CHAPTER 1

Introduction

INTRODUCTION

India has a very rich plant biodiversity and diverse forest resources many of which are medicinally useful (Nayar and Sastry, 1987;Sharma and Thokchom, 2014). Even today, the World Health Organization estimates that up to 80 per cent of people still rely mainly on traditional remedies for their medicines. It is estimated that approximately one quarter of prescribed drugs still contain plant extracts or active ingredients obtained from plants (Tripathi and Tripathi, 2003).

1.1 Medicinal Plants

Medicinal plants are globally valuable sources of herbal products and according to the IUCN (International Union for Conservation of Nature) and the WWF(World Wildlife Fund), there are about between 50,000 to 80,000 flowering plant species which are used for medicinal purposes worldwide (Chen *et al,* 2016). However the distribution of medicinal plants is not uniform across the world (Huang, 2011; Rafieian, 2013), for example, China and India have the highest numbers of medicinal plants used, with 11,146 and 7500 species followed by another 16 countries with percentages of medicinal plants ranging from 7 % in Malaysia to 44 % in India versus their total numbers of plant species (Hamilton, 2003; Marcy *et al,* 2005;Srujana *et al,* 2012; Rafieian, 2013).

Majority of the medicinal plants are higher flowering plants and from the analysis on habits of these plants it was estimated that one third of them are trees, shrubs are with nearly same proportion while remaining one third are herbs and climbers (Fig.1) (GOI, 2000;Sharma *et al,* 2010).Of the 386 families and 2200 genera recorded the families Asteraceae,Euphorbiaceae,Lamiaceae,Fabaceae,Rubiaceae,Poaceae,Acanthaceae,Rosaceae and Apiaceae share the large proportion of medicinal plant species with the highest number of species (419) falling under Asteraceae (FRHLT, 2010).

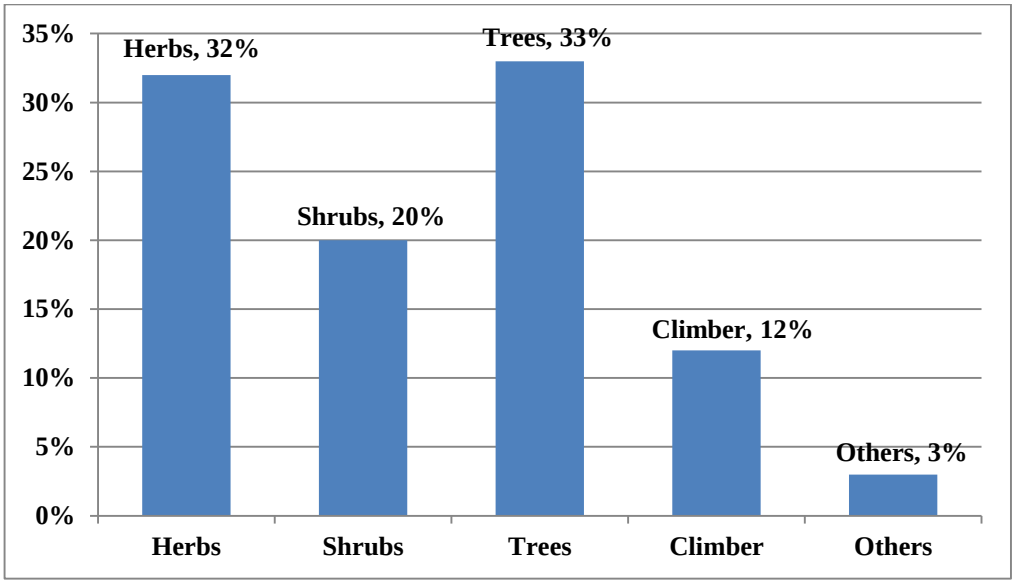

Fig.1: Distribution of medicinal plants by habits (GOI, 2000)

The requirement of medicinal plants high and about 90% of them are collected from the wild for industrial uses. Out of these, less than 20 species of plants are under commercial cultivation and over 70% of the plant collections involves destructive harvesting to obtain their parts like roots, bark, wood, stem and sometimes the whole plant (Fig.2) (GOI, 2000;Sharma *et al*, 2010).

