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*Introduction*

## 1.0 INTRODUCTION

Nanotechnology is an interdisciplinary area of science which has been developing interest across the globe with huge momentum to usher in forming nano revolution. Nanotechnology deals with the synthesis of nanoparticles which has encountered immense progress due to innumerable applications (Cheon and Horace, 2009). Nanoparticles (NPs) are particles less than 100 nm in diameter which exhibit enhanced size-dependent properties compared to its bulk material (Baker and Satish, 2012).

For over 5,000 years ago, the use of nanomaterials for human health was reported in the Ayurveda medicine of Indian system, in which nanoscience technology was applied even before the term “nano” was coined (Dubey *et al.*, 2010). Nanoparticles show completely new and improved properties, such as size, morphology and distribution. Novel applications of nanomaterials and nanoparticles are emerging rapidly on various fields (Kaviya *et al.*, 2011).

Production of nanoparticles can be done through different methods, such as, chemical and photochemical reactions in reverse micelles, reduction in solutions, thermal decomposition of silver compounds, electrochemical, sonochemical, radiation assisted, microwave assisted method and biological routes (Sulaiman *et al.*, 2013).

Nanoparticles synthesis from biological methods using microorganisms, enzyme, and plant or plant extract offers numerous benefits over chemical and physical methods (Sunkar and Nachiyar, 2012). It is cost effective, environmental friendly, easily develop for large scale synthesis (Kouvaris *et al.*, 2012). In this method there is no need to use high pressure, temperature, energy and toxic chemical that may have adverse effect in the medical applications (Sivakumar *et al.*, 2012).

Metal nanoparticles have different properties because of their small sizes when compared to bulk metals and these materials can be employed in various photoelectronic, magnetic, catalytic, sensor and biomedical applications (Maria *et al.*, 2010). Metal nanoparticles have drawn a lot of attention due to their unusual physical and chemical properties, which largely differ from their bulk properties. They show unique properties such as chemical stability, excellent conductivity, and catalytic activity, etc. which are dependent on the particle size, distribution and shape (Zhang *et al.*, 2010).

Silver has the highest electrical and thermal conductivity among all the metals. Silver materials with zero, one, or two-dimensional nanostructures such as nanoparticles, nanowires, and nanocubes have great potential for applications in optics and catalysis (Ahn *et al.*, 2009; Takenobu *et al.*, 2009).

Silver (Ag) is a metal that inactivates the microbes by interacting with their enzymes, proteins, DNA to restrain cell proliferation or cell division. It also prevents the bacterial regeneration by binding to the negatively-charged bacterial cells which change the functionality of the cell membrane. When compared to other usual biocide, silver is a durable biocide with high temperature stability and very low volatility (Maria *et al.*, 2010).

Silver has been recognized as having an inhibitory effect towards many bacterial strains and micro organisms commonly present in medical and industrial processes (Mostafa *et al.*, 2011). Many attempts have been made silver nanoparticles to use as an anti-cancer agent (Vaidyanathan *et al.*, 2009).

Silver nanoparticles (AgNPs) are widely used when compared to other metal nanoparticles, including spectrally selective coating for biolabeling, solar energy absorption, intercalation materials as optical receptors for electrical batteries, antibacterial agents, nonlinear optics and catalysts in chemical reactions (Shameli *et al.*, 2012).

Ag and AgNPs are widely used in medical industry as topical ointments that prevent infections in burns and open wounds (Pasupuleti *et al.*, 2013). The first commercialized NPs are AgNPs that are used as broad-spectrum antimicrobials in consumer products such as cosmetics, clothing, detergents, water filters, dietary supplements, electronics and children's toys (Bondarenko *et al.*, 2013). The silver nanoparticle can be used to fabricate flexible electronics by ink-jet printing due to their low sintering temperatures (Hsu and Wu, 2011).

Research on plants has gone through the remarkable progress with current increase in plant research in synthesis of nanoparticles with controlled size and shape. Synthesis of nanoparticles through plants has become one of the popular alternatives for conventional methods. Heavy metal tolerance by plants is a well-known natural phenomenon which results in phytoremediation and phytomining (Iravani, 2011).

The silver nanoparticles are well-known for their antimicrobial and anti-inflammatory wound healing abilities. The antimicrobial properties of silver ions are widely used as bactericide in surgical prostheses and dental implants (Augustine and Rajarathinam, 2012).

