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The plant kingdom plays an important role in the life of humans and animals. They form the basis for the biological food web and perform a number of environmental services. Plants have played an integral part in the evolution of human cultures, their physical and chemical properties providing not only an invaluable source of food, but also a whole range of material benefits in the form of shelter, clothing and medicine, thus remaining fundamental to their physical, spiritual and social well-being.

Plants have fed and caused the world since began and they have been major source of medicine in all cultures from ancient times. The widespread use of herbal remedies and healthcare preparations, as those described in ancient texts such as the Vedas and the Bible, and obtained from commonly used traditional herbs and medicinal plants, has been traced to the occurrence of natural products with medicinal properties (Hoareau & DaSilva, 1999). Plants are directly used as medicines by a majority of cultures around the world, for example Chinese medicine and Indian medicine. Besides direct usage several medicinal plants form an integrated unit in daily life of humans and other animals in their food system as additives, beverages, as cosmetics, as sweeteners, as biters, as spices, as natural colouring agent and as insecticides.

It has been estimated that of the 2,50,000 higher plant species on earth, more than 80,000 are medicinal, have been used at one time or another for medicinal purposes (Joy et al., 2001). Medicinal plants are the local heritage with global importance because they are most important source of life saving drugs for the majority of the world’s population and they play an important role in the lives of rural
people, particularly in remote part of developing countries with a few healthy facilities. The existence of traditional medicine depends on plant species diversity and the related knowledge of their use as herbal medicine. In addition both plant species and traditional knowledge are important to the herbal medicine trade and the pharmaceutical industry whereby plants provide raw materials and the traditional knowledge prerequisite information (Tabuti et al. 2003).

**Plant -as source of medicine**

Plants can be used as therapeutic resources in several ways. They can be used as herbal teas or other homemade remedies, when they are considered as medicinal plants. They can be used as crude extracts or “standard enriched fractions” in pharmaceutical preparations, such as tinctures, fluid extracts, powder, pills and capsules, when they are considered as phytopharmaceutical preparations or herbal medicines. Finally, plants can be subjected to successive extraction and purification procedures to isolate the compounds of interest, which can themselves be active and used directly as a drug, examples being quinine, digoxin and ergotamine, or they can be used as precursors (e.g. diosgenin) in hemisynthetic processes or as models for total synthesis, with well-defined pharmacological activity or structure–activity relationship studies determining a prototype drug e.g. morphine (Rates, 2001).

The medicinal effects of plants are due to metabolites especially secondary compounds produced by plant species. Plant metabolites include: primary metabolites and secondary metabolites known as phytochemicals. All plants are unlimited source of phytochemicals. Secondary metabolites are biosynthetically derived from primary metabolites such as aminoacids, carbohydrates and fatty acids. These are of major interest because of their different functions ranging from antimicrobial, antibiotic,
insecticidal and hormonal properties to highly important pharmacological and pharmaceutical activities, the more important of these substances are alkaloids, compounds of carbon, hydrogen, oxygen and nitrogen. Besides these substances, glycosides, essential fatty oils, gums, resins, tannins, mucilage, latex, saps are also in large use. These active principles are present throughout the plant body or may be present in storage organs of plant such as root, seeds, bark, leaves, wood. (Roja and Rao, 1998: Khan and Khanum, 1998).

**Importance of traditional medicine**

The traditional medicine is most popular because of holistic approach (Chan, 2005) by a traditional healer. The traditional healer makes a diagnosis based on a thorough examination of the patient and then prescribes a personalized medicine, usually consisting of a mixture of different ingredients. This methodology is in clear contrast with most cases of western medicine, where the same dosage is given to all patients. Only recently a more personalized medication is considered in western medicine, due to the development of novel disciplines such as pharmacogenomics and pharmacogenetics, pharmaceutical disciplines concerned with genetic differences and the effect of medicines. Because of the holistic approach of traditional medicine, a holistic approach to study drug activity of these medicines seems more appropriate than a reductionist approach. Several reasons for a holistic evaluation can be discerned, including the possibility of synergy among plant extract constituents and the occurrence of prodrugs (Verpoorte et al., 2006).

