INTRODUCTION

The globe is rich in enormous diversity evolved during billions of years that plant inhabits a major part of the biota to serve mankind with various “plant derived natural products” being used in traditional medicinal systems all over the world. Hence they revitalize the phenomena of being recognized as “pharmaceutical factories” in recent days. Of the 2, 50,000 higher plant species on earth, more than 80,000 are medicinal (Jawla et al., 2009). Medicinal plants occupy a major counterpart that people still rely upon traditional systems of medicine for various ailments rather than the modern allopathic medicines which in turn rooted into these natural species (Joy et al., 1998). Therefore research on medicinal plant species can reveal hidden facts that might imply in the arena of phytotherapy. The medicinal plants have been used since ancient times for the treatment of human ailments. Over three quarters of the world population relies mainly on plants and plant extracts for health care. The herbal medicines today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment. In the primeval times, the Indian perceptive held the view that herbal medicines are the only resolution to treat numeral health related problems and diseases. Although herbs had been priced for their medicinal, flavoring and aromatic qualities for centuries, the synthetic products of the modern age surpassed their importance, for a while. However, the blind dependence on synthetics is over and people are returning to the naturals with the hope of safety and security (Ibrahim Singab, 2012).

MEDICINAL PLANTS IN INDIA – AN OVERVIEW

India’s diversity is unmatched due to the presence of 16 different agro-climatic zones, 10 vegetation zones, 25 biotic provinces and 426 biomes (habitats of specific species). Our subcontinent is a vast repository of medicinal plants acquainted as one of the world’s 12 biodiversity centres with more than 45000 different plant species, among which 15000-20000 plants have good medicinal value (Joy et al., 1998). However, only 7000-7500 species are used for their medicinal values by traditional indigenous systems such as Siddha (600), Ayurveda (700) and Amchi (600), Unani (700) and the modern allopathy using 30 plant species for ailments (Rabe and Staden, 1997). Eastern Ghats, one of the nine Floristic Zones in India represents a wide range of diverse medicinal plants; in particular the Kolli hills located in southern-eastern ghats harbours some of the rare and endemic plants (Samy et al., 2008).
MEDICINAL PLANTS CHOSEN FOR STUDY

Orthosiphon spiralis (Lour.) Murr.

Orthosiphon spiralis (Limiaceae), commonly called ‘Kidney Tea Plant’ is a medicinal plant widely used in many parts of the world. It is an erect, perennial shrub found in North eastern India and Western Ghats and Nicobar Islands. The herbaceous shrub can grow to a height of 1.5 metres producing a unique white to light violet flowers that looks like cat's whiskers. Common names for this pant are Misai Kuching (Malaysia), Kumis Kucing and Remujung (Indonesia), Java Tea and Kidney Tea (European), Yaa Nuat Maeo (Thailand) and Poonai meesai (Tamilnadu).

The activity of chemical constituents extracted from the leaves of the plant is attributed mostly to the presence of high percentage (0.7 - 0.8) of potassium salts and bitter glycoside, orthosiphonin. The leaves are reported to contain an alkaloid saponin an essential oil (0.2 - 0.6%), tannins, organic acids particularly tartaric, citric and glycolic, urea and greenish fatty oil. The saponin yields arabinose and glucose (or fructose) besides a sapogenin. The essential oil is yellow in color and has a strong unpleasant odour. The unsaponifiable matter of the fatty oil contains sitosterol and a-amyrin (Panda, 2002) (Basheer and Majid, 2010). The plant is also rich in high amounts of flavones, polyphenols, bioactive active proteins, etc. Bioactive pentacyclic triterpenes Betulinic acid, oleanolic acid, ursolic acid and b-sitosterol have been reported from other species of this family (Tezuka et al., 2000). In Orthosiphon stamineus, more than 20 phenolic compounds were isolated from this plant including lipophilic flavones, flavonol glycosides and caffeic acid derivatives such as Rosmarinic acid and 2,3-dicaffeoyltartaric acid, were identified (Sumaryono et al., 1991). The plant is mainly valued for its medicinal values. Leaves are used for the treatment of various kidney and urinary bladder diseases including nephrocirrhosis and phosphaturia. It is also useful in rheumatism and gout. The use of medicine renders the urine clear and help to make it normal. This herbal medicine has the property to increase the alkalinity in blood or making it alkaline normally; and thus usefully suggested in urinary affections. Orthosiphon stamineus and O. aristatus are synonmys of O. spiralis. O. stamineus exhibits hypoglycemic activity and the plant is also used to treat hepato-renal syndrome and renal ischaemia. Scientific studies have found that the plants of this family possess pharmacological properties such as, antioxidant, antibacterial, heptoprotective, anti-inflammatory, cytotoxic,
diuretic, antihypertensive and vasodialative properties (Masuda, et al., 1992; Tezuka et al., 2000; Beaux et al., 1999).