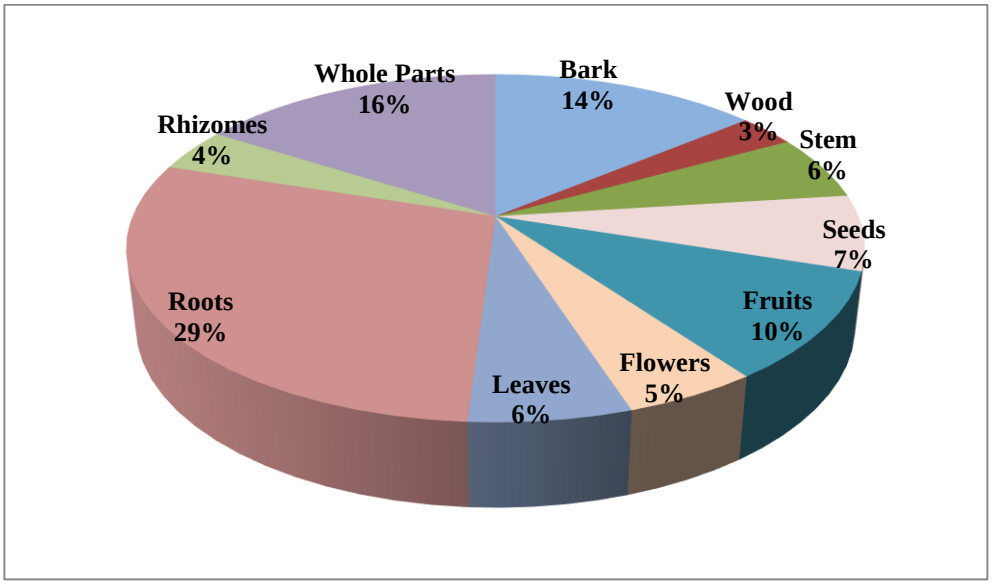

Fig.2: The proportion of different parts of medicinal plants used (GOI, 2000)

Harvesting of whole plant parts or roots is more destructive than collecting their leaves and flowers or buds (Chen *et al,* 2016). Therefore medicinal plants with limited abundance and slow growth, the destructive harvesting generally results in resource exhaustion and even danger of extinction of this species (Larsen and Olsen, 2007;Baker *et al,* 2007).

Hence, the sustainable utilization of medicinal plants should be considered, and good harvesting practices must be formulated (Chen *et al,* 2016). Cultivation of these plants is urgently needed to ensure their availability to the industry as well as to people associated with traditional system of medicine. Increasing demand of these medicinal plants especially for pharmaceuticals is giving warrant for their mass clonal propagation (Chaturvedi *et al,* 2007).

1.2 Importance of forest trees and threats to them

Forest trees are an integral part of human life and vital component of biodiversity that plays a major role in maintaining climatic stability, conserving water and soil, serve as a valuable source of food,fodder,fuel wood, various timber and non timber products. Trees are not only valued for timber but also for a number of other natural products such as fibres, alkaloids, tannins and resins (McCown, 2000). Across the country, the forests are estimated to harbour 90% of India's total medicinal plant diversity and around 10% of the known medicinal plant are restricted to nonforest habitats (Wakdikar, 2004).

Globally 48% of the forest plantation is established for industrial use, 26% for nonindustrial use and remaining 26% is not specified (Yashoda *et al,* 2004).

Due to the rapid growth in the population and the human desire to progress, the pressure on forest and tree-based resources over the years has tremendously increased, resulting in wide scale felling of trees and depletion of natural forest and tree cover from the earth's surface (Giri *et al,* 2004).

IUCN recognises the following different categories of plants on basis of frequency of occurrence in wild: extinct, extinct in the wild, critically endangered, endangered, vulnerable, near threatened, least concern, data deficient and not evaluated. A total of 560 plant species of India have been included in the International Union for Conservation of Nature and Natural Resources (IUCN) Red List of Threatened species, out of which 247 species are in the threatened category. Whereas on a global basis, the IUCN has estimated

that about 12.5% of the world's vascular plants, totalling about 34, 000 species are under varying degrees of threat (Phartyal *et al,* 2002). The species with small populations that are not endangered or vulnerable at present but are at risk in future are called rare (Singh *et al,* 2006). Of the trees on the IUCN Red List about two thirds have been identified as threatened with extinction which resulted in a total number of 9,641 trees threatened with extinction. About one fifth of the threatened trees are Critically Endangered (CR) ie.1, 894, one third are Endangered (EN) ie. 3436 with the rest considered Vulnerable (VU) ie.4311 (ISFR, 2015).

To raise the awareness regarding importance of forests and the major threats they face as well as to strengthen the sustainable forest management, conservation and sustainable development, the year 2011 was declared as the International year of forests by the United Nations.

Due to these threats there is a need to conserve tree ecosystems and necessary to increase propagation and breeding techniques of forest tree species to foster environmental improvement and thus, meet the market demand of wood and wood products (De Gyves *et al,* 2007; Rathore *et al,* 2007).

1.3 Problems in tree propagation

Plant propagation can be carried out through either of the two developmental life cycles the sexual or asexual. In the sexual cycle new plants arise after fusion of the parental gametes and develop from seeds (George, 1984).

Normally trees use seeds as a principal means of establishing their next generation in the natural world. Some trees can easily be grown from seed but, for some trees, it may be much quicker and easier to propagate from cuttings.

Sexual reproduction has many disadvantages like some plants do not produce viable seed or do so only after a long juvenile period (George, 1984). Woody plants are difficult to propagate than herbaceous species as seeds show poor germination and loose viability within short time (Sharma, 2017).

A small seedling can be very tiny and delicate when first germinated and often require much more care than a cutting(Schultz, 2010).Since tree species are out breeders there is a large amount of genetic variation in any seed raised population (Jones and Standen,

1997).Therefore cloning of mature trees is generally preferred over seed raised trees (Bonga, 1987).

Conventional methods of asexual propagation (vegetative propagation) like grafting, layering and cutting also had limited applicability as they are less effective for large scale production. (Yadav and Singh, 2011a; Yadav *et al,* 2012;Sharma, 2017).In many plants species these methods are often too slow or fail completely and also tree breeding efforts are restricted to the most valuable and fast growing species (Giri *et al*, 2004).