Traditional medicine has served as a source of alternative medicine, new pharmaceuticals, and healthcare products and it is also an important source for pharmacological research and drug development, not only when plant constituents are
used directly as therapeutic agents, but also as starting materials for the synthesis of
drugs or as models for pharmacologically active compounds (Mukherjee, 2003).

According to the World Health Organization (WHO), about three quarters of
the world's population currently use herbs and other forms of traditional medicines to
treat diseases. It has been also reported that more than 50% of all modern drugs in
clinical use are of natural products, many of which have been recognized to have the
ability to include apoptosis in various cancer cells of human origin (Rosangkima &
Prasad, 2004). The usage of medicinal plants is popularly known as herbalism, is a
traditional medicinal or folk medicine practice based on the use of plants and plant
extracts. The medicinal plants play a central role not only as traditional medicines but
also as trade commodities, meeting the demand of distant markets.

Modern world is dominated by many traditional herbal systems, and these
systems are Chinese herbs, Ayurvedic medicine, Roman and Greek herbs, and
Shamanic herbs. As a result, according to WHO as many as 80% of all people living
in the world makes use of herbal medicine as their main source of healthcare. It is
estimated that at least 25% of all modern medicines are derived, either directly or
indirectly, from medicinal plants, primarily through the application of modern
technology to traditional knowledge.

In India there are many traditional medicinal systems comes under two
different streams they are the codified system and the folk system. These indigenous
systems were practiced during different regions and cultures. The Folk (tribal) system
includes various practices of tribal communities. All these systems of medicine
provide good base for scientific exploration of medicinally important molecules from
nature (Haque, 2006).
Around 25,000 effective plant based formulations used in folk medicine and known to rural communities was estimated in India. Besides There are over 1.5 million practitioners of traditional medicinal system using medicinal plants in preventive, promotional and curative applications (Ramakrishnappa, 2002). The traditional medicine cures the ailments and also improves the healthy life. Many components of these plants form food itself or used as spices or as flavouring agents . provide immunity against infection and diseases.

**Scenario of Indian medicinal plants:**

In India, the sages mastered in unparallel knowledge of medicinal plants and their medicinal practice is popularly known as Ayurvedic Medicine. The oldest known repository of our Indian culture is *Rig-Veda* (4500-2500 BC) mentions about hundred medicinal plants and is followed by *Yajurveda* 81 species, *Atharva Veda* (2500-2000 BC) describes elaborate description of medicinal plants. Among two important ancient treatise *The Charak Samhita* (1000 BC) written by Charaka describes the use of over 1100 where as *Sushruta Samhita* (1000-800BC) by *Sushruta* describes properties and use of 1270 species and their medicinal practice is popularly known as Ayurvedic Medicine.

India is the largest producer of medicinal plants and thse plants, besides having natural therapeutic values against various diseases, also provide high quality of food and raw materials for livelihood. The subcontinent has 2.4% of world’s area with 8% of global biodiversity. It is one of the 12 mega-diversity hot-spot regions of the world, other countries being Brazil, Colombia, China, South Africa, Mexico, Venezuela, Indonesia, Ecuador, Peru, USA and Bolivia. Across the country, the forests of India are estimated to harbour 90% of India’s medicinal plants diversity in
the wide range of forest types that occur. Only about 10% of the known medicinal plants of India are restricted to non-forest habitats (Pandey et al. 2008).

According to Schippmann et al. (2002), one fifth of all the plants found in India are used for medicinal purpose. The world average stands at 12.5% while India has 20% plant species of medicinal value and which are in use. But according to Hamilton (2003), India has about 44% of flora, which is used medicinally. Although it is difficult to estimate the number of medicinal and aromatic plants present worldwide, the fact remains true that India with rich biodiversity ranks first in percent flora, which contain active medicinal ingredient. (Wakdiar, 2004).

