1. INTRODUCTION

1.1. NANOTECHNOLOGY

Nanoparticles are simply defined as particles in the $10^{-9}$ nm range. Comparing this size, a human hair is said to be approximately 70000 nm in diameter, a red blood cell about 5000 nm and some simple organic molecules fall within 0.5-5 nm range [1]. Nanotechnology/nanobiotechnology, on the other hand, simply denotes the man-made use of these nano-sized particles, for industrial and medical purposes. Application depends on the unique properties of each type of nanoparticle.

Nanotechnology is one of the most active research areas in modern materials science. Nanoparticles exhibit new or improved properties based on specific characteristics such as size, distribution and morphology. There have been impressive developments in the field of nanotechnology in the recent past years, with numerous methodologies developed to synthesize nanoparticles of particular shape and size depending on specific requirements. New applications of nanoparticles and nanomaterials are increasing rapidly [2].

Nanotechnology can be termed as the synthesis, characterization, exploration and application of nanosized (1-100 nm) materials for the development of science. It deals with the materials whose structures exhibit significantly novel and improved physical, chemical and biological properties, phenomena, and functionality due to their nano scaled size (Fig.1.1). Because of their size, nanoparticles have a larger surface area than macro-sized materials. The intrinsic properties of metal
nanoparticles are mainly determined by their size, shape, composition, crystallinity and morphology.

Figure 1.1: Schematic diagram of size distribution of nanoparticles.

Nanoparticles, because of their small size, have distinct properties compared to the bulk form of the same material, thus offering many new developments in the fields of biosensors, biomedicine, and bio nanotechnology. Nanotechnology is also being utilized in medicine for diagnosis, therapeutic drug delivery and the development of treatments for many diseases and disorders. Nanotechnology is an enormously powerful technology, which holds a huge promise for the design and development of many types of novel products with its potential medical applications on early disease detection, treatment, and prevention.
A wide variety of materials can be used to make these nanoparticles, such as metal oxide ceramics and silicates, magnetic materials, liposomes, dendrimers, emulsions, etc. [3]. Engineered NPs are produced by a number of chemical and physical approaches as summarized in Fig.1.2. Traditional technologies use a top-down approach for constructing materials. Most objects are created starting from a bulk materials and then breaking it into smaller pieces using mechanical, chemical or other forms of energy until they precisely form the desired construction (e.g. integrated circuits in microelectronics) [4]. Alternatively, the bottom-up approach recognizes that the building blocks of life (enzymes, and other components of each living cell), already act as machines at the nanoscale. Nanoscale materials are synthesised from atomic or molecular species via chemical reactions, allowing for the precursor particles to grow in size [5]. However, such methods are harmful as the chemicals used are often toxic, flammable, and not easily disposable due to environmental issues, have low production rate and are expensive [6]. Striving for alternative and cheaper pathways for nanoparticle synthesis, scientists contributed to the development of a relatively new and largely unexplored area of research based on the biosynthesis of nanomaterials [7]. A great deal of effort has been put into the search for methods utilizing biological systems in order to produce metal nanoparticles at ambient temperature and pressure without requiring hazardous agents and generating poisonous by-products.
Figure 1.2: Methods for the preparation and manufacture of nanoparticles.

Biosynthesis of NPs is a type of bottom-up approach where the main reaction occurring is reduction/oxidation. Various micro-organisms such as bacteria, fungi and yeasts have been suggested as nanofactories for intra- and extra-cellular synthesis of metals [8]. The use of plants for nanoparticle synthesis is a comparatively new and under-researched technique. Synthesis of metal NPs using plant extracts is very cost effective, so can be used as an economic and valid alternative for the large-scale production of metal nanoparticles [9]. The bioreduction of metal NPs by the combinations of biomolecules found in plant extracts such as enzymes, proteins, amino acids, vitamins, polysaccharides, typically obtained by contact of a broth of
plant leaves with metal salts, has been intensively investigated in recent years [10]. The efficient and rapid extracellular synthesis of Ag, Au and Cu nanoparticles using broth extracts of several plants has been reported, such as *Medicago sativa* [11], *Pelargonium graveolens* [12], wheat [13], lemongrass [14], *Humulus lupulus* [15], *Spinacia oleracea* and *Lactuca sativa* [16]. However, alfalfa (*Medicago sativa*) [17], *Chilopsis linearis* [18] and *Sesbania* seedlings [19] showed the synthesis of silver and gold nanoparticles inside living plant parts.

1.2. HISTORY OF NANOPARTICLES

Recently nanotechnology, nanoscience, nanostructure, nanoparticles are the most widely used words in scientific literature. Nanoscale materials are very attractive for possible machine, which will be able to travel through the human body and repair damaged tissues or supercomputers which small enough to fit in shirt pocket. However, nanostructure materials have potentials application in many other areas, such as biological detection, controlled drug delivery, low-threshold laser, optical filters, and also sensors, among others [20].

In fact, metal nanoparticles have been used a long time ago e.g. Damascus steel which used to make sword and Glass Lycurgus Cup which has unique color [21]. Even though, nanoparticles have been used long time ago, but nobody realized that it reached nanoparticles scale [22]. It is like just unintentionally technique to produce nanoparticles. After the modern device developed to analyze material in nanoscale, scientist can prove nanotechnology has been developed and become an interesting subject for science today.
Blade made from Damascus steel produce from about 500 AD in Damascus [23]. It become renowned because (1) the extreme strength (2) the sharpness (3) the resilience and (4) the beauty of their characteristic surface pattern [24]. The fascinating legend story it can cut clean through rock and still remain sharp enough to cut through a silk scarf dropped on the blade. Many scientists try to reveal this special properties and encounter multiwalled carbon nanotube in steel (MWNTs) [25].