The main constraints in vegetative propagation are as follows:

- In general, trees are slow growing, long-lived, sexually self-incompatible and highly heterozygous plants(Williams and Savolainen, 1996; Singh *et al,* 2002;Giri *et al,*2004). Therefore tree improvement by conventional propagation techniques are slow, time consuming and labour intensive, season specific thus making the availability of a large number of plants for plantation or afforestation a difficult and challenging task (Batra *et al*, 2000;Dhawan and Saxena, 2004).
- In many tree species, the cuttings lose their abilty to root by the time a particular clone is evaluated for its useful traits.
- To propagate forest trees from seeds and storage of the same for longer period is not feasible (Kumar De, 2007).
- The plants which are raised from cuttings tend to form adventitious roots which unlike the tap root of seedlings fail to penetrate very deep inside the ground thereby making the plant highly prone to felling by strong winds.
- Other major constraint is that the methods poses a potential risk for spread of various systemic diseases (Dhawan and Saxena, 2004).

1.3.1 Importance of plant tissue culture in forest trees

To meet the increasing demands and severe shortages, currently practiced tree improvement programmes are not adequate and there is an urgent need to improve the quality and quantity of forest trees. Thus, advances in biotechnological research have opened new avenues for rapid multiplication of forest trees (Kataria *et al*, 2013). *In vitro* regeneration or micropropagation is the best alternative which holds tremendous potential for rapid multiplication and production of high quality medicines from them (Murch *et al*, 2000).

The mass multiplication of important tree species can be carried out through tissue culture techniques(Kaur and Kant, 2000) which has the ability to establish and maintain plant organs (embryos, shoots, roots and flowers)and tissues (cells, callus and protoplasts) in aseptic condition (Biondii and Thorpe, 1981) and regenerate them into entire plants as and when required. Growing plants *in vitro* under controlled conditions and the nature of plant material ensures effective clonal propagation of genetically superior genotypes of important plant species. This technique has advantages in forest tree improvement as it offers multiplication and improvement of trees within a limited timeframe (Chaturvedi and Mitra, 1975;Kumar De, 2007). Morel (1960) was first to utilise this technique for rapid propagation of orchids like, *Cymbidium* and *Odontoglossum* which was the only commercially and found the only commercially viable approach for orchid propagation.

1.4 Tree tissue culture in past decades

In the past two decades, the technique has expanded to include many other ornamentals and some horticultural species. In comparison, tissue culture of forest tree species at that time was comparatively at a developing stage. The main reason which can be attributed to non-commercialisation of tissue culture technique for tree species was that overall research on forest tree species lacked behind as compared to agricultural and horticultural plants of high economic value. The other reason was that as tree species have very long life spans and hence breeding is difficult (Dhawan, 1994). The first complete plants from tissue culture of tree species were regenerated by Winton (1968) from leaf explants of black cotton wood (*Populus trichocarpa*).

According to Abbott (1977) woody plants were assumed to be intractable in culture and research workers therefore paid only little attention to them. An increasing number of papers published emphasized the potential and practical applications of *in vitro* culture to the improvement of a wide range of plants(Conger,1981;Rao,1981;Thorpe,1981;Evans *et al*,1983).The history of tissue culture in tree propagation and its importance in reforestation conservation of genetic resources, forest products and clonal improvement has been reported(Lee and Rao,1980;Rao and Lee,1982).

The rapid and mass multiplication of elite and rare trees was of paramount importance (Bajaj, 1986) and therefore forest tree tissue culture had witnessed remarkable advances and had come of age with a bright future (Bonga and Durzan, 1987). Trees have been

attractive model systems to work, not only because of the challenge they pose, but also because of their economic importance (Lakshmi Sita, 1994). Success in regeneration of plantlets in cultured plant cell and tissues had already been achieved for tree species of high economic value in the generation of somaclonal variation, the formation of haploids, triploids and polyploids, somatic hybrids and cybrids and the introduction of foreign DNA through transformation (Hammat, 1992). Many of the studies were aimed at large scale micropropagation of important trees yielding fuel, pulp, timber, oils or fruits (Fossard, 1987).

Forest biologists are now applying biotechnology in tree species because these methods can help save time, reduce costs and accomplish new goals (David and Strauss, 2010). Tissue culture of tree species is now carried out in several research institutes and universities. The commercialization of *in vitro* propagation of forest trees is being done and has already been proven to be successful in some tree species (Kataria *et al,* 2013). Commercially important forest tree species which had been multiplied by using this technique are: *Eucalyptus* (Lakshmisita, 1979; Grewal *et al,* 1980; Gupta *et al,* 1981); *Melia azaderach* (Raghuraman and Ramanujam, 1998); *Celastrus paniculatus* (Arya *et al,* 2002); *Maytenus emerginata* (Rathore *et al,* 1992); *Zizyphus mauritiana* (Sudershan *et al,* 2000); *Balanites aegyptica, Citrus lemon, Syzygium cuminii* (Rathore *et al,* 2004a,b); *Swietenia mahagoni* (Nagarajan *et al,* 2006).Tissue culture techniques have already revolutionized the mass scale propagation of many horticultural crops and several commercial laboratories have been set up in many parts of world for mass production of elite, cloned plant material. A spectacular progress has been achieved on successful commercial-scale exploitation of tissue culture techniques to forest tree improvement (De Gyves *et al,* 2007; Rathore *et al,* 2007).

