

Review of Literature

2. REVIEW OF LITERATURE

Man has been using herbal medicine for healing right from the beginning of human civilization. Herbal medicines involve the integration of several therapeutic experiences and practices of indigenous systems of medicine that may span many previous generations, which often provides valuable guidelines to the selection, preparation and application of herbal formulation for the treatment, control and management of variety of ailments. According to World Health Organization (WHO, 2001) 60% of the world's population depends on traditional medicine and 80% of the population in developing countries depends almost entirely on traditional medicine practices and herbal medicines for their primary healthcare needs. Alleviation of diseases and maintenance of good health using herbal medicines is as old as mankind and is the most popular form of healthcare practice known to humanity that has been practiced by all cultures in all ages throughout the history of civilization. Herbal medicine has evolved throughout the timeline of human civilization (Chikezie and Ojiako, 2015).

2.1 Ayurveda, The Traditional Medicine System of India:

India has a rich tradition of herbal medicine as evident from Ayurveda, which could not have flourished for two thousand years without any scientific basis. Ayurveda which literally means knowledge (Veda) of life (Ayur) had its beginning in Atharva-veda (Circa 1500-1000 BC) (Pal and Shukla, 2003). In India herbal medicine, the Rig-veda, a collection of Hindu sacred verses, contains most aspects of Vedic science such as yoga, meditation, mantra and Ayurveda, which are still widely practiced today (Grover *et al.*, 2002).

Ayurvedic medicines had an important place in human healthcare since ancient time. The administration of Ayurvedic medicines has become much popular since last few decades. Ayurvedic medicines are herbal, mineral and herbomineral compounds, which are dealt under Rasshastra and Bhaishjya Kalpna (Sharma and Sharma, 2015). Ayurveda bears the testimony of the preservation of available Medical knowledge in India Sub-continent as the

only Surviving system of medicine amongst all over the World, being currently practiced. The indigenous system of medicine is the best accepted method of the treatment and it is widely practiced in Village and Cities of the Countries of the World to-day (Nath and Nath, 2015). An ancient idea is to find healing powers in plants. Dating back to prehistory, people on all continents have long applied poultices and imbibed infusions of hundreds, if not thousands, of indigenous plants. In ethnomedicine, around the world plants are still widely used. Historically, therapeutic results have been mixed; quite often cures or symptom relief resulted (Cowan, 1999).

Currently, 25% - 50% of all pharmaceuticals dispensed have higher-plant origins, very few are intended for use as antimicrobials. Since the advent of antibiotics in the 1950s, the use of plant derivatives as antimicrobials has been virtually nonexistent. Two reasons create interest in the topic of antimicrobial plant extracts: i) phytochemicals will find their way into the arsenal of antimicrobial drugs prescribed by physicians; several are already being tested in humans. ii) Public aware the problems with the over-prescription and misuse of antibiotics. Recently, scientists realize that the effective life span of any antibiotic is limited.

New sources, especially plant sources, are also being investigated. In addition, many people are interested in having more autonomy over their medical care. A multitude of plant compounds (often of unreliable purity) is readily available over-the-counter from herbal suppliers and natural-food stores, and self-medication with these substances is commonplace. The use of plant extracts, as well as other alternative forms of medical treatments, is enjoying great popularity. Approximately one-third of people surveyed in the US used at least one “unconventional” therapy during the previous year (Eisenberg *et al.*, 1993).

The present study focussed on the anti-microbial and anti-inflammatory property exhibited by gold and silver nanoparticles synthesized from methanolic leaf extracts of *Clitoria ternatea* bearing blue and white flowers compared with their respective unconjugated methanolic leaf extracts.

2.2 Nanoparticles (NPs):

Various clinical and epidemiologic studies show that nonsteroidal anti-inflammatory drugs, including aspirin and cyclooxygenase inhibitors helps to prevent cancer (Umar *et al.*, 2015). Uses of plants extracts are found to be more advantageous over chemical, physical and microbial (bacterial, fungal, algal) methods for silver nanoparticles (AgNPs) synthesis (Borase *et al.*, 2014). Bondarenko *et al.* (2013) reported that fifty-five tons of silver nanoparticles (AgNPs) have been produced per year.

The word “nano” is used to indicate one billionth of a meter or 10^{-9} . NPs are clusters of atoms in the size range of 1-100 nm. “Nano” is a Greek word synonymous to “dwarf” meaning “extremely small”. Nanotechnology is a field that is mushrooming, making an impact in all spheres of human life. Nanobiotechnology represents an economic alternative for chemical and physical methods of nanoparticles formation. NPs attract greater attention due to their various applications in different fields including “nanomedicine”. The term Nanotechnology was coined by Professor Norio Taniguchi of Tokyo Science University in the year 1974 (Karthika *et al.*, 2015).

