CHAPTER - I

INTRODUCTION
INTRODUCTION

The plant kingdom represents an enormous reservoir of pharmacological molecules to be discovered (Hostettmann et al. 2000). Over the last decade we have witnessed a substantial acceleration of the changes in the drug discovery process as a whole, and these changes have necessarily had a substantial impact in the area of natural products. Compounds of natural origin play a major role as ‘drugs’ and as ‘lead’ structures for the development of synthetic molecules (Harvey 2000). Natural products are being widely used in the form of medicinal plants. This scenario will definitely demand a revised Research and Development agenda for herbal medicines to be in tune to penetrate an estimated 40% of the synthetic pharmaceutical market by 2010. Importance in potential of plants as a source of an amazing variety of secondary metabolites is undisputed. The global scale of plant derived drugs today stands at around 60 billion $ and is estimated to reach 5 trillion $ scale by 2050. Alkaloids, carotenoids, flavonoids, and terpenes will continue to provide the maximum lead structures for drug production (Edwards and Kauffman 2003).

Consequently, the plants that serve as raw material in herbal drug formulations are now required in much larger quantities. Search for new drugs from plants has been accelerated, especially due to concerns about global diversity. Additional efforts are inevitable to evolve strategies for mass propagation of several precious and endangered medicinal plants to bring about the desired improvement for higher yield. Tissue culture techniques are being used globally for the ex situ conservation of plants. The endeavor is to adopt the method to multiply the medicinal herbs and monitor their secondary metabolites. The technique of plant tissue culture has helped in the regeneration of plantlets through various explants of a number of taxa. An added advantage has been to select stage related higher yield of secondary metabolites for consideration of commercial exploitation. The technique of cell culture offers several advantages over their intact counterpart (field growing plants). These include:

- Cells are uniformly grown in sterile environment under defined conditions.
- Relatively simpler organization permits more precise and deliberate manipulations.
Allow easy dissection of biogenetic steps, particularly the metabolic flux at interphase of primary and secondary metabolisms.

Problems related to long distance transport, permeability and segregation of metabolic pools are minimized.

Shorter life-cycle is free of seasonal or batch-to-batch variation.

Biotransformation through feeding and elicitation is possible at different development stages.

Manipulation of pathways with minimum structural constraints and neighboring cell interferences is possible.

Sterile conditions ensure that biogenesis of target molecules per se is not mediated through associated micro-flora normally prevalent in vivo.

Those biosynthetic steps that are expressed at very low level or for limited period of time in intact plants can be prolonged by media manipulation.

Regeneration is necessary, because manufacturing of highly effective plant based medicines requires the best starting material. Searching for new natural products as starting material for pharmaceuticals, cosmetics, dietary supplements and related products lead to a tremendous increase in bioprospecting that is the collection, investigation and utilization of biological and genetic resources. The great challenge in future will be therefore, the use of biodiversity for selection and breeding of new cultivars rich in desired compounds.

1.1 In Vitro Propagation of Medicinal Plants

Plant tissue culture techniques are now being used globally for multiplication of species and also monitoring for secondary metabolites. It is now a well-recognized fact that plants produce an amazing variety of secondary metabolites and many of these have the potential for clinical use. Till date, many of the medicinal drugs are still harvested from plants grown in the wild or from traditional agricultural practices. To ensure sustain supply of quality raw drugs, it is important to domesticate and cultivate many medicinal plants. Successful in vitro regeneration of medicinal plants could be made possible through the use of varied explants such as leaf and stem segments, shoot buds, hypocotyls, cotyledons, roots, anthers and seedlings. Leaf segments of Echinacea
purpurea (Maxwell et al., 2006, Zeng et al., 2006), Bolbostemma paniculatum (Cui-Qin Li and Zhe-Zhi Wang, 2006), Saccharum spp (Prakash et al., 2006), Gloriosa superba (Sivakumar et al., 2003) and Ocimum basilicum (Gopi and Ponnurugan, 2006) are good examples of successful in vitro cultures. Regeneration through stem is reported from Bolbostemma paniculatum (Cui-Qin Li and Zhe-Zhi Wang, 2006) and Lycopersicon esculentum (kokkiraia et al., 2005). In vitro propagation from flower buds of soyabean (Franklin and Sheeba, 2006) and Phyllanthus (Unander 1991). Other examples of micropropagation are: anthers of Datura innoxia (Srivarastava et al., 1993), Gossypium hirsutum (Ganesan and Jayabalal, 2006 a) and Delonix regia (Gupta et al., 1996); Shoot segments of Taxus chinensis and aloe species (Zhihua et al., 2006); adventitious shoots of Salix nigra (Lyyra et al., 2006), Chlorophytum arundinaceum (Latto et al., 2006) and Plantago major (Ping et al., 2005); shoot tips of Withania somnifera (Singh et al., 2006); shoot buds of Ellettaria cardamomum (Regunath and Bajaj, 1992); hypocotyls of Gossypium hirsutum (Ganesan and Jayabalal, 2006 b). Regeneration through corm is reported from Hypoxis hemerocallidea (Yves et al., 2005).

