INTRODUCTION

The world population is likely to touch the 7.5 billion mark at the current growth rate by the year 2020. Mostly this increase is in the developing or underdeveloped countries, 80% of world population still relies on a traditional system of medicine based on herbal drugs. These folk or household medicines are readily available, cheap and without any side effects. The demand for medicinal plants is continuously increasing not only in developing countries, but also in developed countries as drug, food supplements and cosmetics (Ramawat et al., 2004).

Herbal medicines are as "crude drugs of vegetable origin utilized for the treatment of diseased state often of a chronic nature or to attain or maintain a condition of improved health" (Tyler, 1994). When consider the socio-economic scenario of Asian and African countries, modern medicine is neither affordable nor within the reach of many villagers and tribes inhabiting remote areas and deep forests. There are certain pockets in a country like India where the tribal people have no access to modern amenities like roads, telecommunications and electricity. These communities rely only on their traditional knowledge of medicine for day-to-day requirements (Katewa and Jain, 2006).

India has a well-recorded and traditionally well-practiced knowledge of herbal medicines. There are very few medicinal herbs of commercial importance that are not found in this country. Two of the largest users of medicinal plants are China and India. Traditional Chinese medicine uses over 5000 plant species, while about 7000 are used in India. However, India’s share in the world market is US$ 1 billion, compared to China’s share of US$ 6 billion (Rawat, 2002).

India is one of the 12 mega biodiversity centres, having over 45,000 plant species (17,500 flowering plants, of which 5725 are endemic to India), 8000 of which are
medicinal (Rao, 2006). The flora of India is rich in biodiversity, being a subtropical country, and in Himalaya alone, over 8000 angiosperms, 44 gymnosperms, 600 pteridophytes, 1737 bryophytes and 1159 lichens have been a source of medicine for millions of people in the country and elsewhere in the world (Singh et al., 1996). Some important species have become endangered and need immediate attention for conservation in India.

Medicinal plants and herbs have been used for healing purposes and maintaining good health. Herbal healing has been sustained over a long period since ancient times. There are numerous evidences that herbs and plants have been used in the treatment of diseases and for revitalizing body systems in all ancient civilizations like Indian, Egyptian, Chinese, Greek and Roman. This sector has traditionally occupied an important position in the socio cultural, spiritual and medicinal arena of rural and tribal lives of India. Millions of rural households use medicinal plants in a self-help mode. Medicinal plants constitute a very important bioresource in India because it has one of the richest plant based ethnomedical traditions in the world (Shawl and Qazi, 2004).

About 90% of medicinal plants used by the industries are collected from the wild. While over 800 species are used in production by industry, less than 20 species of plants are under commercial cultivation. Over 70% of the plant collections involve destructive harvesting because of the use of parts like roots, bark, wood, stem and the whole plant in case of herbs. The modern pharmacopoeia still contains at least 25% drugs derived from plants and many others which are synthetic analogues built on prototype compounds isolated from plants (Farnsworth et al., 1985; Astin, 1998; De Silva, 2005).
Industrialization coupled with urbanization is constantly putting pressure on natural resources. Greater demand for these plants especially for the purpose of food and medicine is one of the causes of their rapid depletion from primary habitats. Due to depletion of habitat and ruthless collection, medicinal plants are on the verge of extinction. Many medicinal plant species are disappearing at an alarming rate due to rapid agricultural and urban development, uncontrolled deforestation and indiscriminate collection. Hence, the conservation of these valuable genotypes is imperative (Nalawade and Tsay, 2004).

Plant tissue culture technology holds great promise for conservation and enhancement of the natural levels of valuable secondary plant products and to meet pharmaceutical demands and reduce the in situ harvesting of natural forest resources. There are sufficient reports available for in vitro micropropagation of many threatened medicinal species (Mendelsohn and Balick, 1994). Plant cell and tissue culture are used for clonal propagation, production of disease-free plants, haploid production, triploid production, in vitro pollination and fertilization, embryo rescue, somatic hybridization, somaclonal and gametoclonal variant selection, germplasm conservation, secondary metabolite production and genetic transformation.