Frequent and blind use of antibiotics leads to an significant and serious problem of resistance emergence in disease causing microorganisms to various antibiotics, similarly, insecticides and pesticides, as insects and pests devise strategies to antagonize the toxic effect of commonly used insecticidal preparations (WHO, 1996). To tackle the problem of resistance, research is focused on use of noble metals, mostly silver, by converting bulk silver from silver salts like AgNO_3 and AgCl into nanoform, more particularly NPS of silver. AgNPs have many applications such as targeted drug delivery (Liu *et al.*, 2015), antimicrobial (Patil *et al.*, 2012a), anti-cancer (Jain, 2010), anti-tuberculosis (Mohanty *et al.*, 2013), amoebicidal (Borase *et al.*, 2013a), catalyst (Suja *et al.*, 2012), biosensor (Kirubaharan *et al.*, 2012), in textiles (Vankar and Shukla, 2012), agriculture (Rai and Ingle, 2012), management of insects (Patil *et al.*, 2012b), cosmetics (Borase *et al.*, 2013b), tissue regeneration (Marin *et al.*, 2015) and dye reduction (Borase *et al.*, 2014).

2.2.1 Types of Nanoparticles

Nanoparticles can be broadly grouped into two, namely, organic nanoparticles which include carbon nanoparticles (fullerene) while, some of the inorganic nanoparticles include magnetic nanoparticles, noble metal nanoparticles (like gold and silver) and semi-conductor nanoparticles (like titanium oxide and zinc oxide). There is a growing interest in inorganic nanoparticles i.e. of noble metal nanoparticles (gold and silver) as they provide superior material properties with functional versatility. Metallic nanoparticles are most promising and remarkable biomedical agents. Silver, aluminum, gold, zinc, carbon, titanium, palladium, iron, fullerenes and copper have been routinely used for the synthesis of nanoparticles. Antibiotic resistance is the world's major public healthcare problem. AgNPs and AuNPs particles play a vital role in nanobiotechnology as biomedicine against drug-resistant bacteria (Vadlapudi and Kaladhar, 2014).

2.2.2 Synthesis of Nanoparticles:

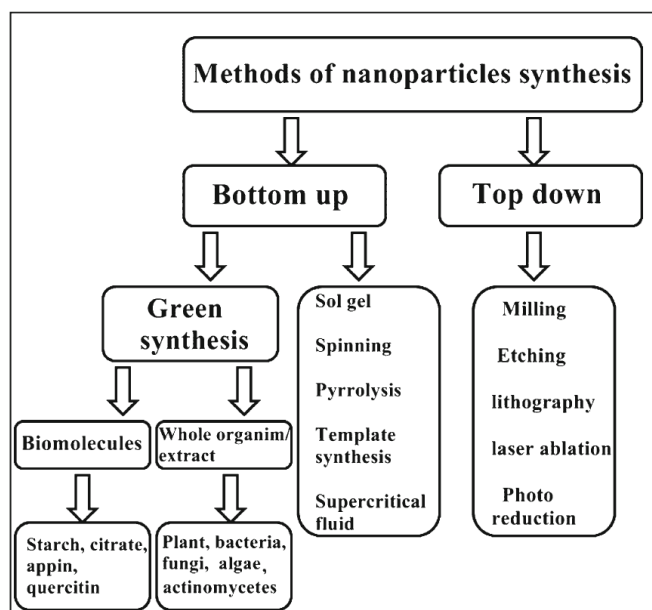
There are three different methods for the synthesis of NPs i.e., physical, chemical and biological. A schematic representation of different methods of nanoparticle synthesis is depicted in Figure 2.1. Each method has its own advantages and disadvantages such as cost, time, use of harmful chemicals and process, quality and stability of synthesized nanoparticles, etc.

2.2.2.1 Physical Methods:

Physical methods include plasma catalysis (Ghorbani *et al.*, 2011), laser ablation (Bae *et al.*, 2002; Malynych and Chumanov, 2007) to name a few. They are costly and trained manpower is required to handle critical instruments, these methods use high temperature and light, which are potentially hazardous and synthesized nanoparticles have less stability (Shahverdi *et al.*, 2007).