**Cleome gynandra** Linn.

*Cleome gynandra* [syn. *C. pentaphylla* Linn., *Gynandropsis pentaphylla* DC., *G. gynandra* (Linn.) Briq] of Cleomaceae (Capparaceae) family is an annual herb, widely spread in many tropical and sub-tropical parts of the world. It is an erect glandular-pubescent annual herb, popularly used in the Ayurveda, Siddha, Folk and Tibetan systems of medicine. It is known as Cat’s whiskers and Spider flower in English; Cararvella, varvar, surjavarta and arkapushpika in Sanskrit; Arkahuli, Karaila, hulhul and churota in Hindi; and Velai keerai, neivayalla keerai and katte kadugu in Tamil.

It is popularly used in the ayurveda, Siddha and Folklore systems of medicine. It has been used for several years in Indian traditional medicine as an anti-helminthic and antimicrobial agent. Leaves are applied externally over the wounds to prevent the sepsis. The whole plant is also used in the treatment of malaria, piles and tumor (Mule et al., 2008). In many cultures, the boiled leaves are regarded as medicinal meal for the treatment of various diseases (Wealth of India, 1956). Bruised leaves are reported to be rubefacient, vesicant, antiseptic, anti-inflammatory and analgesic and hence used to treat local pains, neuralgia, rheumatism and scorpion-sting (Heever and Venter, 2007). Oral administration or an infusion of the boiled leaves or the leaf-juice has been recorded to facilitate child birth, to relieve stomach pain, beneficial in constipation, thread worm infection, conjunctivitis, oral ailments, convulsions and certain bilious disorders (Anbazhagi et al., 2009).

The leaf paste of plant has been used in rheumatism, neuralgia, headache and stiff neck. Its warm juice is a popular remedy for ear disease. The leaf juice is applied in skin disease. Juice of fresh leaves is applied externally during pyorrhoea and it is also used as a Wormicide (Kori et al., 2009). The methanol extract of *Cleome gynandra* possess very good antioxidant property. The plant *Cleome gynandra* also possesses anti-inflammatory and lysosomal stability actions in adjuvant induced arthritic rats (Narendhirakannan et al., 2007). The anticancer effect of *Cleome gynandra* against Ehrlich’s Ascites Carcinoma (EAC) in Swiss albino mice has been reported (Bala et al., 2010). The seed powder is used as a treatment for hemorrhages; the seed oil is used for curing skin diseases, and the aerial parts are reported to heal skeletal fractures. Anticancer
activity has been observed for an alcoholic extract of the plant. Das et al (1999) isolated a Novel Dammarane Triterpenoid, Cleogynol from Cleome gynandra.

**PLANT TISSUE CULTURE**

The over exploitation of medicinal plant leads to habitat loss, extinction and reduces species size. Besides that they are prone to environmental catastrophe, demographic or loss of genetic variations and accumulations of deleterious mutation (Frankham, 1995). Mass propagation of plant species through *in vitro* culture is one of the best and most successful examples of commercial application of plant tissue culture technology. Recently, there has been much progress in this technology for some pharmaceutically important medicinal plants.

Plant tissue culture is a collection of techniques used to maintain or grow plant cells, tissues or organs under sterile conditions on a nutrient culture medium of known composition. Plant tissue culture is widely used to produce clones of a plant in a method known as micropropagation. Plant tissue culture relies on the fact that many plant cells have the ability to regenerate a whole plant (totipotency). Single cells, protoplasts, pieces of leaves, or roots can often be used to generate a new plant on culture media given the required nutrients and plant hormones. Plant growth regulators (PGR) stimulate cell division and hence regulate the growth and differentiation of shoot and roots on explants and embryos in semisolid or in liquid medium cultures. The four major PGR used are Auxins, Cytokinins, Gibberellins and Abscissic acid. Addition of PGR is essential to the culture medium.

The first step in plant tissue culture is to develop a callus culture from the whole plant. Callus is a proliferated mass of cells without any significant differentiation. A callus can be obtained from any portion of the whole plant containing dividing cells. To maximize the formation of a particular compound, it is desirable to initiate the callus from the plant part that is known to be a high producer. The callus tissues can be extracted by suitable solvents to isolate the desired compound (Chattopadhyay et al., 2002).