*Dregea volubilis* Benth. at present better known by the name of wax plant, is one of handsomest of the climbing Asclepiadaceae. It is an officinal drug of India and the leaves are useful in sore fauces and throat. The leaves and flowers and the rind of unripe fruits are boiled and eaten as a vegetable or used in curries, the cooking removes the bitterness and nauseating property of fruits. The seeds are also reported to eaten.

The juice of the plant is used as a sternuataitory. In preliminary investigation, an alcoholic extract (50%) of the plant showed activity on the central nervous system.
It also showed anti-cancer activity against sarcoma 180 in the mice. The leaves are employed in applications for boils and abscesses. The roots and tender stalks are considered emetic and expectorant. The plant yields a very strong fibre. The twining stems are used as a substitute for ropes. It is externally used on various ulcers and oedema by making slurry with water or alcohol and rabies, injury, sore and boils.

**Plant Name and Family**

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<tr>
<th>Botanical name</th>
<th><em>Dregea volubilis</em> Benth.</th>
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<tr>
<td>Synonyms</td>
<td><em>Wattakaka volubils</em> (L.f.) Staff.</td>
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<td><em>Asclepias volubilis</em> (L.f) and</td>
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<td><em>Marsdenia volubils</em> Cooke.</td>
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<td>Family</td>
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**Vernacular names**

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<th>Tamil</th>
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**Plant description**

A tall, woody climber, c. 11 m. high and 958 cm. in girth, with densely lenticellate and pustular branches, common throughout the hotter parts of India and Car Nicobar, ascending to an altitude of c. 1,500 m. Leaves opposite, broadly ovate or suborbicular, cordate, acuminate; flowers bright-yellowish green, in lateral, dropping, umbellate cymes; follicle usually 2, broadly lanceolate, covered with brown,
ferruginous tomentum, turgid, c. 2 cm. long; seed yellowish brown, broadly ovate, or broad-elliptic, winged, comose.

**Chemical composition**

The stems and leaves contain a pigment taraxerol, a triterpenoid, kaempferol, a glycoside of kaempferol and saponins. The stems, leaves and bark contains drevogenin A, drevogenin P, D-cymarose and L-oleandrose. A new genin, drebbysogenin G, has also been reported in the stem. Other constituents present in the bark are kaempferol and kaempferol 3-galactoside (Sauer *et al.*, 1965; Rao and Rao, 1971).

The seeds contain a number of pregnane glycosides and the glycosides separated include drevosides A, B, C and D, reportedly made up of steroidal genins and 2-deoxy sugars. Several aglycones have been isolated from the seeds i.e., drevogenin A, B, C, D and P. The sugars reported are D-cymarose, biose U1, oleandrose, (+)-methyl-pachybioside, digitoxose and an unknown sugar. The seeds also contain the flavonol glycosides, hyperoside and the follicle-hairs contain hyperoside and rutin (Winkler and Reichstein, 1954; Subramanian and Nair, 1968).

The roots contain a glycoside and traces of an alkaloid. The glycoside showed low toxicity in mice and frogs. It lowered the carotid blood-pressure when administrated intravenously, and had a mild stimulant action on organs having autonomic nerve supply (Gupta *et al.*, 1951). The leaves are edible and used as a green vegetable, while the extract from the whole plant or stem parts has been used traditionally as an anti-inflammatory medicine to treat several diseases including eye ailments, tracheitis and stomachache (Sahu *et al.*, 2002; Biju *et al.*, 2007). Nevertheless, the mechanism underlying is still unclear.
Due to uncontrolled harvesting and overexploitation of wild plants from forest and insufficient attempts to either allow its replenishment or its cultivation, *D. volubilis* is rapidly disappearing and is now a threatened plant facing extinction. In nature, this plant propagates *via* seeds, but poor seed germination is one of the major limitations of natural propagation and vegetative stem cuttings, is also rather too difficult. Therefore, it is necessary to device a method for the development of a potentially large scale multiplication protocol for commercial production of this endangered species. *In vitro* propagation methods offer powerful tool for germplasm maintenance and multiplication.