1.5 Applications of Plant Tissue Culture

Tissue culture technology is potent and has opened extensive areas of research for propagation of endangered and superior genotypes of medicinal plants and in conservation of biodiversity. Micropropagation via tissue encapsulation of propagules can not only facilitate storage and transportation, but also promotes higher regeneration rates (Baker *et al,* 2007). When the amounts of normal seeds are insufficient for propagation, synthetic seed technology, defined as artificially encapsulated somatic embryos (or other tissues)

is a feasible alternative (Zych *et al,* 2005;Lata *et al,* 2008).Therefore microrpropagation and synthetic seed are two applications of tissue culture used for conservation of trees which are explained below.

1.5.1 Micropropagation of tree species

Micropropagation of plants is a multibillion dollar industry being practiced in hundreds of small and large nurseries and commercial laboratories throughout the world (Batra *et al,* 2000).As it plays an important role for rapid and mass multiplication of plants under disease free conditions which are true to type (Sharma *et al,* 2010). Micropropagation of tree species offers not only means for the mass multiplication of existing stocks of germplasm for biomass energy production but also for the conservation of important elite and rare and endangered forest tree species with the danger of extinction(Batra *et al,* 2000;Giri *et al,* 2004). It has many advantages over conventional methods of vegetative propagation, which suffer from several limitations. In normal cuttings, each cuttings can result in only one plant, whereas by micropropagation thousands of plants can be produced from a single small piece of plant (explants). The rate of multiplication is increased, and the mean generation time is decreased because the process can continue all round the year under controlled laboratory conditions and is independent of seasonal changes.*In vitro* cultures needs no attention between passages and therefore there is no labor or materials requirement for watering, weeding spraying etc(George, 1984).

The clonal propagation of plant species is achieved through five stages ie. establishment of explants *in vitro,* followed by rapid shoot multiplication, rooting of microshoots and development and finally hardening and establishment of the plantlets under in vivo conditions (Murashige, 1974). The success of micropropagation largely depends on quality of explants and plant growth regulators used in culture media and culture conditions. The following different stages of it is manipulated by media modification. Debergh and Maene (1981) suggested an additional stage 0 for various micropropagating systems.

Stage 0: Selection and maintenance of stock plants for culture initiation

Stage I: Establishment-placing tissue into culture and having it to initiate microshoots.

Stage II: Multiplication-inducing multiple shoot production

Stage III: Root formation-initiating roots on microshoots

Stage IV: Acclimatization-gradually moving plants to open air conditions

Generally *in vitro* propagation of plants can be carried out by the multiplication of shoots from axillary buds or by the formation of adventitious shoots or somatic embryos (George, 1984).Two of the basic strategies used for micropropagation of forest tree species are direct and indirect regeneration via an intermediate callus phase. The direct regeneration or pathway occurs through the continuous development of shoot meristems activity from lateral or axillary buds whereas indirect regeneration often results in somaclonal variation making the strategy less desirable for large scale clonal multiplication (Giri *et al*, 2004).

Nodal segments containing axillary buds have quiescent or active meristems depending on the physiological stage of the plant. These buds have the potential of developing into complete plantlets under suitable conditions. The conventitional method of vegetative propagation by stem cuttings utilizes the ability of axillary buds to take over the function of main shoot in the absence of a terminal bud. In nature these buds remain dormant for specific periods depending on growth pattern of the plant. By the use of tissue culture the rate of shoot multiplication can be enhanced multifold by axillary bud culture in a nutrient medium containing suitable cytokinin and auxin concentrations. Due to continuous availability of cytokinin, the shoot directly develops from axillary buds. Therefore, direct regeneration ie. axillary shoot proliferation without a callus phase is a reliable and preferred method for clonal propagation of forest tree species as the true to type plants are produced and each culture passage makes feasible to obtain as many as possible propagules from a single explants(Batra *et al*, 2000).The use of a protocol to promote axillary and apical shoot bud proliferation *in vitro* has been used for the propagation of forest tree species. Different basal media, plant growth regulators, media additives and carbohydrate sources are being used to manipulate culture conditions *in vitro* for propagation of forest trees.

Plant growth regulators are important in plant tissue culture since they play vital roles in stem elongation, tropism, and apical dominance. They are generally classified into the following groups; auxins, cytokinins, gibberellins and abscisic acid. Moreover, proportion of auxins to cytokinins determines the type and extent of organogenesis in plant cell cultures (Skoog and Miller, 1957).The plant growth regulators such as auxins (NAA; IAA; IBA and GA_3) and cytokinins (BA; KN; TDZ and Zeatin) were used for multiple shoot

induction in trees. Cytokinins (BA, kinetin,zeatin, and 2iP) promote shoot proliferation, and shoot morphogenesis (Miller and Skoog, 1953; Miller, 1961). Thidiazuron (TDZ;N-phenyl-N^1-1,2,3-thiadiazol-5-ylurea) has cytokinin activity and has been effective in low concentrations to stimulate shoot formation (Sankhla *et al,* 1996; Binzel *et al,* 1996; Murthy *et al,* 1998). It has become a necessity to standardize media formulations when dealing with woody forest tree species.