Figure 2.1

Different methods of synthesis of nanoparticles



(Borase *et al.*, 2014)

2.2.2.2 Chemical Methods:

Chemical synthesis of NPs requires three components: precursor (AgNPs usually from silver nitrate AgNO_3 , AuNPs usually from auric chloride HAuCl_4 , TiO_2 NPs usually from titanium tetra isopropoxide), reducing agents such as sodium borohydrate (NaBH_4) sodium citrate, ascorbate, elemental hydrogen, Tollen's reagent, N,N-dimethyl formamide, poly (ethylene glycol) block copolymers, hydrazine, ammonium formate and capping agent like polyvinyl pyrrolidone $(\text{C}_6\text{H}_9\text{NO})_n$ (Hongshui *et al.*, 2005; Ledwith *et al.*, 2007; Xiuyan *et al.*, 2012). In chemical synthesis, there is single reducing and capping agent which allow synthesis of NPs with defined shape and size which is major advantage of this method but use of hazardous chemicals, harsh reaction parameters such as high temperature, pressure and toxic by-product creates environmental concern (Zhu *et al.*, 2000; Wang *et al.*, 2002; Brichkin *et al.*, 2008; Tran *et al.*, 2013; Iravani *et al.*, 2014). There are chances of adherence of toxic chemical residues on surface of NPs and tend

to agglomerate or become insoluble in aqueous system therefore, their application in living system which is aqueous too raise concern about stability and safety. This restricts use of chemically synthesized NPs in medicine and healthcare (Mafune *et al.*, 2000).

2.2.2.3 Biological Methods:

Chemical and physical methods of NPs synthesis uses toxic chemicals, high temperature, pressure and production of hazardous by-products makes it necessary to search for safer alternative method i.e., biological synthesis of nanoparticles (Dhuper *et al.*, 2012). Different microorganisms (bacteria, fungi, algae) and various plants extracts have been reported to synthesize NPs.

a) Nanoparticles from microbial source:

Bacteria such as *Pseudomonas stutzeri* (Klaus *et al.*, 1999), *Bacillus licheniformis* (Kalishwaralal *et al.*, 2008), *Lactobacillus* *sps.* (Nair and Pradeep, 2002); fungi like *Cochliobolus lunatus* (Salunkhe *et al.*, 2011), *Fusariumoxy sporum* (Duran *et al.*, 2007), *Trichoderma* *sps.* (Thakkar *et al.*, 2010), *Aspergillus* *sps.* (Binupriya *et al.*, 2010), *Verticillium* *sps.* (Mukherjee *et al.*, 2001) and *algae* *sps.* such as *Sargassum wightii* Grevilli (Govindaraju *et al.*, 2009), *Chaetomorpha linum* (Kannan *et al.*, 2012), *Padina gymnospora* (Singh *et al.*, 2012) were reported for synthesis of AgNPs.

Microbial mode requires the search for potent strain-producing AgNPs, growth and maintenance of microbial strain on costly media, less flexibility of pH and temperature during nanosynthesis; purification of AgNPs requires cell lysis, adherence of microorganisms on nanoparticles surface create chances of infection (Borase *et al.*, 2014).

b) Nanoparticles from plant source:

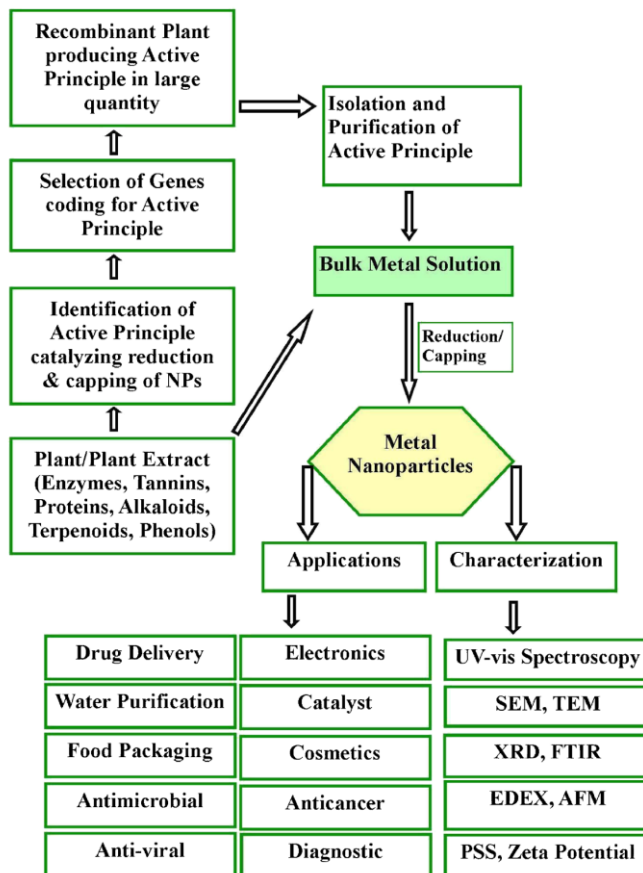
In microbial synthesis, there are chances of infection and contamination during synthesis and applications like antimicrobial, drug delivery, water purification. Microbial synthesis is time consuming and