Recently, the production of secondary metabolites using plant cells has been the subject of extensive research. Many of these compounds are valued for their pharmacological activities and industrial or agricultural properties which increased the commercial value of medicinal herbs (Bingham *et al*., 1998; Paganga *et al*., 1999).

Kaempferol is one of the important polyphenolic flavonoids. It is a natural plant product with potentially useful pharmacological and nutraceutical activities. It is common in vegetable, fruits, plant and herbal medicines. It is known for its health promoting effect and study for its medicinal and nutritional activities. Over the years, Studies have shown that can help treatment of cancers, cardiovascular disease, neuron disorder, cholesterol and serve as antioxidant and anti-inflammatory (Lau, 2008). Also, it has many beneficial biological activities such as antibacterials, restrain cough, anti-spasmodic or spasmolytic, antiulcer, choleric, diuretic and relieve coughing. Kaempferol can be extracted from plant, but only found in small amount (Park *et al*., 2006).
Secondary metabolites are sometimes very low or not detectable in native plant parts and dedifferentiated cells such as callus tissues or suspension cultured cells. For plant cell culture to be economically feasible, certain methods have been discovered and developed that would allow for consistent generation of high yields of secondary metabolites from cultured cells. In order to obtain the desired products at a high enough concentration for commercial purposes, biosynthetic activities of cultured cells were stimulated or restored using various methods (Misawa, 1994).

One of the methods used for obtaining desired secondary metabolites from plants is optimization of cultural conditions like medium, temperature, pH, light and oxygen supply. Several secondary metabolites were found to be secreted in cultured cells at a higher level than those in native plants through these techniques. Other methods employed are selection of high-producing strains, addition of precursors, biotransformation, elicitor treatment, application of immobilized cells and product secretion into the culture media (Misawa, 1994).

Elicitation is also one of the most successful methods employed in the induction of secondary metabolite products. This method uses biotic or abiotic molecule treatment. Effective elicitation has been achieved by Chong et al. (2005) in *Morinda elliptica* cell suspension culture using yeast extract, jasmonic acid, chitosan and glucan at different cell growth cycle. They discovered that different concentration of different elicitors used exerted different effect in cell growth and secondary metabolite, namely antraquinone production.
Aflatoxins are a group of metabolites produced by strains of *Aspergillus flavus* and *A. parasiticus*. Aflatoxin B$_1$ (AFB$_1$) is the most toxic and carcinogenic member of the group and is usually in the highest concentration under natural conditions (Sauer, 1978; Wilson and Payne, 1994). Aflatoxicosis is recently recognized as the sixth amongst the 10 most important health risks identified by (WHO) for developing countries (Williams *et al.*, 2004). Among various mycotoxins, aflatoxins have currently assumed significance due to their deleterious effects on human beings, poultry and livestock. A variety of tissues and organs such as the liver, kidney, nervous system and gastrointestinal system have been reported to be affected by aflatoxin. There are also reports on adverse effects of aflatoxin on germination of seeds (Sinha and Sinha, 1993).

Methods to control aflatoxins are mainly based on chemical strategy (pesticides and fungicides). However, excessive use of chemical treatments has many undesirable consequences: (1) marked pollution of the environment, (2) increased the resistant pathogen populations and (3) presence of chemical residues in food commodities. Thus several research groups are now considering “light” or “natural” food grade products with a high efficacy in the inhibition of mycotoxin production but with a low impact on the environment and on human health (Golob, 2002).

The intake of beta-carotene, ascorbic acid, selenium, uric acid and vitamin E reduced the incidence of AFB1-induced liver cancer in rats (Nyandieka *et al.*, 1990). AFB1-mediated hepatic lipid peroxidation and serum activity of transaminases were reduced by the pretreatment of rats with antioxidants, selenium and vitamin E (Shen *et al.*, 1994). As *D. volubilis* is rich in compounds known to be strong antiinflammatory, it could be expected that extracts obtained from the herb might he haemotoxicity induced by aflatoxin B1. Traditional medicinal plants were applied by
some researchers for their antifungal, antiaflatoxigenic and antioxidant activity (Joseph et al., 2005; Kumar et al., 2007).