1.5.2 Synthetic seed technology

The another method of plant propagation using synthetic seeds has opened new vistas in the field of agriculture. Conservation is an important aspect of encapsulation technology(Engleman *et al,* 2003) and is the fast growing area in plant cell and tissue culture because of its wide use in delivery of tissue cultured plants of commercial and economic importance and conserve a number of medicinal plants(Rai *et al,* 2009). Production of synthetic seeds is effective for conservation and propagation of rare, endangered, critically endangered and threatened plants which are difficult to regenerate through conventional methods and due to low seed set and poor seed germination (Kumari *et al,* 2014). Synthetic seed technology is the most significant applications of plant tissue culture, and has great potential for large scale production of different types of plants at low cost as an alternative to true seeds (Gray,1997;Roy and Mandal,2008). Synthetic seeds would be more applicable in exchange of elite and axenic plant material due to small bead size and ease in handling(Rai *et al,* 2009).

Application of this technology has recently been made for plants ranging from herb to tree to solve problems arising out of:

- Storage
- Viability & germination of plants
- Germplasm conservation represented by seeds or vegetative parts functioning as seeds
- Breeding of plants in which propagation trough normal seeds is not possible.

Many of these native species cannot be propagated vegetatively, or produce very low quantities of seed,for this reason the artificial seed is an alternative for these species. (Ray and Bhattacharya, 2008 ; Dhabhai and Prakash, 2012).

The term "artificial seed", which was first coined by Murashige, is now also known by synthetic seed or synseed. The original definition of an artificial seed, as given by Murashige (1978), was "an encapsulated single somatic embryo" and it is often described as artificially encapsulated somatic embryos, shoot tips, shoot buds, cell aggregate or any other tissue that possess the ability to convert into a plant under *in vitro* or ex vitro condition, that can be used for sowing as a seed, that retain this potential even after storage (Capauno *et al*, 1998).

Synseed are produced through a technique called as encapsulation that enhances transportation of *in vitro* derived plants to the field or to the greenhouse (Piccioni and Standard, 1995). Encapsulation of a plant propagule (axillary buds, shoot tips, nodal segments) in a matrix which provides necessary protection during storage, handling and mechanical planting and allow it to grow into a plant (Moradi *et al*, 2016). The encapsulation matrix is a hydrogel made of natural extracts from seaweed(agar, Carageenan or alginate),plants(Arabic or trgacanth),seed gums(guar, locust bean gum or tamarind)or microorganisms(dextran,gellan or xanthan gum).These compounds will gel when mixed with or dropped into an appropriate electrolyte such as Copper sulphate, calcium chloride or ammonium chloride (Wendy Shu, 2001). During encapsulation, explants are mixed with sodium alginate solution and dropped, using a pipette, into a $CaCl_2.2H_2O$ solution, form round and firm beads due to an ion exchange reaction, sodium ions are replaced by calcium ions forming Calcium-alginate. The hardness or rigidity of the capsule depends open the number of sodium ions exchanged with calcium ions. The size of the beads could be controlled by varying the inside diameter of the pipette (Helal, 2011). The main advantages of sodium alginate are its moderate viscosity, non-toxicity, low cost, the long term storability, quick gellation properties, and hardening of beads at room temperature (Kumari *et al*, 2014).The coating may contain nutrients to provide the energy required for germination which is normally supplied by the endosperm. Plant growth regulators may also be included to aid development during germination. This coating provides necessary protection during storage, handling and mechanical planting (Gupta and Kretinger,1993).

In the present work the tissue culture and synthetic seeds studies in two tree species *Oroxylum indicum* and *Stereospermum suaveolens* were carried out.

1.6 Rational for selection of *O.indicum* and *S.suaveolens* for study

The *O.indicum* and *S.suaveolens* are the ingredients of dashmoola which comprises of group of ten plants whose roots are used for preparing ayurvedic formulations. Dashmoolarisht is one of the herbal formulation in whch five of the species are trees and the other five are shrubs or herbs (Ghate, 1999).The proportion of all the ten plants is given in Fig.3.

- *Aegle Marmelos* – Bilva (Indian Bael)
- *Desmodium gangeticum*-Shalaprni
- *Gmelina arborea*-Gambhari(Kasmari)
- ***Oroxylum indicum*-Shyonaka**
- *Premna serratifola*-Agnimantha(Arani)
- *Solanum indicum*-Brihati(Indian nightshade)
- *Solanum xanthocarpum*-Kantakari
- ***Stereospermum suaveolens*-Patala**
- *Tribulus Terrestris* – Gokshura
- *Uraria Picta* – Prishnaparni

