INTRODUCTION

1.1 Medicinal Plants

Medicinal plants which compose a section of the plants deliver a raw material for the utilization in all the indigenous schemes of drugs in India, where around 1800 species are utilized in traditional Indian schemes of medicines, Ayurveda utilizes 1200, Siddha uses 900, Amchi 600 , Unani 700 and 450 species are utilized by Tibetan. The age of old classical standards appended to the numerous forest types and diversities of forest products (medicinal plants) has increased fabulous significance in the current century (Stein, 2004). As stated by, the World Health Organization (WHO), 80 % of the inhabitants of emerging countries depend on traditional medicines, mainly in the form of plant medicines for their health care requirement (Vines, 2004).

In addition, current medicine includes plant derivatives to the extent of around 25 % on expenses of the detail that spin-offs of medicinal plants are non-narcotic having no side effects, requisition of these plants is an upsurge in both developed and emerging countries. In recent years, developing requisition for herbal items has led to a quantum jump in capacity of plant material trafficked inside and outside the country (Singh and Samuel, 2001).

More than 70 % of the plant gathering includes damaging garnering because of utilize the parts like roots (29.6 %), barks (13.5 %), wood (2.8 %), rhizome (4 %), stalk and entire plant in case of herbs. An estimate of the Export-Import Bank of India (EXIM) bank projects international market of medicinal plants related trade over US $ 60 billion per year that is rising at the rate of 7 % for every year. The global ayurvedic products market is reportedly worth $ 14.2 billion. Planning Commission Task Force

has focused exports of herbal items Rs. 3000 crore by the year 2005 and Rs. 10,000 crore by the year 2010 (Tiwari 2000). WHO has evaluated developing requisition for medicinal plant based raw materials at the proportion of 15 to 25 % yearly and probable to upsurge in excess of US $ 5 trillion in 2050 (Kiran *et al*., 2009).

As per World Conservation Strategy (IUCN- International Union for Conservation of Nature, UNEP- United Nations Environment Programme and WWF- World Wide Fund 1980); Preservation is described as "the administration of human utilization of biodiversity so that it may harvest highest maintainable advantage to the current age group while continuing its possible to encounter necessities and aspirates of upcoming age group" (Udvardy and Udvardy, 1975). To safeguard the existing bioresources for future generations and to accomplish maintainable growth based on the use of available genetic resources, conservation of phytodiversity is of immense importance. Therefore, *in situ* and *ex situ* management programmes are necessary approaches for its conservation and consequently plant improvement. *In situ* preservation includes continuing hereditary resources in their natural habitats/wild whereas, *ex situ* preservation includes conservation external the native habitat and is usually utilized to protection inhabitants in hazard of obliteration, replacement or worsening (Altman, 1999).

1.2 Isolation of active principles from medicinal plants

Segregation of dynamic main beliefs from medicinal plants and characterization can be discovered since the beginning of the 19th century. The big number of medicine from medicinal plants were found and presented in current pharmacopoeias during 1850-1950. Throughout this duration there was excellent participation of fine medicine from better plants similarly deserpidine, reserpine and rescinnamine from *Rouvolfia serpentina*, vincristine and vinblastine from *Catharanthus roseus* (Sharma, 2003).

Ephedrine was isolated from *Ephedra sinica* in 1887 and later presented as medicine in 1925. Similarly, from opium and morphine were quarantined in 1820 and presented as medicine in 1825. In some cases, raw extracts of medicinal plants may be utilized as medicament. In addition, separation and recognizable of dynamic ethics and explanation of the instrument of action of a drug is supreme significance. In excess of 121 most important plant drugs have been ascertained for which no synthetic one is presently obtainable (Kumar *et al*., 1997).

In herbal drugs, raw plant extracts in the formation of decoction, tincture or herbal extract by traditional and utilized for the cure of several illnesses. Even though their effectiveness and mechanisms of activity have not been proved scientifically in most cases, these simple medicinal arrangements frequently mediate useful responses due to their dynamic chemical parts (Barnes *et al*., 2007). Plant-derived items comprise an excessive variety of phytochemicals, which includes antibacterial, antioxidants, antimutagenic, anticarcinogenic, antithrombotic and vasodilatory impacts (Bidlack, 2000).