requires maintenance of potent culture in pure form. Plants are free from such drawbacks and have higher rate of nanoparticles synthesis. Plants extract have several advantages over use of chemical, physical and microbial methods for NPs synthesis. Plants extracts do not require toxic reducing and capping agents, radiation and high temperature, microbial strain and costly media and specific conditions for microbial growth, nanoparticles production, which are very cumbersome (Rajasekharreddy *et al.*, 2010; Salunkhe *et al.*, 2011; Gan and Li, 2012; Sintubin *et al.*, 2012).

Plant extracts could be the best competitor for chemico-physical and microbial methods but it requires easy, large-scale feasible isolation and purification steps for plant metabolites responsible for nanosynthesis and use of genetic engineering tools for enhanced production of reducing and capping agents which generate stable, well-dispersed nanoparticles at the cost comparable to chemical synthesis. Song and Kim (2009) showed that AgNPs synthesized using plant extract requires less time. The rate of reduction of metal ions by neem leaf extract has been found to be much faster compared to that of using microorganisms (Mohanpuria *et al.*, 2008; Iravani, 2011; Patil *et al.*, 2012a).

NPs formed by plants extract remains stable for longer times and are suitable for use in the biomedical field (Patil *et al.*, 2012b; Mohanty *et al.*, 2013; Borase *et al.*, 2013a). Large-scale synthesis of NPs with different shapes and sizes can be obtained by changing different reaction parameters such as temperature, pH, reaction time, varying ratio and concentration of plant extract and precursor which is very tedious or not possible in microbial synthesis (Bar *et al.*, 2009; Tripathi *et al.*, 2009; Iravani, 2011). An economical point of view also gives priority to plant extracts as they are ubiquitous and easily available. Besides this, the methods of extract preparation are cheap and simple. Figure 2.2 shows a representative scheme of plant-extract-synthesized NPs.

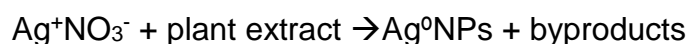
Figure 2.2
Overview of different steps in plant-extract-mediated nanoparticle synthesis



(Borase *et al.*, 2014)

i) Silver nanoparticles of plants:

AgNO₃ reacts with plant extract leads to the formation of AgNPs by following reaction (Tripathy *et al.*, 2010). The quick conversion of solution color showed the formation of silver nanoparticles by observing color change from green to yellowish-brown color. The change in color is primary due to the excitation of surface Plasmon vibrations in silver nanoparticles. Growth of the nanoparticles takes place in the alkaline pH. The alkaline pH is required for the complete formulation and structural data of the nanoparticles. The increased pH indicated the formation of nanoparticles. The reaction is given below.



Mani *et al.* (2015) synthesised silver nanoparticles and found to exhibit an enhanced anti-inflammatory activity due to the synergistic effect of alkaloids of *Piper nigrum* extract and the silver ions. The antimicrobial properties of silver nanoparticles depend on: i. Size and environmental conditions (size, pH, ionic strength) ii. Capping agent (Ahmed *et al.*, 2015).

Ullah *et al.* (2014) studied ethanolic rhizome extract of *Curcuma zedoaria* (family *Zingiberaceae*) for antinociceptive and anti-inflammatory activity and reported one or more secondary metabolite(s) to have central and peripheral analgesic and anti-inflammatory activity both *in vitro* and *in vivo*. Hanprasertpong *et al.* (2014) studied the analgesic activity of the methanol extract of *Cryptolepis buchanani* Roem. and Schult. by acetic acid-induced writhing response in mice, anti-inflammatory activity in ethyl phenylpropionate-induced ear edema and carrageenan-induced paw edema in rats and found analgesic, anti-inflammatory, and chondro protective effects.