It has been estimated that 14 - 28% of higher plant species are used medicinally and that 74% of pharmacologically active plant derived components were discovered after following up on ethno medicinal use of the plants (Ncube et al., 2008). Even until recently, these resources were exploited nearly without any major limitations. The initial basic curative role of Medicinal plants has been maintained, since even today 80 % of the world’s population relies primarily on traditional medicine. India is sitting on a gold mine of well-recorded and traditionally well-practised knowledge of herbal medicine. This country is perhaps the largest producer of medicinal herbs and is rightly called the botanical garden of the world (Dubey et al. 2004). Although India has rich biodiversity of medicinal plants, the growing demand for medicinal and aromatic plants is putting a heavy strain on existing resources, causing a number of species to be threatened or endangered.

**Medicinal Plants –Exploitation & Export:**

The global demand for herbal medicine is not only large, but growing (Srivastava, 2000). The market for Ayurvedic medicines is estimated to be expanding
at 20% annually in India (Subrat, 2002). According to UNESCO (1996) the use of traditional medicine and medicinal plants in developing countries is not only for medicinal purpose and also for manufacture of cosmetics and health care products. Furthermore these nations concentrate the extraction and development of drugs from herbals. (UNESCO, 1998). The wide usage of the herbal medicines and their products throughout the globe leads to be establishment of industries based on medicinal plant.

The forests are the chief source of valuable medicinal plants. Nearly about 400 plants used in regular production of indigenous medicines among them about 75% are from tropical and 25% from temperate forests. The preparations are made from whole plant (16%) or from parts like roots (30%), bark (14%), flowers (5%), fruits (10%), leaves (6%), seeds (7%), wood(3%), rhizomes (4%), stems (6%), and cultivated species (<20%) are used (Anonymous, 1997). During past one century there has been a rapid extension of allopathic medicine in India. It develops a commercial trend in the production of pharmacopocial drugs from the plants. The drugs are directly extracted from the plants or from the chemical compound present in those plants by extraction and also the drugs from inorganic materials have plant based ancestral active compound (Mendelsohn & Balick, 1995). Report of WHO indicates about 25% of modern medicines are from local medicine that are descended from traditional plants and also almost 70% modern medicines are derived from natural products (Choudhary, 2002). All these factors influence the medicinal plant market demand leading to over exploitation of plants in their natural habitat and also brought newer opportunities for the herbal industry.

Today India is the second largest exporter of herbal products/drugs in the world, its market share is only 1 to 2% (Thanuja, 2004) and export share, ±10 %.
India exports crude drugs mainly to developed countries like USA, Germany, France, Switzerland, UK and Japan, who share between them 75-80% of the total crude drugs (EXIM Bank of India, 2003).

Conservation of Medicinal Plants:

India is blessed with varieties of medicinal plants and nearly about 8000 more plants are used in the indigenous traditional medicinal system for primary health care. It is estimated that about 800 to 900 medicinal plants are a part of the trade in India. Most of the plants are collected from the forests by excessive and unscientific harvest methods leads to extinction or genetic loss valuable resources (Haque, 2006). The latest global Red-list of plants brought out by The World Conservation Union (IUCN) presents a shocking picture: nearly 34 000 species or 12.5% of the world’s flora is facing extinction. Based on these figures, it is reasonable to estimate that around 1000 of India's 8000 medicinal plant species are also threatened. Threat Assessment studies carried out in recent years have placed about 200 species on the Red-list (Hariharamurthi, 2000).

The tremendous increase in the practice of herbal medicine and the derivates develops a great demand for the herbals and also helped in generation of big income in terms of rupee as well as foreign exchange but it has also resulted in a different type of problems. It leading to over-exploitation of valuable medicinal plants from their natural habitat by destructive collection techniques, over harvesting and habitat destruction due to increased human activities like settlements, conversion of habitats to crop based agriculture and other developmental activities, has also contributed to this. Finally it leads to a virtual decimation of species in the wild. It leads to depletion of plant resources globally and now hundreds of species are threatened with
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extinction. As a result, 20-25% of existing plant species in India has become endangered (Laloo et al., 2006). Hence, it is necessary to take the steps for conservation of these resources for the future generation.