Various plant components and extracts from plants such as taxol, curcumin, phenolic acids and flavonoids are reported to inhibit tumor growth in many types of cancer (Sartippour et al., 2001; Rajesh Kumar et al., 2002). Several edible botanical extracts have been reported to have antifungal activity (De et al., 1999; Ferhout et al., 1999; Mastura et al., 1999; Pradeep et al., 2003). The inhibitory effects of plant extracts on aflatoxin synthesis have also been examined (Bhatnagar and Cormick, 1988; Zeringue and Bhatnagar, 1990).

Modulation of immune responses to alleviate the diseases has been of interest for many years and the concept of ‘Rasayana’ is based on related principles (Patwardhan et al., 1990). Rasayana, listed as a class in the texts of traditional Indian Medicine literature, consists of a number of plants reputed to promote physical and mental health, improve defence mechanisms of the body and enhance longevity (Bhattacharya et al., 2000).

Fish, like other vertebrates, respond to infectious agents in both nonspecific and specific ways, although they depend to a much greater extent on the nonspecific mechanisms. Immunostimulant is a naturally occurring compound that modulates the immune system by increasing the host’s resistance against diseases that in most circumstances are caused by pathogens (Logambal et al., 2000; Logambal and Michael; 2001; Venkatalakshmi and Michael, 2001).

Recently, number of attention has focused on food-producing animals as one of several potential sources of antibiotic-resistant bacteria in humans. These antibiotic-resistance genes may spread from animals to humans pathogenic bacteria via the food chain, leading to failure of antibiotic treatment for some life-threatening
conditions. At the same time, other side effects, such as the residual impact on the environment, are also risk factors when commercial antibiotics are used for fish diseases. Vaccines are being developed against fish pathogens but these are not yet commercially available (Harikrishnan et al., 2009).

Immunostimulants used for fish disease control are of interest, as they offer an alternative and its cost-prohibitive or limited effectiveness to the drugs, chemicals and antibiotics, currently (Sakai, 1999). The immunostimulants increase resistance to infectious diseases by enhancing both specific and nonspecific defense mechanisms of fish and animals (Siwicki et al., 1994; Cao et al., 1999; Zhou et al., 2003).

Novel cell surface and soluble signaling molecules produced by cells of the immune system have been discovered that regulate host response to microorganisms. Investigations have focused on discovering compounds that positively or negatively modulates the biological responses of the immune cells and enhance the host ability to resist microbial infection (Tzianabos, 2000). Agents that activate host defense mechanisms in the presence of an impaired immune responsiveness can provide supportive therapy to conventional chemotherapy (Dahanukar et al., 2000).

Primary targets of the immunostimulant are T or B lymphocytes or the complement system, an increase in phagocytosis by macrophages and granulocytes plays a central role in immunostimulation (Kuby, 1994). Activation of macrophages is probably important for the stimulating agents to remain in contact with the reactive cell. The second most important role is the stimulation of T lymphocytes, which can be achieved either directly or indirectly via macrophages (Wagner et al., 1985c).

These immunostimulants may directly initiate activation of the innate defence mechanisms acting on receptors and triggering intracellular gene activation that may result in production of antimicrobial molecules (Bricknell and Dalmo, 2005). It leads
to an increase in various components of immunity such as phagocytic activity, complement activities, lysozyme and disease resistance as well as serum Immunoglobulin agents are widely used to improve the impaired immune functions (Jeney et al., 1997; Sahoo and Mukherjee, 2001) and to stabilize the improved immune status. Among these immunostimulants, natural immunostimulants are biocompatible, biodegradable, cost effective and safe for the environment.