The famous Glass Lycurgus Cup from the Romans times (4th century AD) contains silver and gold nanoparticles in approximate ratio 7:3 which have size diameter about 70 nm [26]. The presence of these metal nanoparticles gives special color display for the glass. When viewed in reflected light, for example in daylight, it appears green. However, when a light is shone into the cup and transmitted through the glass, it appears red (Fig.1.3). This glass can still be seen in British museum.

Figure 1.3: Lycurgus Cup (a) green color, if light source comes from outside of the cup; (b) red color, if the light source comes from inside of the cup.
Nanoparticles (1-200 nm) have unique electronic, optical, and catalytic properties. Its properties is also connected to the method how to prepare nanoparticles to control the shape and size of nanoparticles, provide exciting building blocks for nanoscaled assemblies, structure, and devices [27]. Miniaturization of structures by mechanic methods and electron-beam lithography is reaching the theoretical limits of about 50 nm. For further miniaturization of chemical object, alternative approaches must be developed and also to find the applications [28].

1.3. METAL NANOPARTICLES

Nanoparticles of metallic origin have been shown to exhibit unusual properties that they normally will not display in their bulk form [29]. Due to their huge potential and benefits to nanotechnology, they have come under intense scrutiny as far as applications across various disciplines are concerned. In biochemistry, for example, they are considered to be better catalysts [30] and good biological and chemical sensors [31]; in information systems, their size and magnetic properties are being explored in the production of data storage devices where the issue of miniaturization is posing an overwhelming challenge [32]; in medicine their potential as drug delivery agents has being reported [33].

1.3.1. General Methods for the Synthesis of Metal Nanoparticles

Metal nanoparticles are an important tool for actualizing nanotechnology within various areas since they are not only abundant in nature but all living organisms operate at a nanoscale. Since metal nanoparticles display unusual physical
and chemical properties that depend on their size and shape, it then became expedient to synthesize uniform nanoparticles with controlled morphology. Nanoparticles are the product of different physical, chemical and biological processes, some of which have long been used in the past in conventional science and technology and some that are new and radically different [34]. The synthesis of nanoparticles can occur either as part of the top-down or bottom-up process [35]. The former means the production of nanoparticles from its bulk material and involves the breakdown of the bulk material into smaller pieces by the use of chemical or mechanical means. The bottom-up approach, involves the synthesis of nanomaterials by the chemical reactions between atomic or molecular species. This approach allows for the gradual growth of the precursor particles (nucleation). Both methods can be carried out in liquid, gas or supercritical fluids, solids, or in vacuum [36].

1.3.1.1. Chemical Synthesis

The major chemical approaches available for the synthesis of nanoparticles can be grouped into two main areas: sol-gel method and gas phase synthesis. Various types of nanoparticles of diameters in the range of 1 to 100 nm with uniform crystal structure, and a high level of monodispersity have been generated by these methods with about 20% variation in their size; however, for achieving a much better quantum confinement, this value must be reduced to about 5% or less [37]. The chemical method of synthesis depends greatly on the availability of the right metal/metal-organic precursors. The main disadvantage of these approaches is the very high and harsh conditions, such as temperature and pressure and the use of highly flammable
organic solvents that are required for the production of the nanoparticles. These processes may lack scalability and the control over crystalline dispersion is very limited [38].

Sol processing is a wet method of chemical synthesis and differs from other chemical methods as nanomaterials can be produced at low temperatures in direct contrast to the other high temperature methods. Precursors used for sol-gel processing can follow one of two main routes, namely the inorganic or metalorganic routes. The inorganic route uses metal salts in aqueous solutions as raw materials, while the metal-organic route occurs in organic solvents and uses metal alkoxides as starting material. The process consists of sol formation followed by gelling, shape forming, drying and densification. The size distribution of the nanoparticles produced by this method can be controlled by the introduction of a dopant [39] or by treatment with heat.

Gas phase synthesis for the production of metal nanopowders, first reported in 1930 (Luther, 2004), incorporates a vacuum chamber with a heating element, the precursor metal to be made into nanoparticles or nanopowder, equipment for the collection of powder and a vacuum hardware. An inert gas is also used at pressures high enough to boost the formation of nanoparticles while at the same time low enough to enable the generation of spherical nanoparticles. Then, the precursor metal is placed on the already heated element and melted quickly. The melted metal is rapidly adjusted to temperatures far above the melting point of the metal, but less than its boiling point so as to allow enough vapour pressure to be easily attained. At this
point, a continued supply of gas is introduced into the chamber with excess gas removed by pumps in such a way that the gas flow removes the evaporated metal from the heating element. Nanoparticles begin to form as the gas cools the metal vapour though the nanoparticles are still liquid as a result of the very high initial temperature involved. The particles still in the liquid phase collide and fuse together in a fixed environment such that the nanoparticles can grow in a specified manner and thus remain spherical with smooth surfaces. As these liquid nanomaterials continue to cool, growth stops and since they are very reactive they must be coated to prevent aggregation with each other or other materials [40].

Other chemical techniques used in the synthesis of nanoparticles are sonochemical processing [41], cavitation processing [42], microemulsion processing [43], and high-energy ball milling [44].

1.3.1.2. Microwave Synthesis

Microwaves are electromagnetic waves. Microwave heating is used for the development of different materials. In microwave application, heating is caused by the interaction of the permanent dipole moment of the molecule with the high frequency electromagnetic radiation. The conventional method of AgNP synthesis requires the reductive reaction to occur in an oil bath, which must be held at a constant temperature of 80 °C for 5 h (on average). Due to excessive heating and extended reaction time, more eco-friendly means of AgNP synthesis are desired. An alternative green AgNP synthesis technique employs the use of microwaves. In general, microwave (MW) heating is better than a conventional oil bath when it
comes to consistently yielding nanostructures with smaller sizes, narrower size
distributions, and a higher degree of crystallization. Heating samples with microwaves
is a practical method for the greener synthesis of nanomaterials. MW heating is
advantageous as it has shorter reaction times, reduced energy consumption, and better
product yields which prevent the agglomeration of the particles formed.