The most widespread method of metallic nanoparticles synthesis 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 is not suitable for many applications including biological applications like impregnation in wound dressings (Vivekanandhan *et al.*, 2012).

Plants have a major role in the synthesis of nanoparticles to overcome the problem of toxicity in synthesis. The different-shaped polyol and water-soluble heterocyclic components of plant biomolecules have both protective and reductive activity and are mainly responsible for the reduction of silver and gold ions using chemical and radiation methods (Arunachalam *et al.*, 2013). Biological synthesis of nanoparticles is not only a good way to fabricate benign nanostructure materials, but also to reduce the use and generation of hazardous substances to human health and the environment (Jacob *et al.*, 2012).

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, based on their use in traditional medicine. Plants are the basic source of knowledge of medicine. The basic molecular and active structures for synthetic fields are provided by rich natural sources (Preethi *et al.*, 2010).

The medicinal properties of plants have been investigated in the recent scientific development throughout the world, due to their potent antioxidant and antimicrobial activity, no side effects and economic viability (Panda *et al.*, 2011). Medicinal plants are the principle health care resources for the majority of the people all over the world (Kumar *et al.*, 2011). Medicinal plants are those that provide people with medicines to treat illness, maintain and promote health (Sivasankari, 2010). Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases (Dash *et al.*, 2011).

*Ageratina adenophora* (Spreng) (Syn. *Eupatorium glandulosum*, *Eupatorium adenophorum*) is a profusely branching under shrub up to 90-120 cm in height with a few

ascending branches, leaves simple, opposite, subsessile, subentire, lanceolate and glabrous type which belongs to the family Asteraceae. *A. adenophora* is a very common weed and invasion of this weed have replaced the larger part of the vegetation in throughout our country and are considered as a major threat to native plants and animals. It is reported to possess diverse medicinal properties and finds use in traditional medicines. The leaves are used as astringent, thermogenic and stimulant in folklore medicine in India (Vasanthi and Gopalakrishnan, 2013).

*A. adenophora* leaf juice is used to stop bleeding of cut and wounds, forming clots. Root juice is used to treat fever. Pure juice of the leaf is dropped in the eye for insomnia treatment. A decoction of the plant has been recommended to treat jaundice and ulcers (Subba and Kandel, 2012).

Computational (*in silico*) methods have been developed and are widely applied to pharmacology hypothesis development and testing. These *in silico* methods include quantitative structure-activity relationships, databases, pharmacophores, homology models, similarity searching and other molecular modeling, data mining, machine learning, data analysis tools and network analysis tools. Such methods have seen frequent use in the discovery and optimization of novel molecules with affinity to a target, the clarification of distribution, absorption, excretion, metabolism and toxicity properties as well as physicochemical characterization (Noori and Spanagel, 2013).

Over the years, new technologies such as comparative modeling based on natural structural homologues have emerged and began to be exploited in lead design. These together with advances in high throughput screening technologies, combinatorial chemistry and computational infrastructures, have rapidly bridged the gap between medicinal chemistry and theoretical modeling. Docking is used to optimize and identify drug candidates by examine and modeling molecular interactions between ligand and target molecules. Docking is the process by which two molecules fit together in 3D space (Rahman *et al.*, 2012).

Eupalitin is a bioactive compound isolated from *A. adenophora*, a kind of flavonoid, are effective components of some medicines (Wei *et al.*, 2011). Dihydrofolate reductase (DHFR) is an important target for several human diseases, namely, fungal, bacterial infection, psoriasis, protozoal, neoplastic disease and autoimmune diseases. Several DHFR inhibitors are reported as

potential drug candidates in various diseases. Inhibition of DHFR enzyme has found to be used as antiprotozoal, antitropical and antimicrobial agents (Sharma and Chauhan, 2012).

The present study was undertaken to analyze the antimicrobial potential of AgNPs and to analyze the efficacy of eupalitin against antimicrobial target protein dihydrofolate reductase.

The objectives of the present study were:

1. To optimize the method for the synthesis of silver nanoparticles using *A.adenophora* leaf extract
2. To characterise the synthesized nanoparticles
3. To determine the antibacterial potential of the silver nanoparticles
4. To check the potential of the silver nanoparticles against the fungal pathogens.
5. To evaluate the efficacy of the eupalitin against the target protein dihydrofolate reductase of bacterial and fungal pathogens.
6. To check the bioavailability of eupalitin on an *in silico* platform.

The literature pertaining to the present study is briefly reviewed in the next chapter