1.1.2 Advantages of Micropropagation:

Micropropagation has a number of advantages over traditional plant propagation techniques:

➢ It produces disease free plants.
➢ It produces rooted plantlets ready for growth, rather than seeds or cuttings.
➢ It has an extraordinary high fecundity; producing thousands of propagules in the same time it would take a conventional technique to produce tens or hundreds.
➢ It is the only viable method of regenerating genetically modified cells or cells after protoplast fusion.
➢ It is a good way of multiplying plants, which produce seeds in uneconomical amounts.
➢ Micopropagation often produces more robust plants, leading to accelerated growth compared to similar plants produced by conventional methods.
1.2 Importance of Plant Tissue Culture Technique for Production of Secondary Metabolite

Generally, medicinally important plant products are secondary metabolites, which are synthesized at variable periods of growth and development. Plant synthesizes a wide range of chemical substances, many of which are commercially useful compounds. The application of secondary compounds fall into five main categories viz. Flavors, perfumes, pigments, drugs and agrochemicals. Plant cells have the potential to produce, either by de novo synthesis, or by biotransformation of specific precursors, an extensive range of secondary metabolites in culture. Secondary metabolism is the synthesis and metabolism of endogenous compounds by specialized proteins. In other words, they are those products (chemical compounds) of metabolism that are not essential for normal growth, development or reproduction of the plant. The function of these compounds to the organism is usually of an ecological nature as they are used as defenses against predators, parasites and diseases, for interspecies competition, and to facilitate the reproductive processes (coloring agents, attractive smells, etc). Since these compounds are usually restricted to a much more limited group of plant species, they have long been of prime importance in taxonomic research (Luckner, 1986). Contrary to primary metabolites these compounds are not ubiquitous in the plants that produce them or are necessarily expressed continuously. Although plants are better known as a source of secondary metabolites, bacteria, fungi and many marine organisms (sponges, tunicates, corals, and snails) are very interesting sources, too. Secondary metabolites can be classified by their chemical structure or physical properties into one or more of the following groups: alkaloids, terpenoids, polyketides, aliphatic, aromatic and heteroaromatic organic acids, phenols, iridoids, steroids, sapogenins, peptides, ethereal oils, resins and balsams.

They are an expression of cell specialization, which is either triggered by the process of cell differentiation or represents an aspect of the process of plant development. Cell differentiation is itself a basic component of metabolic regulation by higher organisms. It includes all processes, which differentiate cell with same genetic composition. Not all-genetic transformation is actually used in the course of development of cell or plant. Thus the biosynthesis of secondary compounds is usually limited to:
1) Particular development stages

2) Specialized cells.

Under specific culture conditions some plant cells are reported to accumulate large amount of secondary metabolites. Media components, phytohormones, temperature, light, either alone or in combination effect the production of secondary metabolites. One of the most exciting aspects of cell culture technology is potential for producing novel structure not observed in the parent plant. Taking the explants from the parent plant, which accumulates a high level of particular secondary compounds in cell cultures. The synthesis of secondary products are generally associated with specialized cell type which is not as surprising as some of them are not produced in the culture plants. Some times factors like light, temperature, plant hormones as well as the precursors may influence the production of secondary metabolites in intact plants.