Conservation is done by means of *in-situ* and *ex-situ* methods. In *In-situ* conservation a stock of biological communities are protected and preserved by carried out by maintaining the viable breeding population in the wild. *In situ* conservation of medicinal plants can be accomplished through the active support and participation of communities living in and around the protected forest areas. Hence, the government recognized natural sites in the forests areas rich in medicinal flora and has been named as Medicinal Plant Conservation Areas (MPCAs) network. So far 55 MPCAs have been declared in Karnataka, Kerala, Andhra Pradesh, Maharashtra and Tamilnadu. Besides national parks and wild life sanctuaries and sacred groves in many states contribute conservation of several indigenous medicinal floras.

*Ex-situ* conservation involves conservation of medicinal plants at outside their natural habitat in Medicinal Plant Development Areas (MPDA) and also the botanical gardens, biological parks and other suitable sites play a key role in the cultivation and maintaining the valuable medicinal plants. Biotechnological tools play a significant role in the conservation of critical medicinal plants by different techniques such as micropropagation, genetic transformations and cryopreservation methods helps in long term preservation of plant propagules in gene banks such as seed bank, pollen banks (GoI, 2000).

**Conservation and extraction- through *in vitro* technique**

The application of plant cell, tissue and organ culture has proved its potential for the practical application in the improvement of important and threatened
medicinal plants (Remashree et al., 1997; Tiwari et al., 1998). Plant Tissue culture is relatively recent specialization in the field of biotechnology and is being used globally for the *ex situ* conservation of plants through a technique known as micropropagation. Through tissue culture it can be achieved in a short time and space. It needs minimum requirement of plant material for initiation of culture and gives raise plenty of identical clones’ irrespective of season and with good health status. It was initiated by Morel (1960), propagated orchids through micropropagation since then many crop species and also rare and threatened medicinal plants have been micropropagated.

**Importance of Phytochemical studies**

Plants have limitless ability to synthesize aromatic substances, mostly phenols or their oxygen-substituted derivatives (Geissman, 1963). Most of the natural products are secondary metabolites and about 12,000 of such products have been isolated so far. These products serve as plant defense mechanisms against predation by microorganisms, insects and herbivores (Fransworth and Morris, 1976).

Phytochemical analysis of medicinal plants has shown that numerous compounds in plants traditionally used for medicinal purposes have chemical properties effective at treating illness. Knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but also because such information is of value in disclosing new resources of such chemical substances. In addition, the knowledge of the chemical constituents of plants would further be valuable in discovering the actual value of folkloric remedies (Mojab et al. 2003).

The tissue culture is also adopting the method monitor the medicinal herbs for their secondary metabolites. The compounds synthesized in plant parts can also derive
in the *in vitro* callus synthesize these compounds. Hence, *in vitro* culture is used as an alternative to whole plants for the production of useful secondary metabolites (Ammirato, 1987).

**SELECTED PLANTS FOR RESEARCH WORK:**

**Plant 1. Oxystelma secamone (L.) Karst. (Syn. O.esculentum R. Br.)**

Family: Asclepiadaceae

**Botanical Description:**

A twining glabrous, laticiferous climber, roots fibrous from the lower nodes, stems numerous, long, much branched, slender. Leaves lanceolate, acute, green and glabrous. Flowers white or pink, handsome drooping in pedunculate, lateral sub-umbellate or racemose and green glabrous, ovoid-lanceolate follicles.

**Therapeutic uses:**

The plant is reported to have antiseptic, depurative and galactagogue properties. Decoction of the plant is used as gargle in aphthous ulcerations of the mouth and in sore throat and the latex is bitter and said to have marked antiperiodic action. The fruits are bitter, tonic, expectorant, anthelmintic, and the juice is used in gleet, gonorrhoea, pain in the muscles, cough, leucoderma, given to children as an astringent. Fresh roots are used in jaundice. The flowers, fruits and leaves of the plants are reported to be eaten in times of scarcity and roots and leaves furnish fodder in famine areas.
Plant 2. *Tragia involucrata* L.

Family: Euphorbiaceae

**Botanical Description:**

A perennial more or less hispid twiner, with scattered stinging bristles. Leaves linear, oblong to broadly ovate, base rounded or cordate, acuminate, serrate and hairy. Flowers in terminal or leaf opposed usually hairy bracteate racemes. The males in the upper part, yellowish, the females few (rarely many) in the lower part of the raceme. Capsules hispid or strigosely tomentose or nearly glabrous. Seeds globose, smooth, obscurely hairy.