1.3 Phytochemical analysis

Phytochemicals are non-nutritive plant chemicals comportment illnesses resistant characteristics. More than a few thousands of phytochemicals has been recognized in plants, grains, vegetables and fruits, but still there are large numbers of such compounds remains unidentified (Liu, 2004; Arora and Kaur, 2007). It aids in recognizable and description of crude medicine regarding phytochemical constituent. The healing possessions of medicinal plants are maybe due to presence of different secondary metabolites such as alkaloids, phenols, glycosides, flavonoids, steroids, saponins and so on (Britto and Sebastian, 2011). Thus, the initial screening test perhaps valuable in the discovery of the bioactive principles and consequently may lead to the

drug detection and growth (Doss *et al*., 2009). These bioactive mixes have been stated supportive in decreasing chronic illnesses like cancer and cardiovascular sicknesses (Etherton *et al*., 2004). Steroids are known vital for their cardio tonic actions and furthermore utilized in nutrition, herbal drug and cosmetics. Saponins are utilized as mild cleansers and it is used in hypercholesterolaemia, anticancer, anti-inflammatory and antifungal properties. Because numerous saponins show potent antifungal movement and are regularly present in comparatively high levels in healthy plants, these molecules have been embroiled as determinants of a plant's protection from fungal attack. Various different properties are also connected with these mixes, as well as insecticidal, piscicidal and molluscicidal movement. Allelopathic action and anti-nutritional effects (Osbourn, 1996). The antibacterial agents discovered in the plants comprise aminoacids, phlorotannins, acrylic acid, terpenoids, phenolic compounds, steroids, alkanes, halogenated ketones, cyclic polysulphides and fatty acids (Balandrin *et al*., 1985; Kumar and Sharma, 2012).

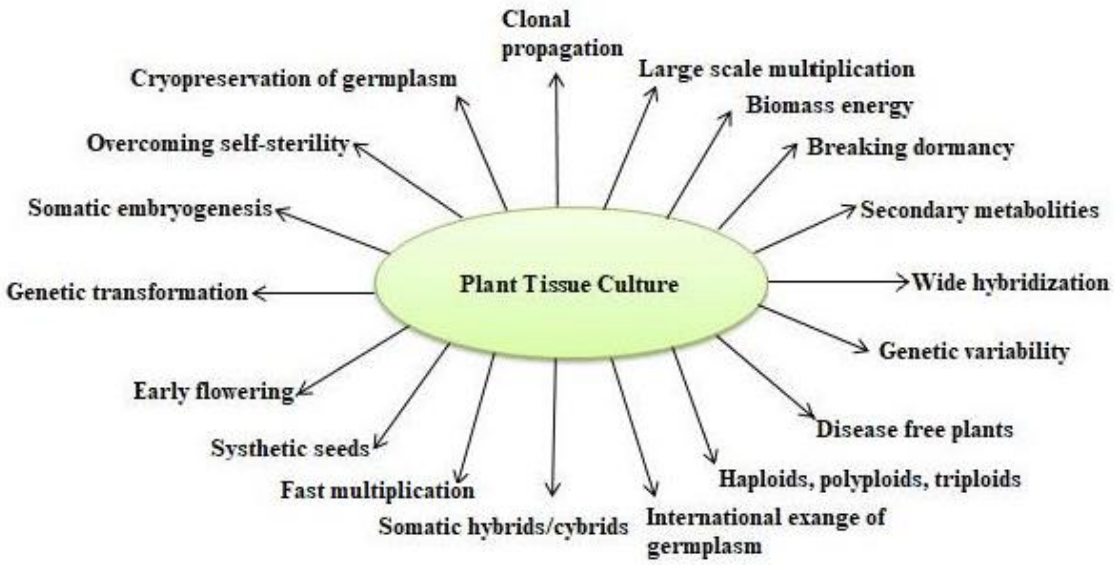
1.4 Tissue Culture

In recent years, plant biotechnology has gained an imposing development as one of the boundaries of biotechnology of scientific and economic significance. To deal with the current alarming circumstances, the latest stimulating growth in plant biotechnology have come as an advantage and has been viewed as a main concern area for technology transfer, because genetically modified food, feed and fibers are vigorous concern to the developed world (Ives and Bedford 1998; Canter *et al*., 2005). The plant biotechnology has delivered a large quantity of tools and techniques which are more efficient in generating novel genetic variability and making selection procedure, more precise and reproducible (Tripathi and Tripathi, 2003). There are four chief parts of biotechnology, which can directly assist plant conservation programmes:

- Tissue culture (*in vitro* technologies)
- Molecular diagnostic
- Molecular marker technology
- Cryopreservation

In vitro culture-a key tool of plant biotechnology, which misuses the totipotency nature of plant cells, an idea proposed by Haberlandt (1902), is the science of developing plant cells, organs or tissues separated from mother plant on artificial media lower than disease-free circumstances. The powerful techniques in plant cell and tissue culture combined with most refined and analytical tools, have offered humanity the great power of misusing the totipotent biosynthetic and biotransformation capabilities of plant cells lower than *in vitro* conditions (Stockigt *et al*., 1985). Plant cell, tissue and organ culture approaches have appeared as escapable biotechnological tool with potential outcomes of complimenting and supplementing the conformist technique in plant breeding, plant improvement, biosynthetic pathways and so on. It plays a main part in preservation of germplasm, rapid clonal propagation and regeneration of hereditarily manipulated l*arger* clones, production of secondary metabolites and *ex vitro* preservation of valuable phytodiversity. Plant improvement through plant tissue culture in different fields has been presented in figure 1.1.