Nadagouda et al., [45] have discussed the production of silver nanostructures
at length through a MW-assisted synthetic approach which shows that the one-pot
synthesis of metallic nanostructures in solutions can be conducted efficiently via MW
heating. Silver, gold, platinum, and gold–palladium nanostructures have been
prepared through this method, which illustrates the generality of this approach.
Through MW heating conditions, spherical nanoparticles can be prepared within a
few minutes and single crystalline polygonal plates, sheets, rods, wires, tubes, and
dendrites can also be formed. By altering some experimental parameters like the
concentration of metallic precursors, surfactant polymers, and solvents, or the
operational temperature, parameters like morphology and nanostructure size could be
controlled. Other than the elimination of the oil bath, MW assisted techniques, in
conjunction with benign reaction media, can also drastically reduce chemical wastes
and reaction times in several organic syntheses and chemical transformations [46].

Vongehr et al., [47] reviewed recent advances in the utilization of various
water-based synthesis routes toward the shape-controlled synthesis of silver
nanoparticles and microstructures. Several one-pot methods employing commercial
MW ovens, inexpensive/low power ultrasound cleaners, or two-electrode
electrochemistry were described. Synthesis of Ag nanostructures with various shapes in solution and their doping on unmodified silica and on/inside carbon spheres were investigated. AgNO₃ in an ethanolic medium along with PVP under MW irradiation was used to synthesize AgNPs; a surface plasmon band at 416 nm indicated that silver nanoparticles have been produced within 5s after being exposed to MW irradiation. The nanoparticles were spherical in shape and had a diameter of 10 ± 5 nm. Their fluorescence band was noticed at 491 nm [48].

Microwave dielectric heating is a fast emerging and widely accepted new processing technology for a variety of inorganic synthesis and biomedical applications [49, 50]. Compared to the conventional heating, MW irradiation shortens reaction times, easy heat control (on and off), low cost and improve yield without causing any appreciable alteration in the composition of products of a chemical reaction.

1.3.1.3. Biological Synthesis

A biological method of synthesis of nanoparticles has several advantages over the previously described chemical approaches. For instance, the chemical methods involve the use of toxic solvents, high energy consumption and generation of hazardous by-products which constitute a high risk to the environment and human health [51]. Furthermore the high cost of production and the generations of limited shapes of nanoparticles (mostly spherical) greatly diminish their potential properties and applications [52].
Consequently, there is an urgent need to design and develop other synthetic methods that will eliminate all of the above listed factors as well as produce the desired results. The biological method seems to be the right approach in achieving this. Various biosynthetic methods for the synthesis of metallic nanoparticles are currently being employed, for example the synthesis of highly stable gold, silver, platinum, palladium, selenium, titanium and other metals/metal alloys have been successfully synthesized by micro organisms such as actinomycetes, bacteria, fungi, viruses and yeasts and plants are listed in the Table-1.1. The reaction condition can be optimized by changing experimental factors such as pH, incubation time, presence of light source, temperature, the composition of the culture medium, etc. This optimization will improve the chemical composition, shape and size of the particles synthesized [53]. The shape of NPs precipitated by bacteria, cyanobacteria, algae, fungi, and plants includes spherical, oval, irregular, triangular, tetragonal, hexagonal, octahedral, rod, cubicl, icosahedral, coil or wire, plate, and thin foil, with size ranging from 1 nm to several mm.
**Table 1.1:** Biological agents used for the biosynthesis of silver and gold nanoparticles.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Biological entity</th>
<th>Nanoparticles</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacteria</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td><em>Pyrobaculum Islandicum</em></td>
<td>Au</td>
<td>[54]</td>
</tr>
<tr>
<td>2.</td>
<td><em>Lactobacillus sp.</em></td>
<td>Au, Ag, Au-Ag</td>
<td>[55]</td>
</tr>
<tr>
<td>3.</td>
<td><em>Shewanella algae</em> ATCC 51181</td>
<td>Au</td>
<td>[56]</td>
</tr>
<tr>
<td>4.</td>
<td><em>Escherichia coli</em></td>
<td>Au</td>
<td>[57]</td>
</tr>
<tr>
<td>5.</td>
<td><em>Rhodopseudomonas capsulata</em></td>
<td>Au</td>
<td>[58]</td>
</tr>
<tr>
<td>6.</td>
<td><em>Pseudomonas aeruginosa</em></td>
<td>Au</td>
<td>[59]</td>
</tr>
<tr>
<td>7.</td>
<td><em>Stenotrophomonas maltophilia</em></td>
<td>Au</td>
<td>[60]</td>
</tr>
<tr>
<td><strong>Fungus</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td><em>Colletotrichum sp.</em></td>
<td>Au</td>
<td>[61]</td>
</tr>
<tr>
<td>9.</td>
<td><em>V. luteoalbum</em></td>
<td>Au</td>
<td>[62]</td>
</tr>
<tr>
<td>10.</td>
<td><em>Thermomonospora sp.</em></td>
<td>Au</td>
<td>[63]</td>
</tr>
<tr>
<td>11.</td>
<td><em>Fusarium oxysporum</em></td>
<td>Au, Ag,</td>
<td>[64]</td>
</tr>
<tr>
<td><strong>Cyanobacteria</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12.</td>
<td><em>Plectonema boryanum</em> UTEX</td>
<td>Au</td>
<td>[65]</td>
</tr>
<tr>
<td>13.</td>
<td><em>Plectonema terebrans</em></td>
<td>Au</td>
<td>[66]</td>
</tr>
<tr>
<td><strong>Algae</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14.</td>
<td><em>Saccharomyces cerevisiae</em></td>
<td>Au</td>
<td>[67]</td>
</tr>
<tr>
<td>15.</td>
<td><em>Sargassum wightii</em></td>
<td>Au</td>
<td>[68]</td>
</tr>
<tr>
<td>16.</td>
<td><em>Fucus vesiculosus</em></td>
<td>Au</td>
<td>[69]</td>
</tr>
</tbody>
</table>