El-Rafie and Hamed (2014) demonstrated a simple and an efficient eco-friendly approach for the biosynthesis of stable, monodisperse silver nanoparticles using aqueous extracts of four *Terminalia* species, (*Terminalia catappa*, *Terminalia mellueri*, *Terminalia bentazoe* and *Terminalia bellerica*) and characterized in terms of synthesis, capping functionalities (polysaccharides, phenolics and flavonoidal compounds) and UV-visible spectroscopy, FTIR and TEM. The results showed a simple and feasible approach for obtaining stable aqueous mono dispersive silver nanoparticles. Dose-dependent antioxidant activity of silver nanoparticles imparted by the plant phenolic and flavonoidal components was evaluated using *in vitro* DPPH assay and found to be comparable to standard ascorbic acid. In case of anti-inflammatory activity, *Terminalia catappa* and *Terminalia mellueri* have a highest inhibition percentage better than that of ascorbic acid in the carrageenan induced hind paw edema. The results also revealed that the aqueous extract

of *Terminallia catapa* and its silver nanoparticles recorded the most potent *in vivo* antioxidant effect.

A detailed analysis done by Borase *et al.* (2014) gives an overall status about AgNPs. Numerous plant extracts have been screened for the production of AgNPs with diverse shape and size. It is clear that proteins, flavonoids, terpenoids and other bio-organics factors were the responsible for formation of AgNPs in majority of the plants. His data also suggests that plants belonging to various families have the potential to synthesize AgNPs of diverse shape and size for their use in variety of applications. The sizes of the plant-extract-synthesized AgNPs range from as small as 2 nm to around 4000 nm, whereas shapes range from spherical to rod and hexagonal. Plant family *Euphorbiaceae* seems to have high potential for synthesis of AgNPs. The plants of *Euphorbiaceae* family are well known for their plant-insect relationship, latex production and pharmacological activities (Patil *et al.*, 2012; Zahir and Rahuman, 2012). AgNPs synthesized by plant extract of this family are mostly spherical in shape with small size and have good antibacterial and insecticidal property (Krishnaraj *et al.*, 2010). Other families like *Rutaceae*, *Poaceae*, *Myrtaceae*, *Solanaceae* and *Asteraceae* also seem to be good candidates for catalysing synthesis of AgNPs. AgNPs synthesized by plant family *Apocynaceae* are reported to be useful against *Plasmodium* and *mosquitoes* acting as vectors for many human and animal diseases. On the basis of information available from different studies, one can synthesize AgNPs for tailor-made specific activity and defined shape and size.

ii) Gold nanoparticles of plants:

Gold nanoparticles have been considered as an important area of research due to their unique and tunable surface plasmon resonance and their applications in biomedical science including drug delivery, tissue/tumor imaging, photo thermal therapy and immuno-chromatographic identification of pathogens in clinical specimens. Both Ag and Au nanoparticles are excellent nano materials providing a powerful platform in biomedical applications of biomolecular recognition, biosensing, drug delivery and molecular imaging

(Khalil *et al.*, 2012). However, plant-based nanoparticle syntheses can be advantageous over other biological methods (microbial) since the reaction rate for the synthesis of nanoparticles is very high and there is no need to grow microbes (Kalishwaralal *et al.*, 2010).

Au⁺ ions reduction into metallic Au⁰ nanoparticles in the presence of metabolites and redox enzymes (Thakkar *et al.*, 2010). The quick conversion of solution color showed the formation of gold nanoparticles by observing color change from colorless to ruby-red color. The reaction is given below.



Leaf extracts of *Coleus aromaticus* (Vanaja and Annadurai, 2012), *Garcinia mangostana* (Karthiga *et al.*, 2012), *Magnolia kobus* and *Diopyros kaki* (Song *et al.*, 2009), *Cassia fistula* (Lin *et al.*, 2010), *Lippia citriodora* (Lemon verbena) (Cruz *et al.*, 2010), herb extract of *Barbated skullcup* (Wang *et al.*, 2009), petal extract of *Rosa hybrid* (Noruzi *et al.*, 2011), and flower extract of *Nyctanthes arbortristis* (Das *et al.*, 2011), have also been used for gold nanoparticles synthesis. Arora *et al.* (2012) demonstrate successful use of gold nanoparticles in enhancing growth and yield of *Brassica juncea*, under actual field conditions and present a viable alternative to GM crops for ensuring food security.

iii) Other nanopartilces:

Metal oxide nanoparticles iron oxide (Fe₃O₄), titanium oxide (TiO₂), copper oxide (CuO), and zinc oxide (ZnO) were also been reported. Most metal oxide nanoparticles exhibit bactericidal properties through reactive oxygen species (ROS) generation although some are effective due to their physical structure and metal ion release (Beyth *et al.*, 2015).

