Review of Literature

2.0 REVIEW OF LITERATURE

NANOTECHNOLOGY

One of the most active research areas in the modern material science is nanotechnology (Sriram and pandidurai, 2014). Nanotechnology is used to describe the utilization and creation of materials with structural features between the atoms and bulk materials with at least one dimension in the nano range (Song and Kiml, 2009).

The field of nanoscience has blossomed over the last twenty years and the need for nanotechnology will only increase as miniaturization becomes more important in areas such as computing, sensors and biomedical application. Advances in the field largely depend on the ability to synthesize nanoparticles of various materials, shapes and sizes, as well as to efficiently assemble them into complex architectures. Nanotechnology provides the ability to engineer the properties of materials by controlling their size and this has driven research towards a multitude of potential uses for nanomaterials (Das *et al.*, 2009).

Nanotechnology is a skyrocketing multidisciplinary field of research that interweaves physics, chemistry, bionanoscience and materials science. Recently, nanobiotechnology is becoming as an emerging and remarkable technology for the production of novel functional materials such as nano-sized particles (Duncan, 2011; Sharma *et al.*, 2009). Bionanotechnology integrates biotechnology and nanotechnology for developing biosynthetic and environmental-friendly technology for synthesis of nanomaterials (Kudle *et al.*, 2013).

NANOPARTICLES

Development of green nanotechnology is generating interest for researchers toward ecofriendly biosynthesis of nanoparticles (Singhal *et al.*, 2011). The application of nanoscale materials and structures, ranging from 1 to 100 nanometers (nm), is an emerging area of nanoscience and nanotechnology. Nanomaterials provide solutions to technological and environmental challenges in the areas of solar energy conversion, catalysis, water-treatment and medicine. The development of techniques for the controlled synthesis of nanoparticles of welldefined size, shape and composition, to be used in the biomedical field and areas such as electronics and optics, has become a big challenge. Development of reliable and eco-friendly processes for synthesis of metallic nanoparticles is an important step in the field of application of nanotechnology. One of the options to achieve this objective is to use 'natural factories' such as biological systems (Gurunathan *et al.*, 2009).

Nanoparticle development has restored interest in the antimicrobial effects of metals, which declined following the widespread application of modern synthetic antibiotics (Kim *et al.*, 2009). Nanoparticles are considered to be the building blocks of next generation of electronics, optoelectronics and various chemical biochemical sensors, in diagnostics and therapeutics (Pavani *et al.*, 2013).

Nanoparticles have been widely used in various real world biomedical, industrial and scientific applications. Several types of metal nanoparticles (MNPs) such as gold, silver, zinc and copper have been successfully synthesized with various anticipated applications that includes nutrition and neutraceuticals, medicine and diagnosis and electronics and optics (Duncan, 2011; Sharma *et al.*, 2009). Various physical and chemical methods have been utilized for the production of silver, gold and other nanoparticles successfully (Bankura *et al.*, 2012; Wei *et al.*, 2012).

APPLICATIONS OF NANOPARTICLES

Nanotechnology and engineered nanoparticles has become an emerging field in the area of materials science. Nanotechnologies manipulate matter at an atomic scale creating new nanoproducts with novel properties. The novel properties of those types of nanoparticles have been widely investigated for their use in medicine, cosmetics, technology and environment. One of the most challenging fields of materials science is the one involving biomaterials. The research takes a lot of effort in order to provide new and improved biomaterials with specific applications in medicine (Hutchison, 2008).

The continuing appearance of antibiotic resistance in microorganisms challenges the scientific community to constantly develop new bioactive compounds and drug targets with high biocompatibility and antibacterial properties (Gutierrez *et al.*, 2010; Jaiswal *et al.*, 2010). Nanoparticles are used as potential drug carriers in the treatment of cancer (Hojo *et al.*, 2011).