1.4. SYNTHESIS OF NANOPARTICLES BY PLANTS SYSTEM

One of the important approaches for biosynthesis of nanoparticles is employing the use of plant extract for biosynthesis reaction. In the case of *Azadirachta indica* leaf extract a competition bioreduction of Au$^{3+}$ and Ag$^{+}$ ions presented simultaneously in solution was observed. A bimetallic Au core-Ag shell
nanoparticles synthesis occurred in solution [70]. *Aloevera* leaf extract has been used for gold nanotriangle and spherical silver nanoparticles synthesis [71]. The kinetics of NPs formation was monitored by UV-vis absorption spectroscopy and transmission electron microscopy (TEM).

It was found that after about 5 h of addition of *Aloevera* extract to $10^{-3}$ M aqueous solution of AgNO$_3$ and HAuCl$_4$ led to the appearance of a yellow and red color in solution. An analysis of the percentage of triangles formed in the reaction medium as a function of varying amounts of the *Aloevera* extract showed that more spherical particles were formed with increasing in amount of *Aloevera* leaf extract. Leaf extracts of two plants *Magnolia kobus* and *Diopyros kaki* were investigated for extracellular synthesis of GNPs [72]. The GNPs were formed by treating an aqueous HAuCl$_4$ solution by the plant extract. More than 90% recovery of GNPs was observed in a few minute of reaction at a reaction temperature of $90^\circ$C.

With the use of *Emblica Officinalis* fruit extract as reducing agent, the extracellular synthesis of highly stable Ag and Au nanoparticles has also been achieved [73]. Adding to the list of plants which are showing potential for nanoparticles production for example *Cinnamomum camphora* leaf extract has been identified very recently for the production of gold as well silver nanoparticles [74]. There was a marked difference of shape control between gold and silver nanoparticles which was attributed to the comparative advantage of protective biomolecules and reductive biomolecules. In this case, the polyol components and the water soluble heterocyclic components were mainly found to be responsible for the reduction of silver ions or chloroaaurate ions and the stabilization of the nanoparticles, respectively.
An overview of some of the reported biological agent synthesizing silver and gold nanoparticles is focused in Table 1.2.

**Table 1.2:** Plants used for the biosynthesis of silver and gold nanoparticles and some of the biomedical applications.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Plant Materials</th>
<th>Nanoparticles</th>
<th>Applications</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Azadirachta indica</em></td>
<td>Ag, Au, AuAg alloy</td>
<td>Antibacterial</td>
<td>[70]</td>
</tr>
<tr>
<td>2.</td>
<td>Aloe vera</td>
<td>AgNP</td>
<td>Antibacterial</td>
<td>[71]</td>
</tr>
<tr>
<td>3.</td>
<td><em>Emblica Officinalis</em></td>
<td>AgNP</td>
<td>-</td>
<td>[73]</td>
</tr>
<tr>
<td>4.</td>
<td><em>Cinnamomum Camphora</em></td>
<td>AgNP and AuNP</td>
<td>-</td>
<td>[74]</td>
</tr>
<tr>
<td>5.</td>
<td><em>Capsicum annuum L</em></td>
<td>Au and Ag NP</td>
<td>-</td>
<td>[75]</td>
</tr>
<tr>
<td>6.</td>
<td><em>Tamarindus indica</em></td>
<td>Au nanoprism</td>
<td>Vapor sensor</td>
<td>[76]</td>
</tr>
<tr>
<td>7.</td>
<td>Coriander leaf</td>
<td>AuNP</td>
<td>-</td>
<td>[77]</td>
</tr>
<tr>
<td>8.</td>
<td><em>Cochlospermum gossypium</em></td>
<td>AgNP</td>
<td>Antibacterial</td>
<td>[78]</td>
</tr>
<tr>
<td>9.</td>
<td>Rosa damascena</td>
<td>Ag and Au NP</td>
<td>--</td>
<td>[79]</td>
</tr>
<tr>
<td>10.</td>
<td><em>Annona squamosa</em> leaf</td>
<td>AgNP</td>
<td>Cytotoxic effect on MCF-7 cells</td>
<td>[80]</td>
</tr>
<tr>
<td>11.</td>
<td><em>Cylindrocladium floridanum</em></td>
<td>Ag and Au NP</td>
<td>Nitrophenol reduction</td>
<td>[81, 82]</td>
</tr>
<tr>
<td>12.</td>
<td><em>Ocimum sanctum</em> (Tulsi)</td>
<td>Ag NP</td>
<td>Antimicrobial</td>
<td>[83]</td>
</tr>
<tr>
<td>13.</td>
<td>Tea leaf</td>
<td>Au, Ag and Fe</td>
<td>Dye removal</td>
<td>[84]</td>
</tr>
<tr>
<td>14.</td>
<td><em>Avena sativa</em></td>
<td>Au NP</td>
<td>--</td>
<td>[85]</td>
</tr>
<tr>
<td>15.</td>
<td><em>Acalypha indica</em></td>
<td>Ag NP</td>
<td>Antimicrobial</td>
<td>[86]</td>
</tr>
<tr>
<td>16.</td>
<td><em>Citrus sinensis</em></td>
<td>Ag NP</td>
<td>Anti-lipid peroxidation assay and anticancer activity</td>
<td>[87]</td>
</tr>
<tr>
<td>17.</td>
<td><em>Rosa rugosa</em></td>
<td>Ag and Au NP</td>
<td>--</td>
<td>[88]</td>
</tr>
<tr>
<td>18.</td>
<td><em>Sorbus aucuparia</em> leaf</td>
<td>Ag and Au NP</td>
<td>--</td>
<td>[89]</td>
</tr>
<tr>
<td>19.</td>
<td><em>Tanacetum vulgare</em></td>
<td>Ag and Au NP</td>
<td>--</td>
<td>[90]</td>
</tr>
<tr>
<td>20.</td>
<td><em>Medicago sativa</em></td>
<td>Ag NP</td>
<td>Antimicrobial</td>
<td>[99]</td>
</tr>
</tbody>
</table>
1.5. THE FORMATION MECHANISM OF METAL NANOPARTICLES