**Therapeutic uses:**

The roots are reported as diaphoretic, alterative and diuretic. Infusion of root is given in ardent fever and in itching of the skin. The root paste is used to aid the extraction of guinea-worm and mixed with *Ocimum sanctum* juice to cure in skin eruptions and itching and also it is given when extremities are cold during fever, also for pains in the legs and arms. The leaves are used in preparation of an errhine, prescribed in cases of headache. The fruits are reported as beneficial in baldness, when massaged locally with a little water.

Thus the present thesis deals with morphogenesis, micropropagation and phytochemical analysis of selected plants *in vitro*. Literature review reveals the analysis of phytoconstituents carried out in different extracts plant material only and *in vitro* studies were not report so far in both the plants. Phytochemically, the air-dried powder of alcoholic and ether fraction of root of *T. involucrata* contains beta sitosterol and beta-sitosterol –beta- D -glycoside (Srinivasan, 1986). Leaf and root
extracts shows multiple pharmacological effects including antibacterial (Perumalsamy et al., 2006b), wound healing (Perumalsamy et al., 2006c), psychopharmacological (Dhara et al., 2002) and a significant analgesic and anti-inflammatory activities (Dhara et al., 2000, Perumalsamy et al., 2006a) were reported. Trivedi et al. (1990, 1989 and 1988) isolated glycosides from in vivo extracts of Oxystelma esculentum and presence of sterols was reported in plant extract by Srinivasan (1986). Keeping in view of medicinal importance these two plants a comparative study of phytoconstituents of in vivo and in vitro were carried out by in vitro culture techniques.

SCOPE OF PRESENT WORK:

India is native to many indigenous tribal groups and it is popularly known as nation of villages because most of the people live in village. According to the Census Data 2001, there are 638,387 villages in India that represent more than 72 per cent of the total population. Most Indian villagers are concentrated in heavily forested areas that combine inaccessibility with limited political or economic significance. The tribes are inhabitants of forests, their food habits, culture, tradition are all dependent on forests resources. Both tribes and the rural communities utilize maximum number of the plants and their products from the wild and their surroundings in their daily diet and for health care. These plants are frequently used by the local inhabitants of the area for treatment of various diseases and also they are exploiting a variety of herbals for effective curing of various ailments. The health practice commonly known as folk or tribal medicine.
The folk system of medicine is practiced by about 5000 ethnic communities across the country. It has been handed on from generation to generation is rich in household remedies and community practice. Folk medicine utilizes more than 4000 plants out of recorded 8000 medicinal plants of India. But still most of the plants practiced under folk medicine are yet to be documented because the information is transformed orally from generation to generation this leads lack of clear knowledge of valuable plant resources. Hence, there is need to identify and conservation of the plants which are popular among tribes. Now a day’s herbal medical system is popular as alternative medicinal system all over the world. Owing to this it is necessary to identify and analyze the chemistry of medicinal plants to extract the hidden active principles. This is responsible for biological activity (Haque, 2006).

Standardization of these products is most essential to compete in the world market by India because, The world market for plant derived chemicals, pharmaceuticals, fragrances, flavors, and color ingredients, alone exceeds several billion dollars per year (Hoareau & DaSilva, 1999).

Owing to the importance of folk medicine for present study two plants such as Oxystelma secamone (Fig. 1) and Tragia involucrata (Fig. 2) which are considered as threatened and highly valuable in tribal medicine were selected (Chatterjee and Pakrashi, 1994; Kirtikar and Basu, 1975; Sivarajan and Balachandran, 1994; www.iucnredlist.org/details/199694/0)
The work is taken with the following major objectives.

- *In vitro* cultures from leaf and stem explants of *Oxystelma secamone* and *Tragia involucrata*

- Histological studies of the callus culture.

- To identify the secondary metabolites from callus suspension.

- Preliminary phytochemical analysis of *in vitro* and *in vivo* extracts
Tragia involucrata L.
Oxystelma secamone (L) Karst