Figure 1.1 Plant improvement through tissue culture technology



The recent development in clonal micropropagation of plants has been of great help in the cultivation of medicinal plants by providing standard quality planting material (George and Sherington, 1984) and widely used for the commercial propagation and re-vegetation of large number of plant species, including various medicinal plants (Naz *et al*., 2011; Perveen *et al*., 2011; Jahan *et al*. 2011a and b; Fatima and Anis 2012a). Numerous medicinal and aromatic plants have been successfully used for *in vitro* regeneration and mass multiplication through the use of various explants (Arya *et al*., 2003; Faisal *et al*., 2005; Chaturvedi *et al*., 2007; Karuppusamy *et al*., 2009; Sharan *et al*., 2010; Siddique *et al*., 2010; Varshney and Anis, 2012).

1.5 Nanotechnology

The science and engineering engaged in design, synthesis, characterization and application of devices and materials containing smallest particle of 1-100 nm range is "nanotechnology". Nanotechnology and nanoscience have proved to bring potential

benefits in various fields like information and communication technologies, water decontamination, drug growth and manufacture of stronger, less weight materials. Revolutionary advances in the areas of communications, medicine, genomics and robotics are thought to be possible as tremendous developments have occurred in nanotechnology from its genesis. A devastating enthusiasm on the research with nanomaterials has been observed in the modern days (Salata, 2004).

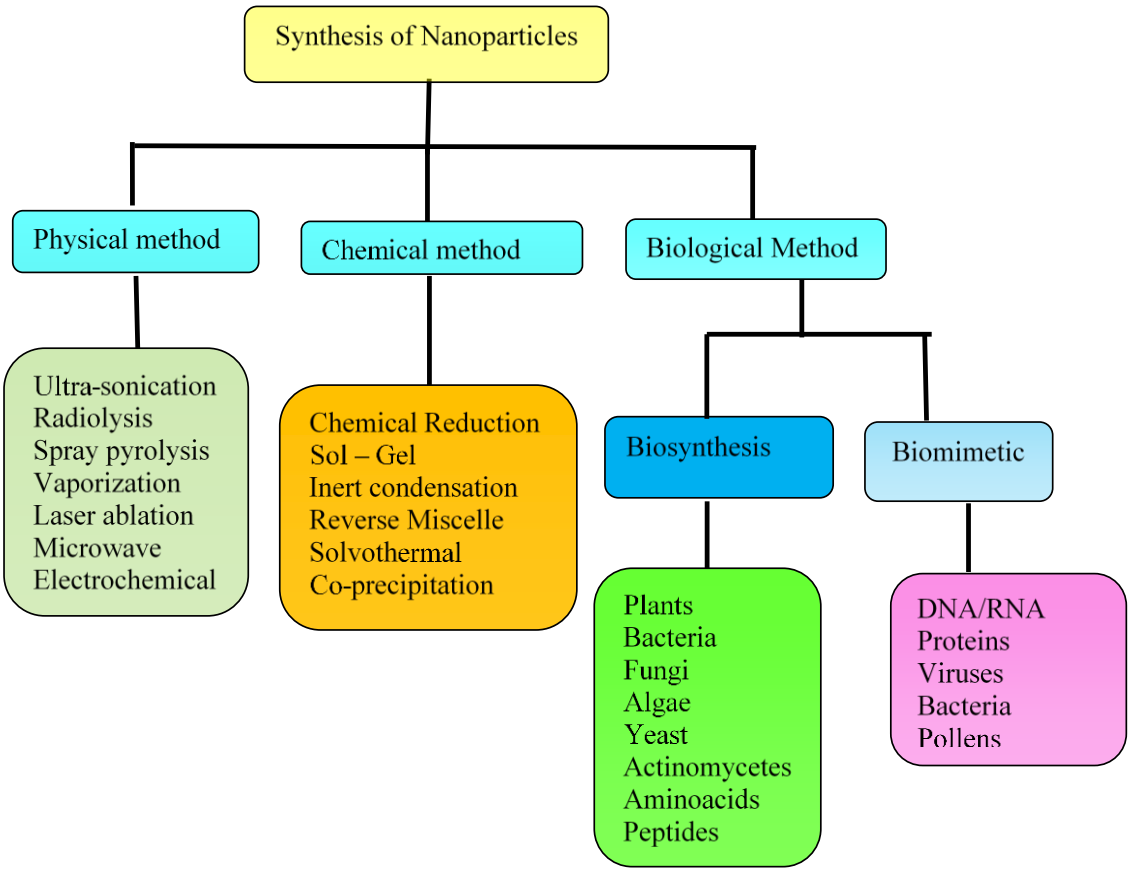
The nanoscale standards and methods to study and transform biosystem and to use biological principles with its materials to manufacture new expedients and structures incorporated with nanoscale are termed "nanobiotechnology". The amalgamation of biotechnology and nanotechnology with information technology has accelerated the evolution in the area of theoretical and applied science world. The union of nanotechnology with recent biology and drug is a pattern that should be mirrored in science strategy decisions.