Biosynthesis is the phenomena which takes place by means of biological processes or enzymatic reactions. These eco-friendly processes are referred as green and clean technology, and can be used for better synthesis of metal nanoparticles from microbial cells [92]. Microorganisms can survive and grow in high concentration of metal ion due to their ability to fight against stress [93]. The exact mechanism for the synthesis of nanoparticles using biological agents has not been devised yet as different biological agents react differently with metal ions and also there are different biomolecules responsible for the synthesis of nanoparticles. In addition, the mechanism for intra- and extracellular synthesis of nanoparticles is different in various biological agents.

According to Beveridge et al (1997), the mechanisms which are considered for the biosynthesis of nanoparticles includes efflux systems, alteration of solubility and toxicity via reduction or oxidation, bioabsorption, bioaccumulation, extracellular complexation or precipitation of metals, and lack of specific metal transport systems [94]. The cell wall of the microorganisms also plays a major role in the intracellular synthesis of nanoparticles. The cell wall being negatively charged interacts electrostatically with the positively charged metal ions. The enzymes present within the cell wall bioreduce the metal ions to nanoparticles, and finally the smaller sized nanoparticles get diffused of through the cell wall.

Mukherjee et al., (2001) reported stepwise mechanism for intracellular synthesis of nanoparticles using Verticillium species. The mechanism of synthesis of
nanoparticles was divided into trapping, bioreduction and synthesis. Similar mechanism was also found in fungus for the synthesis of nanoparticles. Moreover, in the case of bacteria *Lactobacillus* sp, Nair and Pradeep (2002) observed that during the initial step of synthesis of nanoparticles, nucleation of clusters of metal ions takes place, and hence there is an electrostatic interaction between the bacterial cell and metal clusters which leads to the formation of nanoclusters. Lastly, the smaller sized nanoclusters get diffused through the bacterial cell wall. In actinomycetes also, the reduction of metal ions occur on the surface of mycelia along with cytoplasmic membrane leading to the formation of nanoparticles [95].

The mechanism of extracellular synthesis of nanoparticles using microbes is basically found to be nitrate reductase-mediated synthesis. The enzyme nitrate reductase secreted by the fungi helps in the bioreduction of metal ions and synthesis of nanoparticles. A number of researchers supported nitrate reductase for extracellular synthesis of nanoparticles. A similar mechanism was also reported in the case of extracellular synthesis of GNPs using *Rhodopseudomonas capsulata* [96].

### 1.5.1. Mechanism of Gold Nanoparticles (AuNPs)

The bacterium *R. capsulata* is known to secrete cofactor NADH and NADH-dependent enzymes. The bioreduction of gold ions was found to be initiated by the electron transfer from the NADH by NADH-dependent reductase as electron carrier. Next, the gold ions (Au$^{3+}$) obtain electrons and are reduced to elemental gold (Au$^0$) and hence result in the formation of GNPs. Nangia *et al.*, (2009) proposed the synthesis of GNPs by bacterium *Stenotrophomonas maltophilia* and suggested that the
biosynthesis of GNPs and their stabilization via charge capping in *S. maltophilia* involved NADPH-dependent reductase enzyme which converts Au$^{3+}$ to Au$^{0}$ through electron shuttle enzymatic metal reduction process as shown in Fig.1.4.

![Proposed mechanism of gold ions bioreduction via NADPH-dependant reductases.](image)

**Figure 1.4:** Proposed mechanism of gold ions bioreduction *via* NADPH-dependant reductases.

### 1.5.2. Mechanism of Silver Nanoparticles (AgNPs)

The nitrate reductase is responsible for the reduction of Ag$^{+}$ ions and the subsequent formation of silver nanoparticles. The same observation was reported with another strain of *F. oxysporum* and it was pointed out that this reductase was specific to *F. oxysporum*. However *Fusarium moniliforme*, did not result in the formation of silver nanoparticles, neither intracellularly nor extracellularly but contained intra and extra cellular reductases in a similar fashion as *F. oxysporum* [97]. This is an indication that probably the reductases in this kind of *Fusarium* are important for Fe (III) to Fe (II) but not to Ag (I) to Ag (0). Moreover, in *F. moniliforme* anthraquinones
derivatives were not detected unlike the case of *F. oxysporum*. Both *fusariums* were alike in the production of naphthaquinones [98] but differed in the production of anthraquinones. Probably, in our case, Ag (0) reduction was mainly due to a conjugation between the electron shuttles with the reductase participation as shown in Fig.1.5.

![Figure 1.5: Hypothetical mechanisms of silver nanoparticles by biosynthesis method.](image)

1.6. APPLICATIONS OF METAL NANOPARTICLES

Production of inorganic and metal-based nanomaterials has stimulated the development of a new field that links many disciplines of sciences for the quest for different types of nanoparticles with unique properties. Designing and development of novel and affordable techniques for scale-up production of nanomaterials have not only provided an interesting area of study but in future will also address the expanding human requirements including health safety and environmental issues etc.
In industry, the application of nanomaterials is increasing day by day, and they will soon replace the harmful or toxic chemicals conventionally used as antimicrobial agents [99]. Application of nanoparticles and their nanocomposites also offers a sound and relatively safer alternative [100] and, therefore, open up new opportunities for development of antimicrobials. With a variety of unique properties, when NPs are manipulated effectively, it can be applied to many different applications across the field of biology and medicine, environment, and technology [101] (Fig.1.6).