2.2.3 Anti-microbial activity of nanoparticles:

Silver has anti-microbial property. Wiegand *et al.* (2015) also proved silver-containing dressings alginate + ionic-Ag and CMC with Ag⁺ aid wound

healing by antimicrobial effects of silver as well as the establishment of a low physiological pH, also proved that dressings' effects on pH and release of silver ions act synergistically for antimicrobial efficacy. Also demonstrated a gold nanoparticle (AuNP)-based thermal history indicator, afford a proactive way for tracking time temperature history of perishable foods and biomaterials by visual color change, and hence provide objective traceability information to determine quality and/or safety of products. Various clinical and epidemiologic studies show that nonsteroidal anti-inflammatory drugs, including aspirin and cyclooxygenase inhibitors help prevent cancer.

2.2.4 Anti-Inflammatory activity of nanoparticles:

Chronic inflammation, a dysregulated and progressive response resulting in tissue destruction, underlies several “diseases of modern civilization”, such as inflammatory bowel disease, RA, allergies. Three fundamental interdependent evolutionarily conserved, robust and pervasive physiological pathways are relevant in the context of understanding the complex pathology of chronic inflammation are i) immunomodulation (via TH cells), ii) redox modulation (via NF κ B and Nrf2) and iii) metabolic regulation of inflammatory processes (via AMPK). Oxidative stress can activate a variety of transcription factors including NF κ B, AP1, HIF1a, PPARc and Nrf2, leading to gene expression of pro-inflammatory and anti-inflammatory mediators. Nuclear factor (erythroid-derived)-like 2, also known as NFE2L2 or Nrf2, is a redox-sensitive transcription factor that is the “primary cellular defense against cytotoxic effects of oxidative stress”. Natural products seem to be endowed with evolutionarily privileged molecular scaffolds, which can bind to multiple proteins, rendering them innate multiple target ligands that act in moderation (Mathew and Unnikrishnan, 2015).

Wen *et al.* (2014) demonstrated the anti-inflammatory characteristics of 34 novel tylophorine derivatives and discussed their structure-activity relationship in order to explore their therapeutic potentials for inflammatory diseases. Therefore, a growing interest has emerged in using medicinal plants that has attracted a lot of attention globally, and has become the subject of

active scientific investigation in many countries such as Egypt (Sen *et al.*, 2010), Japan (Takaoka *et al.*, 2011), India (Dey and Chandra, 1995; Sivaram *et al.*, 2004), Indonesia (Caruso *et al.*, 2013), Iran (Mousavi *et al.*, 2011), Korea (Harikrishnan *et al.*, 2015), Mexico (Ocampo and Jimenez, 1993), Nigeria (Okeke *et al.*, 2001) and Thailand (Direkbusarakom *et al.*, 1996a; 1996b), because they are cheap and easy to prepare, and are effective with fewer side effects during the treatment of diseases (Jian and Wu, 2003; 2004) and without any environmental and hazardous problems (Citarasu, 2010).

The growing interest in the plants has increased world-wide because they are easy to prepare, cheap and have few side effects on animals and the environment. Medicinal plants include herbs, spices, seaweeds, herbal extracted compounds, ayurvedic medicines and commercial plant-derived products (Hai, 2015).

2.3 The candidate plant - *Clitoria ternatea* L.

Clitoria ternatea Linn. belongs to Leguminosae Family, previously known as Papilionaceae and it seems to be a native of the Caribbean, Central America and Mexico; later distributed to the Indian Sub-continent (Arumugam and Panneerselvam, 2012). It is a perennial twining herb, stems terete, more or less pubescent. Leaves impari pinnate, petioles 2-2.5 cm long; stipules 4mm long, linear, acute. Leaflets 5-7, subcoriaceous, 2.5-5 by 2-3.2 cm, elliptic-oblong, obtuse or acute; stipules filiform. Flowers -axillary, solitary, standard bright or blue or sometimes white, with an orange centre, seed- 6-10, yellowish brown, smooth. Two types, namely white flowered variety and blue flowered variety are used as ornamental plants (Anand *et al.*, 2011).

