous over other biological processes by eliminating the elaborate process of maintaining cell cultures.

• The synthesis and assembly of nanoparticles would benefit from the development of clean, nontoxic and environmentally acceptable ‘‘green chemistry’’.

• To prepare plant extract using ethanol by solvent extraction method.

• To synthesize and characterize gold nanoparticles using different part of medicinal plant using ethanol extract.

• To study anticancer properties of gold nanoparticles using different cell lines in vitro.
2.3. Scope of the Study

Nanotechnology has attracted many researchers from various fields like biotechnology, physics, chemistry, material sciences, engineering and medicine. Nanoparticles are synthesized by physical and chemical methods; these are suffering from drawbacks like expensive reagent, hazardous reaction condition, longer time, tedious process to isolate nanoparticles. Hence, there is scope to develop new methods for the synthesis of gold nanoparticles which should be required inexpensive reagent, less drastic reaction condition and eco-friendly. This study based on the preparation of gold nanoparticles via the green synthesis method, using plant extract. The main focus of the study is to prepare gold nanoparticles using green synthesis method and study its anticancer activities in vitro. This method has merits over other reported methods available as it is inexpensive, non toxic i.e., no hazardous chemical is used and pollution free. The data provide strong evidence that gold nanoparticles should be studied further as potential novel anticancer agents to treat cancer with an interesting mechanism of action.

2.4. Definition of the Problem

Synthesis and characterization of gold nanoparticles using different parts of medicinal plant extract (by ethanol extract method) and study its biomedical applications using different type of cancer cell lines such as SUDHL-4, HCT116 and K-562.