Figure 1.6: Applications of metal (Ag and Au) nanoparticles.
1.6.1. Medical Application

Gold and silver nanoparticles synthesized by various techniques have received special attention because they have found potential application in many fields such as catalysis, sensors, and drugs delivery system. Additionally, silver and gold nanoparticles possess an excellent biocompatibility and low toxicity.

Silver nanoparticles have important applications in the field of biology such as antibacterial agents and anticancer activity. Silver has been known to exhibit strong toxicity to wide range of microorganisms (antibacterial applications). Antibacterial property of silver nanoparticles was tested against the gram negative and gram positive microorganism such as *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* [102]. Silver nanoparticles were found to be cytotoxic to *E. coli* it was showed that the antibacterial activity of silver nanoparticles was size dependent. Silver nanoparticles mainly in the range of 1 -10 nm attach to the surface of cell membrane and drastically disturb its proper function like respiration and permeability. Antiviral behaviour of silver nanoparticles has been investigated [103]. Silver nanoparticles ranging in size from 1 to 10 nm readily interact with the HIV-1 virus via preferential binding to gp120 glycoprotein knobs. Hence, silver nanoparticles could find application in preventing as well as controlling HIV infection. Silver nanoparticles also find application in topical ointments and creams used to prevent infection of burns and open wounds. The *in-vitro* cytotoxicity of the AgNPs was evaluated Hep-2 cell lines at different concentrations by using MTT assay. The result showed that Hep2 cells proliferation were significantly inhibited by AgNPs [104].
From thesis studies it is inferred that plant extract and some of the green method mediated synthesis of gold and silver nanoparticles could be effectively utilised to cure some of the chronic diseases.

Tea leaf extract have been utilised for the synthesis of ZVI (zero valent ion) nanoparticles for the degradation of textile dyes from effluent water. Similar gold and silver nanoparticles synthesised by green method have been utilised for the catalytic reduction of nitrophenol, which is considered to be one of abrasive non degradable pollutant present in various sources. Recently gold nanoparticles synthesised by green method have been utilised for the trace element detection by usual colour changes [105]. Similarly biologically important molecules stabilised metal nanoparticles modified system can be utilised for the targeted drug delivery applications [106].

GNPs due to its biocompatibility and strong interaction with soft bases like thiols play a major role in the treatment of cancer [107]. Epithelial ovarian cancer a common malignancy of female genital tract could be cured with the use of GNPs. Vascular endothelial growth factor (VEGF) performs a vital role in the progression of ovarian cancer and also tumor growth and GNPs possess the capability to inhibit the progression of ovarian growth and metastasis [108]. Also, in case of multiple myeloma (MM), a cancer of plasma cells, GNPs are observed to inhibit the function of VEGF which induces cell proliferation. This inhibition of VEGF further leads to upregulation of cell cycle inhibitor proteins like p21 and p27 which inhibit proliferation [109]. Chronic lymphocytic leukemia (CLL), a cancer caused due to the overproduction of lymphocytes, starts in the bone marrow but could spread to other
organs also. It was reported that as GNPs possess the ability to inhibit the function of heparin-based growth factor, GNPs alone can inhibit the function of factors secreted by CLL cells and induce apoptosis.

Thus, gold nanoparticles have so many advantages in meadiacal field as they are in nanometer-size systems that can get easily into the bloodstream and around cells. Also, the multi-functional gold nanoparticles have been demonstrated to be highly stable and versatile scaffolds for drug delivery due to their properties like unique size, along with their chemical and physical properties. Their ability to tune the surface of the particle provides access to cell-specific targeting and thus controlled drug release [110].

In order to interact with biological target, a biological or molecular coating or layer acting as a bioinorganic interface should be attached to the nanoparticle. Examples of biological coatings may include antibodies, biopolymers like collagen, or monolayers of small molecules that make the nanoparticles biocompatible. In addition, as optical detection techniques are wide spread in biological research, nanoparticles should either fluoresce or change their optical properties. The approaches used in constructing nano-biomaterials are schematically presented below (Fig.1.7).
1.6.2. Environmental Application

Technologies based on NPs are currently being developed for the environmental applications for pollution control and water purification [111]. It has been investigated that bimetallic gold–palladium nanoparticles provides an active catalyst which can be used to degrade trichloroethene (TCE), which is one of the major pollutants in groundwater, into a non-toxic form. NPs incorporated in a water purification device can effectively capture and remove halocarbon-based pesticides from drinking water and can also enhance the oxidation of mercury generated from coal power plants [112]. The use of NPs as a catalyst has a major role to play in green chemistry [113]. Most industrial oxidation processes tend to use chlorine or organic...
peroxides which generates large amounts of chloride salts and chlorinated organic byproducts. NPs supported on carbon active molecular oxygen are found to be able convert alkenes to partial oxidation products such as epoxides at atmospheric pressure and at 60°C-80°C [114]. NPs have been developed for selective oxidation of the biomass-derived chemicals, furfural and hydroxymethyl furfural, to form methyl esters as well as for oxidation of carbon monoxide (CO) and trimethylamine. These chemicals are used for flavor and fragrance applications, in plastics and industrial solvents [115]. Gas sensors based on Au nanoparticles have been developed for detecting a number of gases, including CO and nitrogen oxides (NOx) [116].