❖ Secondary product formation can be considered to be an integral part of the differentiation process. So it can be said as the formation of secondary products includes four major categories. Firstly there are compounds which have a widespread occurrence in the plant kingdom and which do not seem to be associated with specialized structures.

❖ Secondly there are the widely distributed compounds, which are normally associated with the cell types and are frequently found in cultures.

❖ Thirdly very broad category includes compounds which are restricted to the distribution pattern in the plant kingdom, but whose synthesis and accumulation do not appear to be associated with any type of specialized cell types.

❖ The fourth major category includes those compounds whose synthesis and their accumulation is normally associated with specialized cells or groups of cells. They generally include the major part of latex, resins and volatile oils.

With the help of production of secondary metabolites the actual organization of the tissues may be important where the indulge of precursors and the products are synthesized in different tissue of the same organ or in different organs. Differentiation and tissue organization are not only the problems that influence the synthesis of secondary metabolites. Apart from it the environmental factors and the medium constituents affect the production of secondary metabolites. The prerequisite is more
promising the possibilities of using tissue culture for commercial biosynthesis. The success will depend very much on whether mutant strains producing high yields of desired compounds can be isolated with appropriate techniques. A more promising possibility is that tissues may be useful for specific metabolic biotransformation, which is difficult by other methods. It is now a well-recognized fact that plant produces an amazing variety of secondary metabolites and many of these have the potential for clinical use. Till date, many of the medicinal drugs are still harvested from plants grown in the wild from traditional agricultural practices. Plant cell cultures of a many advantages over agricultural sources of raw materials. To ensure sustain supply of quality raw drugs, it is important to domesticate and cultivate many medicinal plants.

1.2.1 Enhancement of Secondary Metabolites

The future of commercial production of secondary metabolites in tissue and cell cultures depends primarily on some strategies, which increase the yield of desired metabolites.

- Selection of high yielding plants.
- Establishment of cell cultures from the selected plants.
- Clonal selection of the desired cells.
- Establishment of stable cell line with a high production capacity.
- Optimization of production medium.

Some other strategies include:

- Media optimization.
- Differentiated cells.
- Regeneration of plants.
- Organogenesis and embryogenesis.
- Differentiation, totipotency and meristems in plants.
- Immobilized cells.
- Micropropagation techniques.
- Elicitation.
- Isolation and culture of single plant cells.
- Metabolic engineering.
  - By increasing carbon flux.
  - Catabolism in plant cells.
  - Antisense genes.
Staba (1985) listed the challenges facing plant tissue culture scientists as:

a) To understand better how to de-repress cells or active dormant genes.
b) To determine how cells are interdependent upon each other.
c) To know how to stabilize genotypes.
d) To grow uniform cell and organ biomass economically.

Plant cell cultures have advantages in metabolite production over intact plants due to the facts:

❖ The ratio of cell growth and biosynthesis in culture initiated from a very small amount of plant material is quite high.
❖ Plant cell cultures are maintained under controlled environmental and nutritional conditions, which ensure the continuous yield of metabolites.
❖ Suspension cultures offer a more effective mechanism of incorporating precursors into cells than in whole plants.
❖ New routes of synthesis can be recovered from deviant and mutant cell lines which can lead to production of novel compounds not previously found in whole plants.
❖ Culture of cells may be more economical for those plants, which take long periods to achieve maturity (*Papaver braceatum*, the source of the base, takes 2-3 seasons to achieve maturity).

1.2.2 Elicitors

Elicitors are the substances that can induce defense responses when applied to plant tissues or cultured plant cells (oligosaccharides, glycoproteins, peptides and lipids) (Bautista Ban et al., 2006). Microbial invasion of intact plants will activate plant defense mechanisms including synthesis of antimicrobial metabolites. Elicitors may form inside or outside plant cells, and are distinguished as endogenously or exogenously inducers. Depending on their origin, they are classified as biotic or abiotic. Elicitors also induce rearrangement of metabolic fluxes between a constitutively expressed pathway and an elicitor-inducible pathway. These differential regulations of branch compound biosynthesis reflect an important feature of elicitor induction of plant secondary metabolism. One cost of the biosynthesis of phytoalexins is the decreased accumulation of other primary or secondary compounds. For example, tobacco suspension cultures can
constitutively synthesize sterols by steadily expressing the isoprenoid pathway, especially the rate limiting squalene synthase gene. A fungal elicitor stimulates sesquiterpene phytoalexin production, but inhibits sterol biosynthesis (Zhao, 2005).