1.5.1 Synthesis of metal nanoparticles

Besides several physical, chemical and biological approaches which have been fixed for making metallic nanoparticles, nanobiotechnology also help as a significant technique in the growth of clean, safe and eco-friendly events for combination and assembly of metallic nanoparticles. Nanoparticle study is unavoidable today not because of only application and furthermore by the method of synthesis. Way of synthesis of nanoparticles may have substantial ecological imperfection, theoretically laborious and financially costly. Numerous scientists have investigated the technological method for the synthesis (Ahmad *et al*., 2010). In the most recent decade numerous effective synthesis techniques have been developed to create a metal nanostructures in an assortment of shapes for example sphere, cube, spheroid, octahedron, bipyramid, plate, tetrahedron, hollow and rod. The approaches for

producing nanoparticles can usually include either a top-down approach or a bottom-up approach.

Figure 1.2: Schematic representation of various methods for the synthesis of nanoparticles



In top-down synthesis nanoparticles are created by size reduced from an appropriate beginning material. Top-down production approaches present defects in the surface structure of the item and this is a noteworthy restriction because the surface chemistry and other physical possessions of nanoparticles are extremely reliant on the surface structure. In bottom-up synthesis the nanoparticles are constructed from smaller entities, for instance by joining atoms, molecules and smaller particles. In bottom-up synthesis, the nanostructured construction blocks of nanoparticles are moulded first and

afterward collected to deliver the final particle. The bottom-up synthesis regularly depends on chemical and biological approaches of production (Ventra *et al*., 2006). The synthesis approaches are comprehensively isolated into physical, chemical and biological approaches. Promote biological approaches are categorized in microbial synthesis, plant mediated synthesis and other dissimilar biological routes of metal nanoparticle synthesis. (Figure 1.2) gives the summary of the various routes of the synthesis process involved in nanoparticle construction.