2.3.1 Phytochemical investigations in the plant:

Phytochemical investigations of *C. ternatea* revealed the presence of flavonoid glycosides such as rutin, delphinidin, kaempferol, quercetin and malvidin, and it has been documented that its leaves contain δ -lactone of 2-methyl-4-hydroxy-n-pentacosanoic acid. This plant is commonly used in the Ayurvedic medicine, as a memory enhancer, nootropic, anxiolytic,

antidepressant, tranquilizing and sedative agent. Its extracts possess a wide range of pharmacological activities including antimicrobial, antipyretic, anti-inflammatory, anti-asthmatic, hepatoprotective analgesic, diuretic, local anesthetic, antidiabetic, insecticidal, blood platelet aggregation-inhibiting and for use as a vascular smooth muscle relaxing properties (Mukherjee *et al.*, 2008, Taur and Patil, 2011; Kalyan *et al.*, 2011; Nithianantham *et al.*, 2013). *Clitoria ternatea* is commonly used as a brain tonic in Ayurvedic system of traditional Indian herbal medicine to ameliorate intelligence and intensify the memory function (Talpate *et al.*, 2014), prevents convulsions as in epilepsy (anticonvulsant) and also as relaxing agent. Aqueous and ethanol extracts of roots, leaves and flowers have been studied for its anti-diabetic potential (Mukherjee *et al.*, 2008; Daisy *et al.*, 2009).

Three flavonol glycosides, kaempferol 3-O-(2"-O- α -rhamnosyl-6"-O-malonyl)- β -glucoside, quercetin 3-O-(2"-O- α -rhamnosyl-6"-O-malonyl)- β -glucoside, and myricetin 3-O-(2",6"-di-O- α -rhamnosyl)- β -glucoside were isolated from the petals of *C. ternatea* by Kazuma *et al.* (2003). Gomez and Kalamani (2003) suggested cultivation of *C. ternatea* in waste lands to narrow down the gap between the demand and supply of forage legumes in live stock production in India.

2.3.2 Antimicrobial activity studies in the plant:

Ponnusamy *et al.* (2010) extracted *Clitoria ternatea* using ethyl acetate, ethanol, acetone and petroleum ether and showed higher antibacterial effects against a range of fish pathogens than that extracted using water. The *in vitro* antimicrobial activity of various extracts of *C. ternatea* Linn. flower was screened against some extended-spectrum beta-lactamases producing enteric and urinary pathogens isolated from patients and found aqueous, methanol and chloroform extracts to exhibit activity against *Typhimurium*, *Klebsiella pneumonia*, *Pseudomonas aureginosa*, *E.coli* of Uropathogenic, Enteropathogenic, Enterotoxigenic and petroleum ether and hexane extracts did not exhibit any activity when compared with standard antibiotics (Uma *et al.*, 2009).

Anand *et al.* (2011) investigate the antibacterial properties of petroleum ether, ethyl acetate and methanol extracts from the leaves of *C. ternatea* and found methanol extract to possess a more potent inhibitory activity effect and also found it possess more potent antibacterial activity compared to other two solvent extracts. Madhu (2013) established a protocol for multiple shoot induction via the culture of nodes of *C. ternatea* L., through callogenesis and organogenesis. Kumari (2012), developed a protocol for rapid clonal propagation of *C. ternatea* L., through *in vitro* tissue culture of embryo explants through callogenesis and organogenesis, and antibacterial study of ethanolic extract of *in vitro* raised plant and callus mass against *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Escherichia coli*, and *Klebsiella pneumonia* and found to have antimicrobial activity against all tested microorganisms.

Sarojini *et al.* (2012) studied the anthelmintic activity of the leaves of the *Clitoria ternatea* and found methanolic extract to have anthelmintic activity, while the ethanolic extract devoid of such activity in adult Indian earthworm *Pheritima posthuma* which is anatomically and physiologically resembles the intestinal roundworm parasite of human beings. Shyamkumar and Ishwar (2012) evaluated the analgesic, anti-inflammatory activity of *C. ternatea* L. flower extract along with its phytochemical study. The study revealed that the petroleum ether extract poses significant anti-inflammatory and analgesic properties and phytochemical investigation revealed the presence of Taraxerol, a penta-cyclic tri-terpenoid.