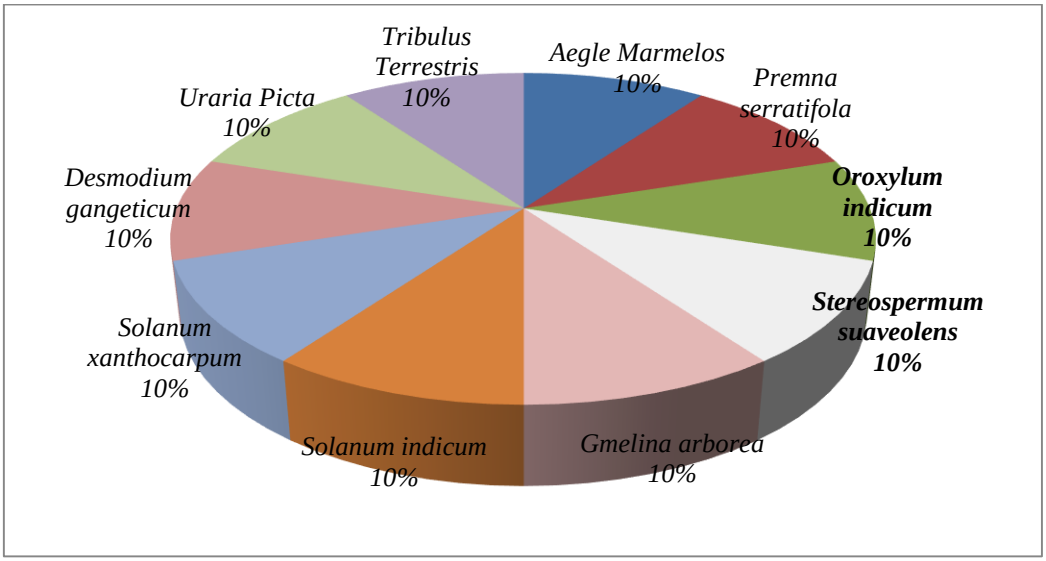

Fig.3: Constituents of Dashmoola (www.ayurtimes.com)

Dashmoola is used for curing many ailments like joints and muscular problems, gastric problems, cough and bronchitis, Gout, Paralysis etc. As it is antiinflammmatory, Antirheumatic, Analgesic, Antioxidant, Anti-paralytic etc.

According to one of the survey the pharmaceutical industries are facing shortage of raw material from species *Aegle marmelos*, *Gmelina arborea*, *Stereospermum suaveolens*, *Desmondium gangeticum*, *Oroxylum indicum*, *Clerodendrum multiforum*, *Solanum*

indicum, Tribulus terrestris, and *Urarua picta* forming the dashmoola group.90 % of these medicinal plants are being collected wild mostly from forest areas and the existing natural resource is not commensurate with the growing demand. About 8000 metric tonnes of roots of dashmoola are used annually by Ayurvedic industry.

Yasodha *et al* (2004) have pointed out the importance of biotechnological research in the tree species of dasamula. In dasamula tree species, where the roots are used in the preparation of ayurvedic formulations, the destruction of plants is severe and planting of seedlings of these species is almost negligible. Clonal propagation through macro and micropropagation techniques is practiced on a limited scale for *Gmelina arborea* and *Aegle marmelos.* Research activities are negligible in species like *Oroxylum indicum, Premna integrifolia and Stereospermum suaveolens.* Hence there is an urgent need to conserve these dashmoola species.

1.7 *Oroxylum indicum* (V.)

O.indicum belongs to family Bignoniaceae and is commonly known as Indian trumphet flower and tree of Damocles and is vernacularly named as Tetu.

1.7.1 Distribution

Oroxylum is a small to medium sized tree which occurs in dry deciduous to moist deciduos forests and found throughout the greater part of India upto an altitude of 1200 m. It is chiefly met with in ravines and moist places in the forests and is rare in the western drier regions (Anonymous, 2001). Globally this tree grows in India, Srilanka, Myanmar,Malysia ,South china, Phillipines and Malacca. In India the species is found throughout the tropical forests ie. North Eastern, Central and Southern India. It is seen to grow naturally in forests near rivers and streams frequent in Vindhyas and southwards in mix deciduos forests, ascending to 1000 m altitude. The species is generally absent in dry climate of Western India. In Karnataka it is recorded in moist deciduos forests of Chikmagalur, dakshina kannada ,uttara kannnada, udupi and coorg districts. In Kerala it is recorded to grow in the lower ghats of Cannanore, Palakkad and Nilabur whereas in Tamilnadu it is reported to grow only from the extreme west of the Thekkady forests.

Naturally grown forests have lost many tree species in which *Oroxylum indicum* Vent. is one of them which is now listed amongst endangered species in many areas in the country

and presently it is known to grow only in gardens. (Gokhale and Bansal, 2006;Najar and Agnihotri, 2012).

1.7.2 Botanical description

Oroxylum indicum is a medium sized soft wooded tree (Fig.4a) attaining a height of 10-16 m. Stem bark is light greyish brown soft and spongy and about 6 mm thick. Leaves are broad opposite,60-120 cm in length and bi or tri pinnately compound with 2-4 pairs of leaflets. These leaflets are ovate or elliptic in shape with acuminate apex and glabrous. Inflorescence is a raceme generally situated at the apices of branches and its length is about 30 cm or more, flowers are numerous violet coloured, bisexual and fleshy having a foul smell. The calyx is leathery oblong-campanulate and glaborus upto 3 cm in size whereas corolla is long bell shaped dark purple colour. Stamens 5 slightly exerted one of them a little shorter than the other 4,filaments are cottony at the base .Capsules are large, sword shaped(Fig.4b,c) upto 90x 9cm flat, tapering to both ends, valves are woody. Seeds are numerous which are flat and thin with broad silvery wing (Fig.4d) (Kirtikar and Basu, 2001; Anonymous, 2001).