Biotic elicitors include polysaccharides derived from plant cell walls (eg. Pectin or cellulose) and microorganisms (chitin, chitosan, glucans), glycoproteins and low molecular weight organic acids. Abiotic elicitors include ultraviolet, salts of heavy metals and chemicals that disturb membrane integrity.

The primary reactions upon elicitation with a biotic elicitor are thought to be composed of recognition of the elicitor and its binding to a specific receptor protein on the plasma membrane. The next step in elicitation is thought to be inhibition of plasma membrane ATPase, which reduces the proton electrochemical gradient across this membrane (Domenburg and Knorr, 1995).

Chitosan has been shown to be a very effective biotic elicitor. Other polysaccharides produced and excreted by plant pathogenic microorganisms, such as xanthan and curdlan or gellan, have also been shown to be active elicitors in plant cell culture systems (Domenburg and Knorr, 1995).

The culture filtrate of Phytophthora megasperma sojae, a fungal pathogen of soyabean, has the ability to stimulate the accumulation of a soyabean phytoalexin in soyabean tissues. The elicitor turned out to be a component of the mycelia cell wall of the pathogen (Rabea, 2003). Treatment of P.somniferum cell suspensions with a homogenate of Botrytis mycelium resulted in a remarkable accumulation of sanguinarine of up to 3% of the cell dry weight (Constable, 1990).

1.2.3 Chitosan

Chitin and chitosan are polysaccharides, chemically similar to cellulose differing only by the presence or absence of nitrogen (Bautista Ban et al., 2006). Chitosan is a polycationic polymer containing more than 5000 glucosamine units. Chitosan is insoluble in most solvents but is soluble in dilute organic acids such as acetic acid, formic acid, succinic acid, lactic acid and malic acid.

The use of chitosan is limited because of its insolubility in water, high viscosity, and tendency to coagulate with proteins at high pH. The characteristics of chitosan that may be varied as required for a particular application are the degree of deacetylation.
(compared to chitin) and the molecular weight. The viscosity of solutions containing chitosan is affected by the degree of deacetylation, the molecular weight, the concentration, the ionic strength, the pH and the temperature. Generally, an increase in temperature causes a decrease in the viscosity of the solution. The effect of pH on the viscosity depends on the particular acid used. Native chitosan is soluble in organic acids when the pH is greater than 6 and insoluble in water, in alkaline medium, or in organic solvents. However, water-soluble salts of chitosan may be formed by neutralization with acids such as hydrochloric acid, acetic acid, lactic acid and formic acid.

Chitosan is inexpensive and nontoxic and possesses reactive amino groups. It has been shown to be useful in many different areas as an antimicrobial compound in agriculture, as a potential elicitor of plant defense responses, as a flocculating agent in wastewater treatment, as an additive in the food industry, as a hydrating agent in cosmetics and more recently as a pharmaceutical agent in biomedicine (Rabea, 2003). The degree of acetylation of chitosan or chitin was found to be important in inducing defense metabolites in the plant cell cultures (Dornenburg and Knorr, 1995).

Chitosan is also important component of cell wall of fungi such as one belonging to class of Zygomycetes (Bartniki-Gracia, 1968). In tomato plants, the production of phenolics, phytoalexins or related compounds, induced by chitosan, precedes or coincides with the action of hydrolytic enzymes of F. oxysporum f. sp. Radicislycopersici (Benhamou and The' rtluaire, 1992).
## Table 1: Some biotic elicitors used for production of secondary metabolites in plant suspension cultures