A number of herbal immunostimulants such as *Viscum album*, *Urtica dioica* and *Zingiber officinale* (Dugenci et al., 2003), *Radix astragalin* and *R. angelicae* (Jian and Wu, 2003; Jian and Wu, 2004), *Astragalus radix* and *Scutellaria radix* (Yin et al., 2006), *Achyranthes aspera* (Rao et al., 2006), *Eclipta alba* (Christybapita et al., 2007), *Astragalus membranaceus* and *Lonicera japonica* (Ardo et al., 2008), *R. officinale* (Xie et al., 2008), *A. radix* and *Ganoderma lucidum* (Yin et al., 2009), *Azadirachta indica*, *Oscimum sanctum*, and *Curcuma longa* (Harikrishnan and Balasundaram, 2005; Harikrishnan et al., 2009) have been reported to enhance the innate immunity of fishes. Historically, studies have used hematological indices and immunological assay to determine the health status of fishes (Harikrishnan et al., 2003; Harikrishnan et al., 2009).

**Vibrio** species that play role in distribution of cholera outbreak around the world include *Vibrio cholerae* causes diarrhea disease that infects thousands of people yearly. Accordingly, *V. cholerae* was frequently detected in estuary and coastal waters and its growth in filtered natural seawater under defined laboratory conditions has been reported (Louis et al., 2003; Binsztein et al., 2004; Worden et al., 2006).

The vector-borne diseases (VBDs), malaria, filariasis, Japanese encephalities, dengue, etc., are increasing and have been spreading to newer areas recently due to the increased risk of transmission fuelled by developmental activities, demographic
changes and introduction of new products. All over the world, more than 50% of persons with filariasis receive their infections from the bites of mosquitoes, very particularly *Culex quinquefasciatus* (Southgate, 1984). This species of mosquito and the incidence of filariasis are quite abundant in India, particularly in Tamil Nadu.

Human filariasis is a major public health hazard and remains a challenging socioeconomic problem in many of the tropical countries (Udonsi, 1986). Lymphatic filariasis caused by *Wuchereria bancrofti* and transmitted by mosquito *Culex quinquefasciatus* is found to be more endemic in the Indian subcontinent. It is reported that *C. quinquefasciatus* infects more than 100 million individuals worldwide annually (Rajasekariah et al., 1991). *W. bancrofti* is the most predominant filarial nematode, which is usually characterized by progressive debilitating swelling at the extremities, scrotum, or breast (elephantiasis) in an infected individual (Myung et al., 1998). Vector control is an important compound of the public health programme for malaria and filariasis prevention. The main objective is to break the transmission of parasites popularly known as “Emergency Insectisides” (Verma and Rathman, 1986).

Mosquitoes constitute a major public health menace, serves as a vector for transmitting diseases to humans. Control of such mosquito-borne diseases is becoming more and more difficult because of increasing resistance to pesticides, lack of effective vaccines and drugs against disease-causing mosquitoes. Hence, an alternative approach for mosquito control is the use of extracts of plant origin (El Hag et al., 1999). Search for natural insecticides, which do not have any ill effects on the non-target population and are easily degradable, remains to be one of the top priority issues for the tropical countries (Redwane et al., 2002).

Attempts has been failed to use synthetic insecticides due to development of resistance among mosquito species (Kalyanasundram and Babu, 1982; Ansari et al.,
Safe and efficacious insecticides of plant origin have gained importance in recent years and are considered as less hazardous to human and cattle health. Studies on the larvicidal action of terrestrial plant extracts against the mosquito larvae were carried out tremendously (Dharam Shaktu and Menon, 1983; Kalyanasundram and Das, 1985; Saxena and Sumithra, 1985). Many authors have studied the larvicidal action of terrestrial plant extracts against different mosquito larvae (Kalyanasundaram and Babu, 1982; Saxena and Sumithra, 1985) at very high concentrations of the plant extracts for achieving significant mortality of mosquito larvae. Further more, Phytochemicals may serve as suitable alternatives to synthetic insecticides in future as they are relatively safe, inexpensive, and are readily available in many areas of the world. According to Bowers et al., (1995) the screening of locally available medicinal plants for mosquito control would generate local employment, reduce dependence on expensive imported products and stimulate local efforts to enhance public health.