1.7.3 Chemical constitiuents

The tree is medicinally important and the different part contains various active ingredients as follows:

- The stem bark contain three flavones colouring matters oroxylin-A (stem bark-0.65%;root bark-0.86 %),baicalein(stem bark-0.5%) and chrysin(stem bark-0.35%)(Anonymous, 2001).
- It also contains tannic acid, scutellarein-7-rutinoside (Grampurohit *et al,* 1994) and ρ-coumaric acid.Root bark contains oroxylin-A and ellagic acid; heart wood contains prunetin and ß-sitosterol9, (Bisht *et al,* 2011)
- Leaves show presence of baicalein-6-glucuronide, baicalein-7-glucuronide,scutellarein, scutellarein-7-glucuronide5, alo-emodin6. They also contain a flavone glucuronide – orxindin,,baicalein 7-O-β-gentiobioside,fixed oil(25%) and crude proteins(7.9%) (Rastogi and Mehrotra, 1993;Grampurohit *et al,* 1994).
- Seeds contain oroxindin, baicalein-6-glucoside, tetuin, a glucoside andfixed oils 10-12 (Bisht *et al,* 2011).

1.7.4 Medicinal uses

O. indicum is extensively used in Indian System of Medicine as an important ingredient of Dashmula which is a compound decoction of 10 roots. It is a medicine of repute in the treatment of remittent fever, otorrhoea, bronchitis, leucoderma, diarrhoea, inflammation and in acute rheumatism (Bisht *et al*, 2011).

- The root bark is a well known drug in ayurvedic system and is prescribed fresh as it is astrigent, tonic, antidiarrhoeal and diuretic, acrid, bitter, pungent, , cooling, aphrodisiac ,increases appetite, useful in vata, fevers, intestinal worms, vomiting, dysentery, asthma, inflammation etc.(Anonymous, 2001).
- The roots are one of the ingredients of the well known ayurvedic formulation dashmoola which is used as anti-inflammatory, appetizing, digestive, carminative, tonic, anthelmintic. Also used for treatment of sprains, cough, asthma, indigestion, dysentery, wounds and fever.
- The roots skin of *O. indicum* is used externally as a paste of its skin of roots, it dries up the discharges and promotes the wound healing, and is also useful in dressing the wounds.
- The decoction of the roots is commonly used for arthritis,in diarrhea and dysentery the decoction is combined with mocarasa (gum of samali *Bombax malabaricum*)or honey (Sharma and Thokchom, 2014).
- The stem bark is more leathery or tough and it is antirheumatic and an infusion of bark powder is diaphoretic.
- The leaves are used externally for enlarged spleen, headache and ulcers.
- Tender fruits have carminative and stomachic properties. The fruit is acrid, sweet, stomachic, anthelmintic, good in diseases of heart and throat, piles, bronchitis. Also an expectorant improves the appetite useful in leucoderma and in treating cough, bronchitis, indigestion and leucoderma.Mature fruits are used in the treatment of intestinal worms, bronchitis and bleeding piles.The seeds are purgative(Anonymous, 2001; Kirtikar and Basu, 2001).
- Young shoots and unripe fruits are edible as vegetable. Flowers and bark of the tree are also reported to be eaten. The tree is lopped for fodder. Thin light seeds are said to be used as stuffing material for hats and umbrellas. Bark and fruits may be used as mordant in dyeing and tanning (Anonymous, 2001).

Fig.4: *Oroxylum indicum*

a. Habit

b. Tree with capsule fruit

c. Single capsule

d. Winged seeds

1.7.5 Mode of propagation and its problems in natural regeneration

Propagation can be done by seeds and root suckers. But the natural propagation of this tree is difficult as the seed set is poor and seed viability is also low (Ankurdeep and Sharma, 2016). Also the indiscriminate over exploitation and uprooting of whole plants for taking out roots for medicinal purpose has pushed this valuable tree to the list of endangered plant species of India. It has become vulnerable in Karnataka and Andhra Pradesh and endangered in Kerala, Maharashtra, Madhya Pradesh and Chattisgarh (Darshan and Ved, 2003; Jayram and Prasad, 2008] and is feared to become endangered soon in other states too (Najar and Agnihotri, 2012).Destructive and non-sustainable collection methods coupled with low regeneration and habitat destruction have posed serious threat to the survival and availability of this highly useful tree (Yasodha *et al,* 2004).Hence there is an urgent need to conserve this tree species which can be achieved through tissue culture.

1.8 *Stereospermum suaveolens* DC.

S.suaveolens belongs to family Bignoniaceae. It is commonly known as Rose Flower Fragrant and vernacularly named as Patla.

1.8.1 Distribution

It is a large deciduous medicinal tree species(Fig.5a) reaching upto 18 m high and 1.8 m in girth found throughout the moist parts of India (Anonymous, 1998).It is also a native to Bangladesh and Myanmar (Troup, 1986) and is mostly distributed in the tropical Africa and Asia. It is found in Western Ghats and also in deciduous forests, in the hills of Mysore, Malabar and Travancore, Scarce. It is found throughout the greater part of India in mixed deciduous and sal forests and is common in the subhimalayan tract ascending to an altitude of 1500 m. In Rajasthan, Chota Nagpur, Central India and in many parts of the peninsula it occurs chiefly in valleys and on plateaux and plains (Anonymous, 1998).