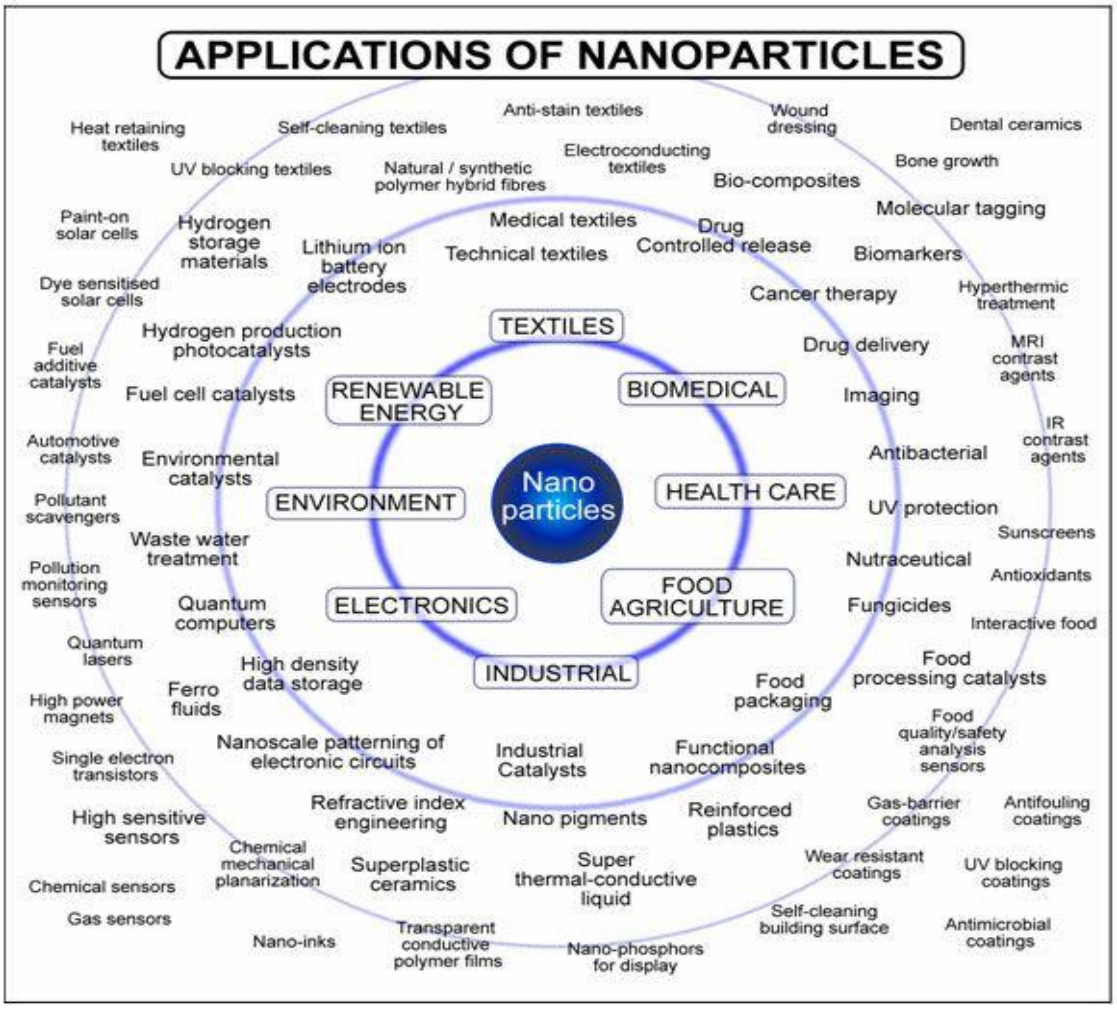
1.5.2 Applications of nanoparticles

Nanotechnology products have become increasingly useful in biomedicine and have led to the advent of a hybrid science called nanobiotechnology (Saji *et al*., 2010). Nanomaterials have notable applications in nanobiotechnology, particularly in analysis, medicine delivery systems, prostheses and implants. Nanoscale materials assimilate well into biomedical tools because most biological systems are furthermore nanosized. The materials generally utilized to improves these nanotechnology items are inorganic and metal nanoparticles, carbon nanotubes, liposomes and metallic surfaces (Faraji and Wipf, 2009). Recently, scientists have found that nanoparticles collaborate with a various gathering of solvable biomolecules when they move in a biological environment. Biomolecule-nanoparticle contacts lead to the creation of an adsorbed biomolecular corona. The mechanism of contact amongst nanoparticles and cells, alongside with the resulting cellular response is typically studied using *in vitro* cell culture systems (Liu *et al*., 2016a).

In most recent couple of years, biosynthesis of nanoparticles have acquired important thought due to developing needs in nontoxic chemicals, antiviral, diagnostics, antibacterial, anticancer, directed medicine conveyance environmental

agreeable solvents and renewable materials which is shown in figure 1.3 (Gericke and Pinches, 2006).

Figure 1.3: Applications of nanoparticles



(Source: https://www.pinterest.com/pin/86975836532997854/)

1.5.3 Biomedical applications of metal oxide nanoparticles

The electronic structure of zinc oxide (ZnO) nanoparticles is useful for biomedical applications; for example, the intrinsic fluorescence of ZnO nanowires has been utilized to image cancer cells. To this end, functionalization of the surface of ZnO nanowires increases their solubility in water and their biocompatibility and decreases their cellular poisonousness. Functionalization of the ZnO surface with particular

biomolecules creates photosensitive biosensors (Ramos *et al*., 2017). Cerium oxide (CeO_2) nanoparticles, otherwise called nanoceria, have been utilized for decades for various applications. Nanoceria turn as superoxide dismutase and catalase mimetics, scavenging of nitric oxide radical, oxidative stress-mediated disease, *in vitro* cell culture and *in vivo* models (Walkey *et al*., 2015). Cerium oxide nanoparticles possess antibacterial activity, cytotoxicity, genotoxicity, neurotoxicity and antioxidant activity (Rajesh kumar and Naik, 2017).

1.6 *In vitro* cytotoxic activity

Cancer is most important perilous illnesses in persons and chemoprevention has been a promising anticancer approach aimed at reducing the morbidity and mortality of cancer by delaying the process of carcinogenesis (Goodwin and Brodwick, 1995). Cancer is a collection of ailments described by uncontrolled development and spread of irregular cells. In the event that the spread is not controlled, it can outcome in demise. Cancer illnesses are described by unusual expansion of cells. They establish the second reason of humankind behind cardiovascular sicknesses in advanced countries and the third after communicable and cardiovascular sicknesses in emerging countries (Mbaveng *et al*., 2011).