Green chemistry is the design, development and implementation of chemical products and processes to reduce or eliminate the use and generation of substances hazardous to human health and the environment. Strategies to address mounting environmental concerns with current approaches include the use of environmentally benign solvents, biodegradable polymers and non toxic chemicals. In the synthesis of metal nanoparticles by reduction of the corresponding metal ion salt solutions, there are three areas of opportunity to engage in green chemistry: (i) choice of solvent, (ii) the reducing agent employed, and (iii) the capping agent (or dispersing agent) used. In this area, there has also been increasing interest in identifying environmentally friendly materials that are multifunctional. Mallikarjuna et al., [117] have reported the caffeine/polyphenols used to study functions both as a reducing and capping agent for Ag and Pd nanospheres. In addition to its high water solubility, low toxicity, and biodegradability, caffeine is the most widely used behaviourally active drug in the world which is environmentally safe.
1.6.3. Catalytic Application

Nanocatalysis has recently been a rapidly growing field which involves the use of nanoparticles as catalysts. The catalysis properties of gold and silver nanoparticles varied from their sizes and synthesis method. It is well-known that metals such as Au, Ag and Pt and metal ions can catalyze the decomposition of $\text{H}_2\text{O}_2$ to oxygen. In addition, these metal ions can catalyze luminal-$\text{H}_2\text{O}_2$ systems. It was observed, when the Ag colloid was injected, chemiluminescence emission from the luminal-$\text{H}_2\text{O}_2$ system was greatly enhanced [118]. Silver is also the most popular catalyst for the oxidation of ethylene to ethylene oxide and methanol to formaldehyde. When Au nanoparticles less than 5 nm in size are supported on base metal oxide or carbon, very active catalysts are produced. Understanding the interaction between Au nanoparticles and their support material is a key issue [119]. Au nanoparticle catalysts are highly active for the oxidation many compounds, particularly CO and trimethylamine.

The most catalytical active material has a Au core (submonolayer Pd shell) nanostructure. Pd-coated silver nanoparticles are very effective catalyst for remediation of trichloroethene (TCE) and common organic pollutant in ground water [120]. One of the potential advantages that Au catalysts offer compared with other precious metal catalysts is lower cost and greater price stability, Au being substantially cheaper and considerable more plentiful than Pt. The extraordinary optical properties of noble metal nanoparticles have been taken advantage of optical biosensors and chemosensors.
It is a well known fact that AgNPs and their composites show greater catalytic activity in the area of dye reduction and removal (Fig.1.8). Silver nanoparticles act as an electron relay, aiding in the transfer of electrons from the BH$_4^-$ ion to the dyes, and thereby causing a reduction of the dyes. BH$_4^-$ ions are nucleophilic while dyes are electrophilic. It has been proven that nucleophilic ions can donate electrons to metal particles, while an electrophilic can capture electrons from metal particles [121]. It has been shown that BH$_4^-$ ions and dyes are simultaneously adsorbed on the surface of silver particles, when they were present together.

Pal et al., studied the reduction of methylene blue by arsine in the presence of silver nano [122], while Witcomb et al., studied the catalytic activity of AgNPs on the reduction of phenosaffarin dye [123]. Ashokkumar et al., reported the reduction of methylene blue by the natural green aqueous extract of Gloriosa superba leaf extract containing AgNPs [124]. Chien et al., reported a novel magnetically recoverable Au nanocatalyst was fabricated by in situ formation of Au nanoparticles on the surface of GA-modified Fe$_3$O$_4$ nanoparticles with GA (Gum Arabic) as a reducing and stabilizing agent simultaneously. The resultant Au nanoparticles formed a 2 nm-thick layer on the surface of GA-modified Fe$_3$O$_4$ nanoparticles. Their catalytic ability was demonstrated by the study on the reduction of 4-NP to 4-AP with NaBH$_4$ [125].
1.7. PLANTS USED FOR PRESENT RESEARCH

The wider potential application of green synthesis method, we have chosen four medicinally important plants to synthesis gold and silver nanoparticles. Among the various medicinally important plants we have chosen four important species like *Aloevera, Phyllanthus niruri L, Ficus Carica* and *Cardiospermum Helicacabum L.* These species are considered important medicinally important plant to cure various diseases. Based on literature report, we have taken an initiate to isolate and identify some of the biologically active compounds including flavanoids, aloin, emodine, etc.

1.7.1. *Aloevera* Plants

*Aloevera* (family, Xanthorrhoeaceae) is a species of succulent plant that probably originated in northern Africa. Nowadays it is more cultivated in south India.
(Fig.1.9). Tamil name is Kathazhi [126]. The species is frequently cited as being used in herbal medicine since the beginning of the first century AD. Extracts from *Aloevera* are widely used in the cosmetics and alternative medicine industries, being marketed as variously having rejuvenating, healing or soothing properties. There is, however, little scientific evidence of the effectiveness or safety of *Aloevera* extracts for either cosmetic or medicinal purposes, and what positive evidence is available is frequently contradicted by other studies [127,128]. Medical uses of *Aloevera* are being investigated as well. Among the most recent properties studied for these related compounds, the effects of aloin derivatives against some human breast cancer cell lines have been reported [129]. Other activities found for these compounds include several antimicrobial properties [130] and some oxidant and antioxidant properties on free radical-induced DNA breaks [131]. The species has a number of synonyms: *A. barbadensis* Mill., *Aloe indica* Royle, *Aloe perfoliata* L. var. *vera* and *A. vulgaris* Lam. Common names include Chinese Aloe, Indian Aloe, True Aloe, Barbados Aloe, Burn Aloe, First Aid Plant [132].

*Aloevera* leaves contain phytochemicals under study for possible bioactivity, such as acetylated mannans, polymannans, anthraquinone C-glycosides, anthrones, anthraquinones, such as Aloin A, Aloin B, Aloe emodin, and various lectins [133, 134]. Some of these compounds are used to manufacture insecticides.
Figure 1.9: Medicinal plants of *Aloevera* with different images.