SILVER NANOPARTICLES

Metal nanoparticles have a high specific surface area and a high fraction of surface atoms. Because of the unique physicochemical characteristics of nanoparticles, including optical properties, catalytic activity, antibacterial properties, electronic properties, and magnetic properties, they are gaining the interest of scientist for their novel methods of synthesis.

Silver is well known for possessing an inhibitory effect toward many bacterial strains and microorganisms commonly present in medical and industrial processes. In medicines, silver and silver nanoparticles have a wide application including skin ointments and creams containing silver to prevent infection of burns and open wounds, medical devices and implants are prepared with silver-impregnated polymers. In textile industry, silver-embedded fabrics are now used in sporting equipment (Singhal *et al.*, 2011).

Silver nanoparticles play a tremendous role in the field of biological system, living organisms and medicine when compared with any other nanoparticles (Gurunathan *et al.,* 2009; Jain *et al.,* 2009; Parashar *et al.,* 2009). Silver nanoparticles are a good choice of inorganic nanomaterials in combination with various classes of antibiotics for use against pathogenic micro-organisms because of their surface chemistry and chemical stability of the silver nanoparticles, their appropriate size and the capacity to maintain a constant shape and size in solution (Ghosh *et al.,* 2012).

Ag nanoparticles (AgNPs) are the first commercialized NPs that are nowadays used as broad-spectrum antimicrobials in over 300 consumer products including clothing, cosmetics, detergents, water filters, electronics, dietary supplements, and children's toys (Marambio-Jones and Hoek, 2010; Cerkez *et al.*, 2012). Actually, colloidal silver, e.g., protein-stabilized nanosized Ag particles, has been used for numerous medical purposes (Nowack *et al.*, 2011).

Silver nanoparticles play a significant role in the field of biological systems, living organisms and medicine (Gurunathan *et al.*, 2009; Jain *et al.*, 2009; Parashar *et al.*, 2009). In addition, silver-containing consumer products such as colloidal silver gel and silver-embedded fabrics are now used in sporting equipment (Song and Kim, 2008).

METHODS FOR SYNTHESIS OF SILVER NANOPARTICLES

Reducing the particle size of materials is an efficient tool for improving their bioactivity. A number of preparation methods have been described for the synthesis of metallic nanoparticles (Pal *et al.*, 2007) such as, reverse micelles process (Xie *et al.*, 2006), salt reduction (Pillai and Kamat, 2004), microwave dielectric heating reduction (Patel *et al.*, 2005), ultrasonic irradiation

(Salkar *et al.*, 1999), radiolysis (Karim *et al.*, 2007; Remita *et al.*, 2007), solvothermal synthesis (Starowicz *et al.*, 2006), electrochemical synthesis (Liu *et al.*, 2001) and biological techniques (Naik *et al.*, 2002). The most widespread method of synthesis of metallic nanoparticles is based on the chemical reduction of metal salt solution by a reducing agent (Szczepanowicz *et al.*, 2010).

But the chemicals used in such processes are quite often toxic and flammable which make them unsuitable for many applications including biological applications like impregnation in wound dressings (Maneerung *et al.*, 2008; Vivekanandhan *et al.*, 2012) and sutures (Augustine and Rajarathinam, 2012). Biological syntheses of nanoparticles are not only a good way to fabricate benign nanostructure materials, but also to reduce the use or generation of hazardous substances to human health and the environment (Jacob *et al.*, 2012). These biological methods are regarded as safe, sustainable, cost-effective and environment friendly as well as they do not require any special culture preparation and isolation techniques (Gardea *et al.*, 2003). Biosynthesis of silver nanoparticles using microorganisms like bacteria (Saifuddin *et al.*, 2009), fungi (Duran *et al.*, 2009) and yeast (Kowshik *et al.*, 2003) are already reported. However, exploitation of the plant extracts as the potential agents for the biosynthesis of nanoparticles has opened a new way- the green synthesis of nanoparticles (Augustine *et al.*, 2013).