Based on the origin, dissimilar types of cancer exist, for instance prostrate, thyroid, breast cancer, bladder cancer, pancreatic, melanoma, kidney cancer, leukaemia along with numerous kinds are oral cancer, colono-rectal combined cancer and so on. Cancer is a main public health issue worldwide with several of new cancer patients analysed each year and numerous deaths resulting from this illness (Engel *et al*., 2011). Around 14.1 million cancer cases were analysed around the world in 2012, of this 52.5% were in males and 47.5% in females, and is anticipated to growth up to 21.7 million by 2030. This ailment contributed to total 8.2 million deaths worldwide in 2012

and predictable to upsurge to million by the end of 2030 (Burney *et al*., 2014). Two factors are involved which can caused cancer. External factors are added chemicals, radiation, tobacco, and viruses. Internal factors which includes immune conditions, hormones and transformations. This may demonstration together or in arrangement to initiate or support carcinogenesis. Cancer is basically an illness of regulation of tissue growth (Croce, 2008).

Breast cancer is the most commonly recognized and the second foremost reason of tumour demise amid women. Every year, in excess of one million cases and half million deaths are recorded around worldwide (Ardebil *et al*., 2011). Age, family history, regenerative irregularities, exogenous hormones (contraceptives or hormone replacement therapy) and topographical sites are particular portion of the danger factors (American Cancer Society, 2007). Breast cancer is a heterogeneous sickness which is represented by propagation and nonstandard difference of dangerous and not fully formed cells that frequently carry abnormalities that decontrol the hundreds or even thousands of genes (Ghojazadeh *et al*., 2013).

In excess of 3000 diverse plant species have purportedly been shown to treat cancer around the world (Mukherjee and Wahile, 2006). Utilization of botanical items in mainstream drug improvement includes the distinguishing proof and extraction of dynamic mechanisms of plants or crude extracts and, in some cases, synthesis of equal active mixes (Littleton *et al*., 2005). The rationale for this method is to decrease or remove the erraticism of chemical composition and concentration that subsists in crude plants and can give exact dosages of known mixes for management to patients. In addition, botanicals are inexpensive and less poisonous than the conformist anti-cancer therapeutics (Butler, 2004; Butler, 2005).

Experimental agents derivative from natural items offer a great chance to assess not only new chemical classes of anticancer agents, as well as novel and possibly pertinent mechanisms of activity. Plant derivatives the anti-cancer drugs performance by multiple targets at the same time and/or synergistically. Many of these drugs are chemo preventive which prevent both primary and secondary recurrence of the disease. It may be indicated that several chemotherapeutic drugs utilized in conformist cancer treatment have been technologically advanced from botanical sources, to give some examples are camptothecin, taxol (Oberlies and Kroll, 2004), topotecan, irinotecan and taxotere (Shrivastava *et al*., 2005 One of the best known instances is etoposide, derivative from the may apple plant (*Podophyllum peltatum*) and two of the most significant chemotherapeutic medicines, vincristine and vinblastine, presently utilized were initially created from a folk remedy comprising rosy periwinkle plant (*Vinca rosea*) (Cragg and Newman, 2005).

1.7 Antimicrobial activity

Bacteria are common disease causing agents in humans, as shown by the wide clinical use of antibiotics, however, a new worldwide health issue has ascended as in discriminant antibiotic utilize and extraordinary capability of bacteria to obtain opposition (via genetic mutation or gene acquisition) lesser these medicines efficiency. Undoubtedly, new classes of antibiotics with novel structures are desirable to battle this tendency (Nair and Chanda, 2005).

Hence, there is an emergency necessity to found a new antimicrobial mixes with varied chemical structures and novel devices of activity for new and re-rising communicable sicknesses. The new therapeutic agents ought be operational and have a novel method of activity that renders them impermeable to existing obstruction devices. Not only medicines from natural sources have new structural highlights, with novel

biological action but phytochemicals derivative from them are likewise tremendously valuable as main structures for synthetic modifications and optimizations of bioactivity (Sung and Lee, 2007).

The number of multi-drug impermeable microbial straining and presence of strains with condensed vulnerability to antibiotics are constantly increasing. This upsurge has been credited to indiscriminate utilize of broad-spectrum antibiotics, intravenous catheters, immunosuppressive agent, organ transplantation and ongoing epidermis of HIV infection (Dean and Burchard, 1996).

Over the past 20 years, there has been a lot of interest to investigate natural materials as a source of new antibacterial agents. A number of natural items have been permitted as new antibacterial medications but there is an emergency necessity to detect novel substances which can performance in contradiction of pathogens with great resistance (Jantova *et al*., 2000). A wide variety of compounds derived from plants conventionally utilized in ethnomedicine have the antimicrobial action in contradiction of numerous microorganisms (Cowan, 1999). In the previous couple of years local infections has improved to a great extent. After the advent of antibiotics many of the pathogenic bacteria have now become resistant to some antibiotics (Yadava and Jharbade, 2008). Natural products of greater plants may proposal a new source of antibacterial agents for external utilization Such as cataplasms, compresses, gargles and ointments (Brantner and Grein, 1994).