1.8.2 Botanical description

The tree is more or less pubescent, young parts are viscous hairy. Bark grey or dark brown with horizontal furrows exfoliating in large flat scales leaves (Anonymous, 1998). Leaves are long 0.3-0.6m with simply pinnate 3-4 pairs of leaflets which are broadly

elliptic in shape, usually acuminate apex often serrulate. Flowers are sweet fragrant dull purple in colour (Anonymous, 1998) which are arranged in large lax trichotomous viscidly hairy panicles. Calyx are long campanulate 1cm in size that are viscidly hairy, lobes are 3-5 short and broad and corolla are infundibuliform long 2.5-3.8 cm in size. Capsules are cylindric (Fig. 5b,c)0.3-0.6 m by 1.7 cm in size, slightly ribbed, somewhat rough with elevated whitish specks, valves are thick and hard. Seeds long with a membranous wing at each end (Fig.5d) (Kirtikar and Basu, 2001).

1.8.3 Chemical constituents

S.suaveolens is also a medicinal plant with its parts known to possess several active ingredients as follows:

- Both the timber (Sandermann and Dietrichs, 1957) and the root heartwood (Joshi *et al,* 1977) of *Stereospermum suaveolens* were found to contain lapachol,a known elicitor of contact dermatitis(Schulz *et al,* 1977).Wood contains lapachonone. (Anonymous, 1998).
- Plant contains naphthoquinone lapachol. Lapachol shows highly significant activity against walker 256 carcinoma. Root bark contains 6-sitosterol, n-triacontanol. Root, heart-wood also contains, dehydro-a-lapachone and dehydrotectol and Leaves contain flavone glycoside scutellarein, dinatin-7-glucuronide.
- Seeds contain non-drying oil.

1.8.4 Medicinal uses

- Barks, flowers, roots and leaves of *S.suaveolens* are used by traditional healers, rural communities and pharmaceutical companies for remedies of diseases like heating, vomiting, piles, acidity, diarrhoea, gonorrhoea, loss of taste, malaria and other fevers(Troup, 1986).
- The root bark is is an astringent and constituent of an ayurvedic compound Dashmoola. It is regarded as cooling, diuretic and tonic and is generally used in combination with other medicines. The root is bitter, heating useful in kapha and vata, vomiting, asthma, blood diseases, thirst, loss of taste (Kirtikar and Basu, 2001).

Fig.5: ***Stereospermum suaveolens***

a. Habit

b. Tree with capsule fruit

c. Single capsule

d. Winged seed

- A decoction of the roots is used in intermittent and puerperal fevers inflammatory affections within the chest affections of the brain and many other diseases. The bark is considered to possess diuretic and tonic properties. The bark yields a dark coloured gum .It is also reported to contain a bitter substance (Anonymous, 1998).
- The flowers are acrid with a flavour, useful in kapha and vata, bilious diarrhoea, burning sensations (Kirtikar and Basu, 2001)and are given along with honey to control hiccups. The leaves are much lopped for fodder (Anonymous, 1998).
- The fruit is useful in hiccough, leprosy and strangury (Kirtikar and Basu, 2001).

1.8.5 Mode of propagation and its problems in natural regeneration

Can be propagated from seeds and by suckers. But this whole plant is uprooted for taking out roots which are used in preparation of medicine. Also the plant possess low percent of seed germination. The current destructive harvesting practice is seriously reducing seed production, introducing pathogenic infections to standing healthy trees and causing physiological stresses resulting in gradual erosion of the natural populations of this medicinal tree species. Continuation of existing harvesting practice may lead to extinction of this tree species in wild (Baul, 2006).

Fruits of *Stereospermum suaveolens* are capsules usually dehisce on the tree and the light winged seeds escape and carried some distance by the wind (Troup, 1986) which makes collection of seed from forest floor very difficult. The dangerous and difficult task of seed collection by climbing the trees is a disincentive for farmers to cultivate this species. Propagation of the species by seeds is time consuming, troublesome and expensive (Baul, 2006). Hence, steps have to be taken to conserve this tree of great economic value. Therefore in order to propagate the tree faster, tissue culture serves an effective tool.

Since this plants are overexploited for their roots for preparing dashmoola and chywanprash and keeping in mind the problems related with the propagation of two tree species the *O. indicum* and *S. suaveolens* species were selected for the present studies which were carried out with the following hypothesis and objectives.

Hypothesis for the present studies

In vitro regeneration of *O.indicum* and *S.suaveolens* shoots can be induced from different

explants when placed on a medium fortified with PGRs followed by rooting and hardening of plantlets.The synthetic seed production of these species can be through encapsulating *in vitro* nodes in appropriate matrix and regenerative medium.

1.9 Objectives of the study

- Establishing shoot cultures utilizing suitable explants
- Multiplication of shoot cultures in MS and WPM media
- Rooting of *in vitro* shoots
- Hardening of plantlets
- Regeneration of plantlet from synthetic seed