Nanoparticles can be synthesized using various approaches including chemical, physical, and biological. Although chemical method of synthesis requires short period of time for synthesis of large quantity of nanoparticles, this method requires capping agents for size and stabilization of the nanoparticles. Chemicals used for nanoparticles synthesis and stabilization are toxic and lead to non-ecofriendly byproducts. The need for environmental non-toxic synthetic protocols for nanoparticles synthesis leads to the developing interest in biological approaches which are free from the use of toxic chemicals as byproducts. Thus, there is an increasing demand for ''green nanotechnology''. Many biological approaches for both extracellular and intracellular nanoparticles synthesis have been reported till date using microorganisms including bacteria, fungi and plants (Singhal *et al.*, 2011).

Nanoparticles can be prepared using a variety of chemicals and physical methods, including chemical reduction, photochemical reduction, electrochemical reduction and heat vaporization. The reagents can be inorganic compounds, such as hydrazine, sodium/potassium borohydrate and salts of tartrate or organic compounds, like ascorbic acid, sodium citrate and amino acids, which are capable of being oxidized. Because noble metal nanoparticles are now widely used in areas of human contact, there is a need to develop environmentally friendly processes that do not use toxic chemicals in their synthesis. A quest for an environmentally sustainable synthetic process has led to several biometric approaches, one of the fundamental processes of which involves bioreduction (Arunachalam *et al.*, 2013).

There are various choices for the biological synthesis of nanoparticles. For example synthesis of particles using microorganisms, enzymes, fungus and plants or plant extracts (Dubey *et al.,* 2009; Jae and Beom, 2009) have been suggested as alternatives for chemical and physical methods. At times it is advantageous to synthesise nanoparticles using plants or parts of plants over other biological processes (Ponarulselvam *et al.,* 2012).

PLANT MEDIATED SILVER NANOPARTICLE SYNTHESIS

Plant mediated nanoparticles synthesis has led to a remarkable progress via unfolding a green synthesis protocol towards nanoparticles synthesis. It seems to have drawn quite an unequivocal attention with a view of reformulating the novel strategies as alternatives for popular conventional methods. The production of nanoparticles by plants relies on various factors among which, type of processing with optimized parameters is very much essential towards synthesis of nanoparticles such as growing plant in a media incorporated with raw material for the synthesis of nanoparticles, use of dried powdered plant material which is employed in the synthesis of plant material, drying plant material and evaluating nanoparticles synthesis and employing fruits and flowers in the synthesis of nanoparticles (Baker *et al.*, 2013).

The recent development and implementation of advance technologies have emerged the nano-revolution which provides the tools and technology as platforms for the investigation of biological entities which offer inspiration models for bio-assembled components toward synthesis of nanoparticles. Biosynthesis of nanoparticles is a type of bottom up approach which employs a biological system or its components for the formation of nanoparticles, where the main reaction is reduction of raw metal into nanoparticles (Li *et al.*, 2007).

The production of nanoparticles by plants were known to be stable than the nanoparticles synthesized by microorganisms. The use of plants in synthesis of nanoparticles has become one of the popular alternatives for conventional methods. In recent years, epoch research on plants has gone through the remarkable progress with current upsurge in plant research in synthesis of nanoparticles with controlled size and shape. Thus, these unique properties of metal tolerance by plants have been exploited with respect to nanoparticles synthesis (Iravani, 2011).

Plant mediated synthesis of nanoparticles is conferred due to the presence of biomolecules such as proteins, amino acids, vitamins, polysaccharides, polyphenols, terpenoids, and organic acids such as citrates etc. present in the plants as their phyto chemicals. Apart from mediating the synthesis, these molecules also stabilize the nanoparticles formed with desired size and shape (Baker *et al.*, 2013).