The progress of new antimicrobial medicines has been utilized to overcome resistance. Though, plant-derived medicines have been piece of traditional heath care in best fragment of the world and the antimicrobial possessions of plant determined

composites is well documented. There is cumulative the enthusiasm in plants as sources of antimicrobial agents (Perumal and Kala, 2009).

1.8 *Hybanthus enneaspermus* L. F. Muell

H.enneaspermus (L.) F. Muell. is a perennial herb, which has place with the family Violaceae. It is a rare, small suffrutescent everlasting herb identified in some of the warmer portions of the Deccan peninsula in India, Ceylon, Tropical Asia, Africa and Australia. *Hybanthus enneaspermus* (L.) F. Muell called as Lakshmisheshta, Padmavati, Padmacharini or Purusharathna in Sanskrit, it is a significant plant in the Indian structure of drug. It is called as ‘Amburuha in Ayurvedic, Orithal thamarai in Siddha/Tamil and commonly called as spade flower plant and Ratanpuras in Hindi. *Ionidium suffruticosum* DC is the synonym. It matures 15-30 cm in height with numerous diffuse or arising branches and is pubescent in nature. It has narrow, 4-5 cm long, stipulate, entire and alternate leaves. The stems are slightly hairy. The plant has small pinkish flower with darker patterns, solitary, axillary inflorescence, pubescent flowers with five sepals and five petals, unequal, upper pale pink and oblong. It is broadly disseminated throughout the tropical region. The natural redevelopment prospective of this herb is exceptionally poor because of low seed viability. Because the seeds and emerging capsules are frequently identified on the ground, loss owing to rodents and inundation is substantial. Increasing humanoid and livestock inhabitants shave already affected the status of wild plants, particularly those used in herbal drug (Shantha *et al*., 2000).

1.8.1 Medicinal Properties of *H. enneaspermus*

This ethnobotanical herb is famous to have inimitable medicinal properties. The arrangements prepared from leaves and tender stalks of the plant are utilized in herbal drug for its aphrodisiac, demulcent and tonic properties. The root is diuretic and

managed as an imbuement in gonorrhea and urinary contagions (The Wealth of India, 1959). The ovaries and leaves are utilized as remedies for scorpion bites and cobra stings by tribes (Sudarsanam and Sivaprasad, 1995). Generally the plant is utilized as an aphrodisiac, tonic, demulcent, diuretic, urinary infections, diarrhea, dysuria, leucorrhoea and sterility (Yoganarasimhan, 2000). In addition, the plant is stated, in ancient ayurvedic writings, to cure situations of "kapha" and "pitta", urinary calculi, painful dysentery, strangury, vomiting, wandering of the mind, burning sensation, blood troubles, urethral discharges, asthma, epilepsy, cough and to provide tone to the breasts (Kirtikar and Basu, 1991). The plant is pondered to have uppermost medicinal worth which is utilized by the traditional specialists for the cure of sicknesses like malaria (antiplasmodial activities), diabetes, male sterility, urinary tract infections and water retention. The fond leaf stalks are utilized as demulcent; the roots are antigonorrhoeic, diruretic bowel grievances and urinary issue (Deshpande, 2006). Orithazh thamarai chooranam is a powder type of Siddha formulation, which is broadly utilized for increasing the secretions of milk in women (lactation) and progresses the worth of the semen men (Das *et al*., 2004).

1.8.2 Chemical constituents of *H. enneaspermus*

Various phytoconstituents viz. dipeptide alkaloids, isoarborinol and β-sitosterol, aurantiamide acetate have been quarantined from various portions of this plant (Khare, 2007). Even though chemical research were seemed for the existence of flavonoids, alkaloids, triterpene and steroids in this species, but no information are accessible regarding its definite structural information and its biological actions. *H.enneaspermus* is of substantial chemotaxonomic significance, as it comprises the first described of a Violaceae plant to intricate a peptide alkaloid.

1.9 Reasons for choosing *H.enneaspermus* for the studies

This plant has been a source of medicine right from the ancient time. So, many books and articles have been written so far on the medicinal and other values of this plant. It is highest popular herbs in all deliberations, symposia and seminars and so on associated to herbal preservation. This wonderful herb was found in profusion a many years ago. Over harvesting for medical utilize, poor seed viability and germination, sporadic distribution are the main intimidations to this plant.