**PLANT SECONDARY METABOLITES**

Plant secondary metabolites are a diverse group of molecules that are involved in the adaptation of plants to their environment but are not part of the primary biochemical pathways of cell growth and reproduction. In general, the terms plant secondary compounds, phytochemicals, antinutritional factors, and plant xenobiotics have been used in the literature to refer to this group
of compounds. There are over 24,000 structures, including many compounds that have antinutritional and toxic effects on mammals. This number does not include the oligomeric polyphenolic compounds (proanthocyanidins and hydrolyzable tannins) that are just now being more accurately described and will increase the number by several thousand. Some major plant secondary metabolites or phytochemicals that occur in plants include protease inhibitors, lectins, alkaloids, nonprotein amino acids, cyanogenic glycosides, saponins, and tannins. These compounds are involved in defense against herbivores and pathogens, regulation of symbiosis, control of seed germination, and chemical inhibition of competing plant species (allelopathy), and therefore are an integral part of the interactions of species in plant and animal communities and the adaptation of plants to their environment (Makkar et al., 2007). Humans use some of these compounds as medicines, flavorings, or recreational drugs.

Medicinal plants serve as a rich source of exploring nature’s chemodiversity, the secondary metabolites which act as leads in drug development strategies. The plant is a biosynthetic laboratory, not only for chemical compounds, but also a multitude of compounds like glycosides, alkaloids etc. These exert physiological and therapeutic effect. The compounds that are responsible for medicinal property of the drug are usually secondary metabolites (Kraisintu, 1997). Many higher plants are major sources of useful secondary metabolites which are used in pharmaceutical, agrochemical, flavour and aroma industries. Several formulations of these plants has been used since folklore traditional systems for therapeutics and improving health such as herbal teas, extracts, decoctions, infusions, tinctures, etc are prepared from medicinal plants (Hassanpour et al., 2011). Some of the more potent and active metabolites includes many alkaloids such as morphine (pain killer), codeine (antitussive), papaverine (phosphodiesterase inhibitor), caffeine (stimulant), various types of cardiac glycosides (heart insufficiency), terpenoids etc. (Wink et al., 2005). Recent times, plants and plant cell cultures have served as resources for flavours, aromas and fragrances, biobased fuels and plastics, enzymes, preservatives, cosmetics (cosmeceuticals), natural pigments, and bioactive compounds (Karuppusamy, 2009).

**ISOLATION AND CHARACTERIZATION OF SECONDARY METABOLITES**

Secondary metabolites are natural products extracted from different parts of the plants like leaf, stem, root, bark, seed etc. A partially purified (untreated) extract from any one of these sources typically contains novel, structurally diverse chemical compounds. The collected plant
materials can be washed, air dried in shade and ground into powder. Many methods are available to extract the metabolites from the powdered plant material: infusion, decoction, digestion, maceration, cold percolation and hot percolation. Hot percolation is done by using a soxhlet apparatus. Different solvents like polar, nonpolar solvents were used to extract the metabolites. The extracts were evaporated using rotary vacuum evaporator and dried by vacuum pump. The dry residues were weighed and reconstituted in the respective solvent. Phytochemical analysis for the presence of various secondary metabolites like alkaloids, flavonoids, terpenoids, etc. was done for the extracts. To isolate different compounds, the extracts were subjected to different chromatographic techniques. Qualitative analysis of the extracts was done by Thin Layer Chromatography (TLC). TLC serves as one of the many methods in providing a chromatographic plant extract fingerprint (Wagner and Bladt, 1996). Column chromatography is a method used to purify individual chemical compounds from the mixtures of compounds. After the separation of individual chemical compounds, they were subjected to quantitative analysis by using High Pressure Thin Layer Liquid Chromatography (HPTLC). HPTLC is a universally accepted analytical method for applications including separation, identification, purification and quantification of various compounds. For the structural identification of the compound they were subjected to UV, IR, GC-MASS and NMR spectral analysis.