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Plant Species</th>
<th>Elicitor used</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroxycinnamic derivatives</td>
<td><em>Phoenix dactylifera</em></td>
<td><em>Fusarium oxysporum f.sp. albedinis</em></td>
<td>Daayf et al (2003)</td>
</tr>
<tr>
<td>Indole alkaloid</td>
<td><em>Catharanthus roseus</em></td>
<td><em>Aspergillus niger</em></td>
<td>Zhao et al (2001)</td>
</tr>
<tr>
<td>Rosmarinic acid</td>
<td><em>Ocimum basilicum</em></td>
<td><em>Phytophthora cinnamoni</em></td>
<td>Bais et al (2002)</td>
</tr>
<tr>
<td>Taxol</td>
<td><em>Taxus chinensis</em></td>
<td>Fungi isolated from bark of plant ant salicylic acid</td>
<td>Yu et al (2001)</td>
</tr>
<tr>
<td>Taxol</td>
<td><em>Taxus baccata</em></td>
<td><em>Rhizopus stelonifera</em>, Methyl jasmonate and Salicylic acid</td>
<td>Khosroushahi et al (2006)</td>
</tr>
</tbody>
</table>
1.3 Biological Significance of Saponins:
Saponins constitute a vast group of glycosides, which are ubiquitous in plants but also by lower marine animals and some bacteria. They are characterized by their surface-active properties; they dissolve in water to form a foamy solution. Most saponins have hemolytic properties and are toxic for cold-blooded animals, especially for fish. They deserve attention because of their industrial applications, as some of them are the starting material for semi synthesis of steroidal drugs. Several saponin-containing drugs are also used in the pharmaceutical industry to prepare galenicals and other applications in phytotherapy. Saponins consist of a sugar moiety generally linked with glucose, galactose, glucoronic acid, xylose, rhamnose or glycosidically linked to a hydrophobic aglycone which may be a triterpenoid or steroid in nature. The great complexity of saponin structure arises from the variability of the aglycone structure, the nature of the side chains and the position of attachment of these moieties on the aglycone. Triterpenoid saponins are generally predominant, while steroid saponins are common in plants used as herbs or for their health promoting properties. A number of factors, such as physiological age, environmental and agronomic factors have been shown to affect the saponin content of plants. Generally immature plants of a species have been found to have higher saponin contents than more mature plants of the same species.

1.3.1 Role in plants: The physiological role of saponins in plants is not fully known. Many of them are known to be antimicrobial, to inhibit mould and to protect plants from insect attack. They may be considered as a part of plants defense system and as such have been included in a large group of protective molecules found in plants named phytoanticipins or phytoprotectants. The first term describes those saponins, such as A and B avenacosides in oat, that are activated by the plant’s enzymes in response to tissue damage or pathogen attack. The second describes those saponins that have a general antimicrobial or anti-insect activity. A glycosylated triterpenoid saponin from Pisum sativum was purified and characterized as a specific inhibitor of diguanylate cyclase, a key regulatory enzyme in the synthesis of cellulose. It has also been suggested that saponins could be a source of monosaccharide.
1.3.2 Biological effects in animals

1.3.2.1 Cell membranes:

The interactions between saponins and biological membranes were found to be more complex when the insertion of the aglycon present in the lipid bilayer was independent of the presence of cholesterol (Brain et al. 1990). Cholesterol enrichment was shown to have an inhibitory effect on membrane ATPase that directly interacts with the boundary lipid and alters the intermolecular hydrogen bonds of the protein (Choi et al. 2001). The side chains present on the aglycon such as sugar chains, acyl residues shows a hemolytic activity and were able to alter the membrane activity with the increase number of polar groups attached with them in aglycon moiety (Segal et al. 1974, Oda et al. 2000, Namba et al. 1973).