H.enneaspermus, is an infrequent medical plant under danger in the natural habitat because of over utilization by natural therapists, overgrazing by seasonal habitat, animals, sporadic delivery and poor germination frequency of seeds (Prakash *et al*., 1999; Sonappanavar and Jayaraj, 2011). Eventhough, the seeds and emerging capsules are frequently identified on the ground, loss by reason of rodents and accumulation is substantial (Prakash *et al*., 1999).

H. enneaspermus has tremendous medicinal properties found only during the monsoon season. When the season is over, the plant gets dried up. The germination of its seed is very difficult or impossible because of susceptibility to many pests and over-exploitation in habitats for medicinal purpose has pressed this taxon to endanger. In order to deliver sufficient plant material for commercial manipulation, the farming of the plant is not adequate. Thus, mass propagation through *in vitro* techniques of this plant is immediately required not only to preserve but also to meet the request for this medicinal plant as a source of secondary metabolites and to make available throughout the year.

In vitro culture of plants is progressively utilized in preservation of biodiversity, particularly for uncommon and endemic species and contemplates as a significant

element of plant genetic resource management. The plants growing in the wild are dependent on soil, seasons and weather conditions. Hence, they may not be available throughout the year. Growth of mass propagation methods and alternative methods for the making of raw drugs/metabolites should assuredly increase the threat rank of the plant. Study on the micropropagation of *H. enneaspermus* accessible in the literature is insufficient. A prior published report on *in vitro* shoot redevelopment is from seed-derived callus (Prakash *et al*., 1999). Sonappanavar and Jayaraj (2011) proved that the rapid callogenesis from leaf explants utilizing MS supplemented with 0.5 mg L^{-1} 2, 4-D. Usually, maximum of the *in vitro* redevelopment research have managed with assessment of plant growth regulators (PGRs) with a single nutrient formulation system. Evaluation of various nutrient media is likewise very essential particularly under states of poor multiplication rate. Optimum response of explants in culture is reliant on the basal medium and PGR. As stated by Bejoy *et al*., (2006) ideal nutrient states are necessary to permit explants to fast their swarming potential. Moreover, Preece (1995) has likewise recommended that the tissue culture medium is vital and it can in part substitute PGRs. It is essential to research such factors for enhancing *in vitro* regeneration systems.

In perspective of its ethno medical significance, there is a necessity to preserve the wild stock of *H. enneaspermus*. Plant tissue culture is a beneficial tool for the preservation and quick propagation of a valuable beneficial ethnobotanical plant (Sanyal *et al*., 1998). Due to these reasons, the plant was selected for the study and makes it an important issue so that conservationists, botanists, entrepreneurs and NGOs come forward to rescue and save this plant.

In the recent investigation, *Hybanthus enneaspermus* L.F. Muell has been carefully chosen based on their medicinal properties which can be utilized for various

treatments of diseases and also for its large scale propagation which can be used for re-introduction and conservation purposes through various biotechnological approaches and few applications were studied. In consideration of the prospects of multifarious possessions of the herb, additional scientific research are needed to utilize the unnamed potential. Hence, the recent research was planned to assume a penetrating examination into the anticancer and antimicrobial potential of *H. enneaspermus*.

1.10 Aim of the study

The aim of this study was made to increase the *in vitro* production of *H. enneaspermus* by using various strategies. We have also studied the phytochemical analysis and antimicrobial action of numerous extracts of *H. enneaspermus*. In this present work we have synthesized zinc oxide and cerium oxide nanoparticles from *H. enneaspermus*. The significant effect of synthesized nanoparticles on cell proliferation and cell death of human breast (MCF-7) cancer cell lines was investigated using MTT assay and fluorescent microscopy.

1.10.1 Objectives of the study

The aspects discussed above forms the basis of the present work. Hence, *H.enneaspermus* (L.) was selected to carry out the following objectives:

- Collection and identification of medicinal plant *H. enneaspermus* (L.)
- To standardize the protocol for indirect organogenesis *in vivo* leaf explants of using *H. enneaspermus*.
- To investigate the phytochemicals present in the *in vivo* and *in vitro* leaf of *H. enneaspermus*

- To identify various bioactive compounds present in the *in vitro* leaf extract of *H. enneaspermus* by GC-MS
- To evaluate the antimicrobial activity of numerous extracts of *H. enneaspermus*
- To biosynthesize zinc oxide (ZnO) and cerium oxide (CeO_2) nanoparticles utilizing the aqueous leaf extract of *H. enneaspermus*
- To characterize the biosynthesized nanoparticles using UV, FTIR, XRD, XPS, SEM-EDX, TEM and SAED
- To determine the cytotoxic activity of the biosynthesized zinc oxide and cerium oxide nanoparticles against MCF-7 cell lines.
- To observe the cytotoxicity (MCF-7) effect of synthesized zinc oxide and cerium oxide nanoparticles using fluorescence microscopic analysis