**IMPORTANCE OF BETULINIC ACID AND ROSMARINIC ACID**

Betulinic acid (BA) (3β-hydroxy-lup-20(29)-en-28-oic acid), is a widely distributed pentacyclic lupane-type triterpene in the plant kingdom, and has been shown to exhibit a variety of biological activities including inhibition of Human Immunodeficiency Virus (HIV), antibacterial, antimalarial, anti-inflammatory, anthelmintic and antioxidant properties (Yogeeswari and Sriram, 2005). A researcher at the University of Illinois reported that Betulinic acid killed melanoma cells in mice (Pisha et al., 1995). Since then, a number of researchers have conducted laboratory tests on Betulinic acid to determine antitumor properties, especially with respect to melanoma cells. Betulinic acid has recently been selected by the National Cancer Institute for addition into the RAID (Rapid Access to Intervention in Development) program.
Rosmarinic acid (RA) is an ester of caffeic acid and 3, 4 dihydroxyphenyllactic acid. It is commonly found in species of the Boraginaceae and the subfamily Nepetoideae of the Lamiaceae (Petersen and Simmonds, 2003). RA possesses a multitude of biological activities, e.g. antiviral, antibacterial, anti-inflammatory and antioxidant, which makes it a valuable product for the pharmaceutical and cosmetic industries (Li et al., 2005). The presence of RA in medicinal plants, herbs and spices has beneficial and health promoting effects. In plants, RA is supposed to act as a preformed constitutively accumulated defense compound (Petersen and Simmonds, 2003).

ROLE OF SECONDARY METABOLITES AS ANTIOXIDANTS AND ANTICANCER AGENTS

Plants continue to be important sources of new drugs, as evidenced by the approvals in the United States in 1983 of two new plant-derived drugs. Etoposide, a semisynthetic antineoplastic agent derived from the mayapple (*Podophyllum peltatum*), is reported to be useful in the chemotherapeutic treatment of refractory testicular carcinomas, small cell lung carcinomas, nonlymphocytic leukemias, and non-Hodgkin's lymphomas. Atracurium besylate, a skeletal muscle relaxant, is another secondary metabolite; it is structurally and pharmacologically related
to the curare alkaloids. Vinca alkaloids are derived from the *periwinkle* plant, *Catharanthus roseus* (*Vinca rosea*). Vinca alkaloids are useful in treating leukemias. They are effective in the M phase of the cell cycle and work by inhibiting tubulin assembly in microtubules. Vincristine, Vinblastine, Vinorelbine, and Vindesine are some of the popularly used vinca alkaloid chemotherapy agents used today. Major side effect of vinca alkaloids is that they can cause neurotoxicity in patients (Balandrin *et al.*, 1985).

A more recent addition of plant secondary metabolite based chemotherapeutic agent is the taxanes (Kingston, 2005). Paclitaxel (taxol®) initially was isolated from the bark the Pacific Yew, *Taxus brevifolia* Nutt. (Taxaceae), as part of a random collection program for the NCI by the U.S. Department of Agriculture (Cragg and Newman, 2005). The use of various parts of *Taxus brevifolia* and other *Taxus* species (e.g., *Taxus canadensis* Marshall, *Taxus baccata* L.) by several Native American tribes for the treatment of some non-cancerous conditions has been reported, while the leaves of *Taxus baccata* are used in the traditional Asiatic Indian (Ayurvedic) medicine system, with one reported use in the treatment of “cancer” (Hartwell, 1982). Paclitaxel, along with several key precursors (the baccatins), occurs in the leaves of various *Taxus* species, and the ready semi-synthetic conversion of the relatively abundant baccatins to paclitaxel, as well as active paclitaxel analogs, such as docetaxel (Taxotere®), has provided a major, renewable natural source of this important class of drugs. Paclitaxel is used in the treatment of breast, ovarian and non-small cell lung cancer (NSCLC), and has also shown efficacy against Kaposi sarcoma, while docetaxel is primarily used in the treatment of breast cancer and NSCLC. Paclitaxel has also attracted attention in the potential treatment of multiple sclerosis, psoriasis and rheumatoid arthritis. In addition, 23 taxanes are in preclinical development as potential anti-cancer agents. Another important addition to the anti-cancer drug armamentarium is the class of clinically active agents derived from camptothecin, which is isolated from the Chinese ornamental tree, *Camptotheca acuminata* Decne (Nyssaceae) (Rahier *et al.*, 2005). Camptothecin (as its sodium salt) was advanced to clinical trials by the NCI in the 1970s, but was dropped because of severe bladder toxicity, but extensive research led to the development of more effective derivatives, Topotecan and Irinotecan (CPT-11; Camptosar). Topotecan is used for the treatment of ovarian and small cell lung cancers, while Irinotecan is used for the treatment of colorectal cancers.
FUNGAL ENDOPHYTES