Green nanoparticle synthesis has evolved into an important branch of nanotechnology because of its potential application in the biomedical, magnetics, energy science and aerospace industries. Large amounts of nanoparticles can be easily synthesized from plants and the majority of these are nontoxic (Braditchote *et al.*, 2009). They have been used in pharmaceutical drug-based industries to treat B-chronic lymphocytic leukemia, for detecting DNA, to inhibit bacteria and fungi (Parashar *et al.*, 2009).

ANTIMICROBIAL ACTIVITY

The size and the specific surface area of the silver nanoparticle affect the antibacterial activity. Specifically, the toxicity of AgNPs to *E. coli* is proportional to the relative surface area of silver oxide monolayers, which dissolve and release Ag ions upon contact with water (Sotiriou *et al.*, 2012).

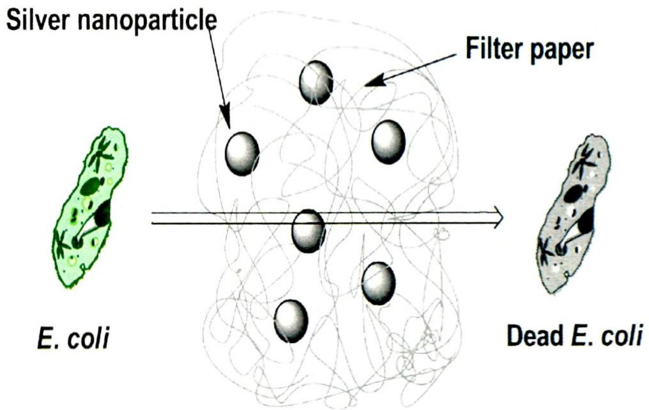
The application of silver nanoparticles as an antimicrobial agent was investigated and exhibited better antimicrobial activity against all human pathogens. Additionally, the silver nanoparticles showed good inhibition activity towards *C. albicans*. The mechanism of inhibitory action of silver nanoparticles on microorganisms is not very well known (Shameli *et al.*, 2012).

However, several mechanisms have been proposed to explain the inhibitory effect of silver nanoparticles on bacteria. It is assumed that the high affinity of silver towards sulfur and phosphorus is the key element of the antimicrobial effect. Due to the abundance of sulfur containing proteins on the bacterial cell membrane, silver nanoparticles can react with sulfur-containing amino acids inside or outside the cell membrane, which in turn affects bacterial cell viability. It was also suggested that silver ions (particularly Ag^+) released from silver nanoparticles can interact with phosphorus moieties in DNA, resulting in inactivation of DNA replication, or reacting with sulfur containing proteins, leading to the inhibition of enzyme functions which results in loss of cell viability and eventually resulting in cell death (Kim *et al.*, 2011).

Escherichia coli, *Bacillus subtilis*, *Vibrio cholerae*, *Pseudomonas aeruginosa*, *Syphilis typhus*, and *Staphylococcus aureus* are some of the organisms against which the silver nanoparticles show a potential antibacterial effect (Iravani and Zolfaghari, 2013).

Figure 1

Effect of AgNP on *Escherichia coli*



The growth inhibition of *C. albicans* was double in silver nanoparticles when compared with gold nanoparticles. The antibacterial effect was more pronounced in Gram-negative bacteria than Gram-positive ones. The antimicrobial activity of colloidal silver particles is influenced by the particle dimensions (Kaviya *et al.*, 2011). Silver has long been recognized as having an inhibitory effect on microbes present in medical and industrial processes. Gold nanoparticles are harmful to bacteria and fungi. They bind closely to the surface of microorganisms, causing visible damage to cells, with complete destruction of flagella, stimulated production of biofilm, and aggregation within the biofilm. Comparison of gold and silver nanoparticle sizes indicates the latter to be smaller than the former. The smaller particles have greater antimicrobial effects (Geethalakshmi and Sarada, 2012).