1.7.2. *Ficus Carica* Fruits

*Ficus carica* (Syn: *Ficus sycomorous*; family: *Moraceae*), which is commonly referred as "Fig", Tamil name is Athi fruit, grows in tropical and subtropical regions of India (Fig.1.10). The fruit can be used to treat hypoglycemic, hepatoprotective, hypolipidemic, scavenging and immune response, anti-inflammatory, anticancer, antioxidant, antipyretic, antibacterial and antifungal activities as well as used in Indian homeopathic medicine [135]. In traditional medicine the roots are used in treatment of leucoderma and ringworms and its fruits which are sweet, have antipyretic, purgative, aphrodisiac properties and have shown to be useful in inflammations and paralysis [136]. In addition, several therapeutic effects have been shown for different parts of *Ficus carica*, such as hypoglycemia, cancer suppressive [137], anthelmintic, hypotriglyceridemia, hypocholesterolemia and bovine papilomatosis.
*Ficus carica* has been reported to have numerous bioactive compounds were flavonoids such as quercetin and arabinose, β-amyrins, β-carotines, glycosides, β-setosterols and xanthonoxol [138]. Earlier chemical examination of this plant have shown the presence of psoralen, bergapten, umbelliferone, campesterol, stigmasterol, fucosterol, fatty acids, 6-(2- methoxy-Z-vinyl)-7-methyl-pyranocoumarin and 9,19-cycloarlane triterpenoid as an anticancer [139] and antiproliferative agent: 6-O-acyl-β-Dglucosyl -β-sitosterol [140], calotropenyl acetate, and lupeol acetate.

![Figure 1.10: Medicinal fruits of *Ficus carica* with different images.](image)

### 1.7.3. *Cardiospermum Helicacabum* Plants

*Cardiospermum helicacabum* (Linn), family *Sapindacea*, is a decdous, branching, herbaceous climber, which is distributed through out the plains of India (Fig.1.11). English name is Baloon vine, winter cherry, hearts pea, heart seed and Tamil name is Muttakathan. The whole plants has been used for several centuries in
the treatment if rheumatism, stiffness of limbs, snake bits [141]. Its roots for nervous
diseases, as a diaphoretic, diuretic, emetic, emmenagogue, laxative, refrigerant,
stomachic and sudorific [142]. Its leaves and stalks are used in the treatment of
diarrhoea, dysentery and headache and as a poultice for swellings. The whole plant,
rubbed up with water is applied to rheumatism and stiffness of the limb. The juice of
the plant promotes the catamenial flow during the menstrual period. It is also a
demulcent in gonorrhoea and in pulmonary affection. *Cardiospermum halicacabum*
can use for antioxidant and Hepatoprotective effect due to the presence of flavanone
[143]. In Indo-China the plant is considered anthelmintic and anti-blenorrhagic.
*Cardiospermum halicacabum* is cyanogenic due to the presence of hydrocyanic acid
releasing cyanolipids.

The plant contains cyanogenic glycosides, saponins, flavones, sterols, tannins,
alkaloids, reducing sugars, and triterpenes are reported [144]. Leaves contain pinitol,
gluconides of apigenin, chrysoeriol and luteolin. [145]. Fatty acids of the seed lipid
include arachidic, linoleic and stearic acids [146]. The major bioactive compound
present in this extract is *Cardiospermin* (2-glucopyanosyloxy-3-hydroxymethyl-3-
butenylnitrile) [147]. Seed oil also contains β-sitosterol, cyanogenic glycoside and
luteollin glucurinide-β-sitosterol is also present in the roots.
1.7.4. Phyllanthus Niruri Plants

*Phyllanthus niruri* L., (Syn. *P. fraternus* Webster), Euphorbiaceae family, is a common kharif (rainy season) weed found in both cultivated fields and wastelands, Tamil name is Kizhanelli (Fig. 1.12). Although considered a problematic weed for farmers it is a valuable medicinal for herbalists [148] and holds a reputed position in both Ayurvedic and Unani systems of medicine. Recently it has attracted the attention of researchers, because of its hepatoprotective properties [149]. No effective specific therapy is available for viral hepatitis but *Phyllanthus* niruri has shown clinical efficacy in viral Hepatitis B [150]. Its root, leaves, fruits, milky juice, and whole plants are used as medicine. The fresh root is believed to be an excellent remedy for jaundice. In many parts of India, it is commonly used for the treatment of snake bite. It is a major component of many popular liver tonics in India including Liv.-52.
The active compounds phyllanthin and hypophyllanthin have been isolated from leaves. Recently, lignans niranthin, nirtetralin, and phyltetralin have been isolated from leaves [151].

Figure 1.12: Medicinal plants of *Phyllanthus niruri* with different images.

1.8. STRUCTURE OF BIOACTIVE COMPOUNDS

The *Alovera*, *Ficus carica*, *Cardiospermum helicacabum* and *Phylanthus niruri* are used as phytomedicine because the bioactive molecules present in the plants. Some of structure of the compounds is listed in the Fig.1.13.
**Figure 1.13:** Structure of bioactive compounds for *Aloe vera* extract (a) Aloin, (b) Aloe-emodin, (c) Aloesin; *Ficus carica* extract (d) Psoralen, (e) Bergapten, (f) Quercetin; *Cardiospermum helicacabum* extract (g) Cardiospermin, (h) Pinitol, (i) Luteolin; *Phyllanthus niruri* extract (j) Phyllanthin, (k) Hypophyllanthin, (l) Phyltetraline.
1.9. LOCATIONS FOR PLANTS AND FRUIT COLLECTIONS

_Aloevera_ plant was collected from well grown _Aloevera_ plant from harvested in Thuraipakkam area, Chennai and _Ficus carica_ fruit was collected from well grown tree in Madras University campus, Guindy, Chennai, Tamil Nadu, South India. _Cardiospermum helicacabum_ and _Phyllanthus niruri_ plants were harvested from well grown plants in Nedungadu village, Kariakal, Pondicherry. The location was identified and drawn in the map in Fig.1.14.

**Figure 1.14:** Map for plants and fruit collections from various locations.