Endophytes are fungi or bacteria occurring inside plant tissues without causing any apparent symptoms in the host that are extremely common and highly diverse microorganisms that live within plant tissues, but usually remain asymptomatic. Endophytes traditionally have been considered as plant mutualists, benign commensals or latent pathogens. Hawksworth (2001) estimated that there are 1.5 million species of fungi; of these, only 75,000 species have been so far described. Fungal endophytes play a major role in secondary metabolite production as they co-evolve with pathways of the plant system to produce these natural products. Also, horizontal gene transfer mechanisms or transfer of metabolites to other symbiont following its production by either plant or fungi are few assumptions extensively researched in recent times. They can influence the distribution, ecology, physiology, biochemistry of plants and confer fitness benefits and ecological adaptations of plants. They contribute towards nutrient cycling, drought – survival, fecundity and general fitness in their host plants (Hundley, 2005). They do involve in stress tolerance mechanisms in plants particularly in indirect defence mechanisms against the host pathogens (Rodriguez et al., 2009). They serve as tremendous source of potent novel bioactive metabolites in various clinical ailments.

ELICITORS AS ENHANCERS OF SECONDARY METABOLITES

Plant cell culture systems are viable alternatives for the production of secondary metabolites that are of commercial importance in food and pharmaceutical industries. However, relatively very few cultures synthesize these compounds over extended periods in amounts comparable to those found in whole plants. Various strategies have been employed to increase the production of secondary metabolites in cell cultures as well as in hairy root cultures for commercial exploitation. These include manipulation of culture media (hormonal and nutrient stress) and environmental conditions (temperature, pH and osmotic stress), precursor addition, elicitation and combination of these strategies. Enhancement of secondary metabolites by elicitation is one of the few strategies recently in commercial application (Singh, 1999).

The recent developments in elicitation of cell cultures have opened a new avenue for the production of these compounds. Secondary metabolite synthesis and accumulation in cell cultures can be triggered by the application of elicitors to the culture medium. Elicitors can be defined as signaling molecules triggering the formation of secondary metabolites in cell cultures by inducing plant defense, hypersensitive response and pathogenesis related proteins. Depending on the
origin, elicitors can be classified into two classes: biotic and abiotic. Elicitors of biological origin are called biotic elicitors. These include polysaccharides, proteins, glycoproteins or cell-wall fragments derived from fungi, bacteria and even plants. Among these, fungal elicitors have been most widely studied for enhancement of synthesis of commercially important compounds from plant cell cultures. Elicitors of non-biological origin are called abiotic elicitors, which include metal ions, UV light and chemically defined compounds. Recently, the term ‘abiotic stress’ is also being used for abiotic elicitors (Baldi et al., 2009).

**PLANT SECONDARY METABOLITES INDUCING DNA FRAGMENTATION**

Apoptosis, or programmed cell death, has recently been focussed where the cancer cells are being targeted in inducing their self-destruction thereby cancer growth can be slowed, and even regressed. Although apoptosis is characterized by cell shrinkage and fragmented apoptotic bodies, detection of DNA fragmentation through electrophoresis makes a promising feature for studying dead apoptotic cancer cells (Bakshi et al., 2010). Several plant extracts constitute polyphenolics, terpenoids, tannins, flavanoids etc., unraveling their underlying mechanisms in inducing apoptosis in cancer cell lines. Cells undergoing apoptosis exhibit shrinkage, loss of cell-to-cell contact in organized tissues, and orderly fragmentation of nuclear DNA at internucleosomal sites. The fragmented DNA may then be organized into sharply defined membrane-bound bodies referred to as apoptotic bodies. Studies reveal various metabolites like Betulinic acid, Proanthocyanidins, taxanes, triterpenes, etc. inducing apoptosis evidently observed via DNA fragmentation patterns indicating cytotoxicity (Jarvis et al., 1994).

**PRESENT STUDY**

The chosen medicinal plants were propagated using tissue culture for enhancement of secondary metabolites using elicitors inducing biotic and abiotic stress. The study follows recent approaches in inducing the biotic stress via endophytic fungal counterparts which makes the study a different perspective. Comparative studies were undertaken among the secondary metabolites extracted from wild plants and tissue culture propagates. In particular, Betulinic acid and Rosmarinic acid were purified and subjected for anticancer and antioxidant assays. Their role in inducing apoptosis was also analyzed via DNA fragmentation assay in cancer cells. The study becomes novel for the enhancement of Betulinic acid and Rosmarinic acid among the chosen plants that impacts a futuristic approach for better prospects as drug lead targets and natural anticancer compounds.