MICROORGANISM – CAUSATIVE AGENT OF INFECTIOUS DISEASES

The development of antimicrobial agents has been undeniably one of the greatest accomplishments of modern medicine. In recent years, multiple drug resistance in both human and plant pathogens has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases (Rai *et al.*, 2011).

Bacterial and fungal pathogens are the important cause of morbidity and mortality worldwide and have a major impact on public health. They are clinically important bacterial pathogens that cause food poisoning and gastroenteritis in millions of people worldwide every year (Vasudha *et al.*, 2011).

BACTERIAL STRAINS

Pseudomonas aeruginosa is a Gram negative, aerobic, rod shaped bacterium with unipolar motility. It is an opportunistic human pathogen (Bennasar, 2010).

Escherichia coli, a Gram negative, rod shaped bacterium that is commonly found in the lower intestine of warm – blooded organisms (Bradley and Williams, 2011). *E. coli* strain can be used as a probiotic agent in medicine, mainly for the treatment of various gastroenterological diseases (Berger and Sodha, 2010).

Staphylococcus aureus, a member of the family *Micrococcaceae*, is a Gram-positive bacterium that normally colonizes the epithelial surface in 30 to 40% of humans. Despite advances in antimicrobial therapy, *S. aureus* remains a major cause of infections in the hospital setting (Ingavale *et al.*, 2005).

Salmonella typhi belongs to the family Enterobacteriaceae. It is a motile, facultative anaerobe that is susceptible to various antibiotics. *Salmonella typhi* leads to the development of typhoid or enteric fever. It is characterized by the sudden onset of a sustained and systemic fever, severe headache, nausea and loss of appetite (Mandal *et al.*, 2011).

Shigella is Gram negative, non motile, non spore forming, rod shaped bacteria, very closely related to *Escherichia coli.* It is a genus in the family Enterobacteriaceae. Shigellosis is an infectious disease caused by various species of *Shigella* (Watson, 2011).

FUNGAL STRAINS

The polymorphic yeast *Candida albicans* is an important opportunistic human pathogen causing infections that range from superficial mucosal lesions to life-threatening systemic disease. It is by far the most common cause of fungal invasive infections, which could be attributed to the little immunosuppression required to predispose an individual to invasive *Candida* infections (Correia *et al.*, 2010).

Aspergillus flavus is a saprotrophic and pathogenic fungus with a cosmopolitan distribution. It is an opportunistic human and animal pathogen causing aspergillosis in immunocompromised individuals (Gomi and Machida, 2010).

Aspergillus niger is a fungus and one of the most common species of the genus *Aspergillus*. It is one of the most common causes of otomycosis. *Aspergillus fumigatus* is currently the most important air-borne fungal pathogen causing different kinds of disease depending on the immune status of the host (Mulder, 2009).

Mucor is a microbial genus of about 3000 species of moulds commonly found in soil, digestive system, plant surfaces, and rotten vegetable matter. *Mucor* are unable to infect humans, due to their inability to grow in warm environments (Chandra and Woodgyer, 2008).

IN SILICO APPROACHES

A convergence of biochemical, mathematical and computational approaches is being applied to evaluate protein-ligand interactions for identifying pharmacological targets to modulate protein activity. Protein-ligand interactions are increasingly employed to derive three dimensional structures of protein complexes. Computational techniques have become important to understand the molecular mechanisms of biological systems, as well as in obtaining leads for therapeutic agent identification (Sridhar *et al.*, 2012).

Advances in computational techniques have enabled virtual screening to have a positive impact on the discovery process. Virtual screening utilizes docking and scoring of each compound from a dataset and the technique used is based on predicting the binding modes and binding affinities of each compound in the dataset by means of docking to an X-ray crystallographic structure. Some recent studies have focused on certain factors such as the size and diversity of the ligand dataset, wide range of targets and the evaluation of docking programs.