Silva *et al.* (2015) showed, for the first time, that the water extract of roots of *C. ternatea* exhibits dose-related potent antibacterial activity against bacterial pathogen, *B. cereus* while other four plants extracts ie, stem of *Stereospermum suaveolens*, *Oroxylum indicum* and whole plant of *Bacopa monnieri* and *Alysicarpus vaginalis* possessed no antibacterial activity against any of the bacterial strains tested. The antibacterial activity of *C. ternatea* roots is attributed to flavonoids, polyphenols including tannins, and saponins present in the extract and concluded that roots of Sri Lankan medicinal plant

C. ternatea can function as a potent antibacterial agent against common food poisoning bacterial pathogen, *B. cereus*.

Padmavairavasundaram and Senthil (2014) investigated the amount of quercetin in plant parts of *Clitoria ternatea* and its antifungal activity on dermatophytes and found greatest amount of flavonoid in methanolic extracts of *C.ternatea* flowers while the lowest level in the *C. ternatea* stem extracts, whereas HPLC fingerprinting of flavonoids in flowers was significantly different from stem and leaf extracts. Also differ in their fungicidal capacity against dermatophytes tested, although they all showed significant antifungal activity against *Candida* *sps.* and *Aspergillus* *sps.* flower extracts showed concentration dependent strong inhibition on growth of *Microsporum gypseum* and *Epidermophyton floccosum*, and weak inhibition on *Trichophyton metagraphytes*. Arumugam and Panneerselvam (2012) compared the antibacterial property between tissue cultured and wild plants of *Clitoria ternatea* and found to exhibit antibacterial activity against certain pathogenic bacteria.

2.3.3 Antioxidant activity studies in the plant:

C. ternatea is one among the medicinal plants used for the treatment of snakebite by folk medicinal practitioners in the twelve districts, Bangladesh (Hasan *et al.*, 2015). Jadhav *et al.* (2013) studied the antioxidant activities of different fractions from different extracts (leaves, stem and root) of *Clitoria ternatea* using antioxidant assay like DPPH, FRAP, metal chelating ability and reducing power assay. The results obtained in this study proved antioxidant properties which provide a basis for the traditional use of plant and could be harnessed as drug formulation. Nithianantham *et al.* (2013) evaluated the hepato protective and antioxidant activity of *C. ternatea* flower extract against acetaminophen-induced liver toxicity in mice and confirmed the hepato protective effect against model hepato toxicant acetaminophen.

Zingar *et al.* (2013) reported that *C. ternatea* is a rich source of phytochemicals, with high levels of phenolic compounds and antioxidant

activities and also indicates that the leaf and flower extracts have a hypo glycaemic effect. Effective in regulating the biochemical indices associated with diabetes mellitus and possesses strong hepato protective potential. Lakshmi *et al.* (2014) carried out phytochemical screening of the methanolic extract of leaves and flower of *Clitoria ternatea* by FTIR, as well as quantitative analysis of total phenolic content, total flavonoid content and also *in vitro* antioxidant activity. Concluded that the significant amount of phytochemicals, phenolic and flavonoid contents present both in leaves and flowers are responsible for the *in vitro* antioxidant activity of *C. ternatea*, and it is potential to be an alternative source of natural antioxidants.

Phrueksanan *et al.* (2014) found phenolic compounds, flavonoids, and anthocyanins in *Clitoria ternatea* flower petal extract. In addition, showed antioxidant activity as measured by oxygen radical absorbance capacity method and DPPH radical scavenging assay and remarkably protected erythrocytes against AAPH-induced hemolysis at 4 hours of incubation and reduced membrane lipid peroxidation and protein carbonyl group formation and prevented the reduction of glutathione concentration in AAPH-induced oxidation of erythrocytes. It also effectively protected AAPH-induced morphological alteration of erythrocytes from a smooth discoid to an echinocytic form.

Vivek *et al.* (2014) evaluate the antiulcer activity of petroleum ether, chloroform and ethanol extract of *Clitoria ternatea* (Linn.) leaves in experimentally induced animal models. Preliminary phytochemical screening was also undertaken and it revealed the presence of alkaloids, tannins, flavonoids, glycosides and steroids and concluded that the plant have potent antiulcer activity that may be due to the presence of flavonoids, tannins or antioxidants. Sushma *et al.* (2015) biologically synthesized MgO NPs from ethanol extract of *Clitoria ternatea* and found to exhibit good antioxidant activity by DPPH assay, an *in vitro* antioxidant studies.

In the lights of literature study presented, the present work was designed with the initial screening of different parts of *Clitoria ternatea* bearing

blue colour flowers and white colour flowers, using different solvent systems for their potential antioxidant and antimicrobial activity. From the potential extracts, green synthesis of silver and gold nanobioconjugates was carried out followed by characterization. Then they were screened for antibacterial and anti-inflammatory activity *in vitro*. *In vivo* studies were performed to confirm the results obtained *in vitro*. The plan of work and the methodology adopted are presented in the next chapter.