Plant products a diverse array of secondary metabolites which include alkaloids, flavonoids, terpenoids and other compounds. Natural compounds are likely to provide effective, environmentally safe, easily bio-degradable and narrow spectrum control agents in insects like mosquitoes. The common therapeutic properties of plant extracts like antifungal, anti-bacterial, anti-parasitic, anti-neoplastic and immunomodulator activities have been well exploited, but the search for new compounds with larvicidal and insecticidal activities is becoming more important as the region is currently experiencing a dramatic increase in the incidence of insect-transmitted diseases (Omena et al., 2007).

There are more than 3, 08, 000 plant species, among which 2400 have been reported to possess insecticidal properties. The insecticidal plant receiving global
attention for the last few decades in the wonder tree in Indian origin neem (Babu and Murugan, 1998).

Use of biologically active plant materials with antilarval properties has attracted considerable interest (Kalyanasundaram and Babu, 1982). Because of this, bio-degradable nature and being relatively safer to human beings and non-target organisms in the environment. Indigenous plants, wildly used for flavoring foods and for folk medicinal purposes, are numerous and diverse. In recent years, the uses of plant in processed food are of intense interest in the food industry as an alternative to synthetic antimicrobials and other purposes (i.e., antioxidant activity and other food additives etc.). A large number of plants in different location around the world have been extracted and semi-purified to investigate individually their antimicrobial activity (Draughon, 2004).

In recent years, drug resistance to human pathogenic bacteria has been commonly and widely reported in literature (Mulligen et al., 1993; Davis, 1994; Robin et al., 1998). Because of the side effects and the resistance that pathogenic microorganisms build against antibiotics, many scientists have recently paid attention to extracts and biologically active compounds isolated from plant species used in herbal medicines (Essawi and Srour, 2000). Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world (Saxena, 1997; Nimri et al., 1999; Saxena and Sharma, 1999).

The antimicrobial compounds from plants may inhibit bacterial growth by different mechanisms than those presently used. Antimicrobials therefore, may have a significant clinical value in treatment of resistant microbial strains (Eloff, 1988). In particular, the antimicrobial activities of plant oils and extracts have formed the basis
of many applications including raw and processed food preservation, pharmaceuticals, alternative medicine, and natural therapies (Hammer et al., 1999).

The medicinal value of plants lies in some chemical substrates that produce a definitive physiological action on the human body. The use of plant extracts and phytochemicals, with established antimicrobial properties, could be of great significance in preventive and/or therapeutic approaches. The most important antimicrobial compounds of plants are alkoloids, flavonoids, tannins and phenolic compounds (Ates and Erdogru, 2003; Duraipandiyan et al., 2006; Mahasneh and El-Oqlah, 1999; Nascimento et al., 2000). The increasing prevalence of multi-drug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raised the specter of ‘untreatable’ bacterial infections and adds urgency to the search for new infection-fighting strategies (Zy et al., 2005; Rojas et al., 2006). Contrary to synthetic drugs, antimicrobials of plant origin usually are not associated with many side effects and have an enormous anti-infective potential in numerous infectious diseases.

There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action because there has been an alarming increase in the incidence of new and reemerging infectious diseases. Another big concern is the development of resistance to the antibiotics in current clinical use (Rojas et al., 2003).

In light of the recent emergence of the bacteria that are resistant to multiple antimicrobial drugs posing a challenge for the treatment of infections, the need to discover new antimicrobial substances for use in combating such microorganisms becomes pertinent. Resistant bacteria representing a challenge in the treatments of various well-known infections necessitated the need to find new substances with
antimicrobial properties to be used in the combat against these microorganisms (Martins et al., 2001).

Besides small molecules from medicinal chemistry, natural products are still major sources of innovative therapeutic agents for various conditions, including infectious diseases (Clardy and Walsh, 2004). Current research on natural molecules and products primarily focuses on plants since they can be sourced more easily and selected on the basis of their ethno-medicinal use (Verpoorte et al., 2005). The antimicrobial compounds produced by plants are active against plant and human pathogenic microorganisms (Mitscher et al., 1987). There are several reports in the literature regarding the antimicrobial activity of plant crude extracts and the bioassay-guided fractionation to yield active principles (Palombo and Semple, 2001; Zgoda-Pols et al., 2002; Parekh et al., 2005; Nair and Chanda, 2006; Parekh and Chanda, 2007a).