However, in general, it is important to visualize the docked poses of high-scoring compounds because many ligands are docked in different orientations and may often miss interactions that are known to be important for the target receptor. This study becomes more difficult as the size of the dataset increases. Therefore, another approach is to eliminate unpromising compounds before docking by restricting the dataset to drug-like compounds; by filtering the dataset based on appropriate property and sub-structural features and by performing diversity analysis (Ammiraju *et al*., 2012).

Molecular docking can be defined as an optimization problem, which would describe the "best-fit" orientation of a ligand that binds to a particular protein of interest (Jubie *et al*., 2011). Docking allows the scientist to virtually screen a database of compounds and predict the strongest binders based on various scoring functions. It explores ways in which two molecules, such as drugs and a receptor, fit together and docks to each other well. The molecules binding to a receptor inhibit its function and thus act as drug. The collection of drug and receptor complex was identified via docking and their relative stabilities were evaluated using molecular dynamics and their binding affinities, using free energy simulations (Sivakumar *et al*., 2011).

PLANTS AS A SOURCE OF MEDICINE

Researchers are increasingly turning their attention to natural products and looking for new leads to develop better drugs against cancer, as well as viral and microbial infections. Several synthetic antibiotics are employed in the treatment of infections and communicable diseases (Malar *et al*., 2011).

Medicines have made large contributions to human health and well being. In our country we are using crude plants as medicine since Vedic period. A major part of the total population in developing countries still uses traditional folk medicine obtained from plant resources. In the present era, plant and herb resources are abundant, but these resources are dwindling fast due to the onward march of civilization (Molla *et al*., 2010).

Medicinal plants represent a rich source from which antimicrobial agents obtained. The antimicrobial activities of plants may reside in a variety of different compounds. Antimicrobial drugs have caused a dramatic change not only for the treatment of infectious diseases but also for the fate of mankind. Antimicrobial chemotherapy made remarkable advances, resulting in the overly optimistic view that infectious diseases would be conquered in the near future (Saga and Yamaguchi, 2009).

India has great diversity of medicinal plants and these medicinal plants are used in Ayurvedic, Unani medicines and a number of pharmaceutical products (Jain *et al.,* 2013). Application of green chemistry to the synthesis of nanomaterials has much importance in basis of medicinal and technological aspects (Begum *et al.,* 2009; Mondal *et al.,* 2011).

Ageratina adenophora (Spreng.) is a perennial, herbaceous invasive plant which has invaded around 30 countries in tropical and subtropical zones of the world (Wang, 2005).

A. adenophora is seldom attacked by bacteria, fungi and insects, suggesting that rich bioactive secondary metabolites that might be defense related might exist in this plant. Previously, structurally diverse chemicals including (mono-, sesqui-, di-, and tri-) terpenoids, phenylpropanoids, flavonoids, coumarins, sterols and alkaloids were reported from this species some of which were shown to possess allelopathic, phytotoxic and antifeedant activities (Zhao *et al*., 2009; Zheng *et al*., 2012).

Presence of flavonoid glycosides in the leaves has been reported. Many flavonol glycosides: 4′- methylquercetagetin 7-O-(6"-E-caffeoyl-β-d-glucoside) and quercetagetin 7-O-(6"-acetyl-β-d-glucoside), 7-*O*-glucosides of 6-hydroxykaempferol, quercetin and quercetagetin, 3,5,7-Trihydroxy-6,4′-dimethoxyflavone (betuletol and its 3-galactoside) have been isolated and characterized from the leaves of *A. adenophora* (Yang *et al*., 2013). A new quinic acid derivative, 5-*O*-*trans*-*o*-coumaroylquinic acid, was isolated from the aerial parts of the invasive plant *A. adenophora* (Spreng.), along with chlorogenic acid methyl ester, macranthoin F and macranthoin G. All the compounds showed *in vitro* antibacterial activity towards the assayed bacterial strains (Zhang *et al*., 2013).

The methodology adopted in the present study is presented in the next chapter.

PLATE 1

Ageratina adenophora