1.3.2.2 Reproduction:

Saponins were found to be extremely strong stimulators of luteinising hormone release from cultured hypophysical cells, but their action was neutralized in the presence of serum indicating passive membrane permeability (El Izzi et al. 1989, Benie et al. 1990). The butanoic extract of Mussaenda pubescens was capable of terminating pregnancy in rats within few hours of administration, which resulted a highly potent contraceptive drug (Chou et al. 1971). Saponins have been shown to have both positive and negative effects on the viability of human sperm cells in vitro with some ginseng saponins their was an increase motility as well as progression of sperm (Chen et al. 1998). Saponin rich extracts of Turnera diffusa and Pfaffia paniculata improved the copulatory performance of sexually sluggish or impotent rats but they were found to be opposite in case of sexually potent rats (Arletti et al. 1999). Saponins are also reported to affect the functioning of male reproductive system. The gonado-somatic index of 6-month-old male tilapia fed diet containing Quillaja saponin during the initial part of the life cycle was significantly higher than that of control, where as male tilapia receiving a continuous supply of dietary saponins tended to have lower gonado-somatic index. The in vivo effects of saponins on the reproductive function seem to indicate more than a single permeabilising effect on secretory cell membranes and could possibly be linked to interactions between saponins and steroid receptors given the similarities between the basic chemical structures of saponins and steroid hormones (Punnonen and Lukola. 1980). Ginsenoside Rg3 (20(s)
protopanaxadiol type) exhibited a dose dependent inhibitory activity on the expression of marker genes coding for androgen receptors and 5α reductases that converts testosterone into the more potent dihydrotestosterone, which in turns bind to androgen receptors for eliciting its action (Liu et al 2000).

1.3.2.3 Immune System:
Saponin based adjuvant have a unique property to stimulate the cell line immune system as well as to enhance antibody production and also has the advantage of low dose for its potential (Oda et al 2000). Quillaja and other saponins either as crude mixtures or as purified compounds have been reported to increase immune-cell proliferation in vitro (Chavali et al 1987, Plohmaim et al 1997). The mechanism of immune-stimulating action of saponins has not been so clearly justified, but explanations were there to be put forward. Saponins induced the production of cytokinins such as interleukins and interferons that mediate the immunostimulant effects to produce the significant activity (Jie et al 1984, Kensil 1996). The activity of saponins were thought to be because of the adjuvant branched sugar chains or aldehyde groups or an acyl residue bearing the aglycon moiety, they were responsible for the increase or decrease of the activity possessed by them (Boniford et al. 1992). But sometimes it has been noticed that the stimulatory effects on specific immunity components, saponins have also been shown to be able to prevent non specific immune reactions like inflammation and monocyte proliferation (Delmas et al. 2001).

1.3.2.4 Virucidal Activity:
Some saponins and sapogenins have been shown to be capable of deactivating viruses; i.e. purified saponin mixture from Maesa lanceolata, maesasaopnins with 21, 22 diacylation had shown the virucidal activity. Triterpenoid sapogenin oleanolic acid inhibits HIV-I virus replication probably by inhibiting HIV-I protease activity (Sindambiwe et al 1998, Mengoni et al 2002).

1.3.2.5 Anti oxidant Activity: The importance of antioxidants contained in foods is well appreciated for both preserving the foods themselves and supplying essential antioxidants in vivo. It describes chain-breaking inhibitors of lipid peroxidation as free radicals generated in vivo damage many targets other than lipids, including proteins, DNA and small molecules. These oxidations reactions might lead to an array of adverse biological
effects. Some of the protection mechanisms afforded by saponins have been effective to cure diseases. A group of saponins produced by legumes that is namely as group B soyasaponins contain an antioxidant moiety which is attached at C23 allows to scavenge superoxides by forming hydroperoxide which prevents the damage by the mechanism of free radicals (Hu et al 2002).

1.3.2.6 Nervous System Functioning:
Ginseng extract has always been highly successful in proving neutrophic and neuroprotective effects as it increases learning ability and cognitive functions in brain damaged rats in a dose dependent manner and there by enhances the strategic performance of normal rat (Rudakewich et al 2001). When ginseng total saponin was injected intracerebroventricularly it inhibited stress induced hypothalamic-pituitary-adrenal response by inducing nitrous oxide production in the brain (Kim et al 1998). When ginseng saponins were administered intraperitoneally they inhibited the abnormal increase in platelet aggregation and platelet adhesiveness in rats subjected to permanent occlusion of the middle cerebral artery. The anti ischemic effect was mainly due to the changes in the rank and structure of functional membrane proteins induced by fluidity of membranes that leads to a significant change in the protein activities (Ma and Xiao, 1998). Thus these potential functions of saponins suggest that they play an important role in biological system.

Polyvalency is defined as the range of biological activities that an extract may exhibit which contribute to the overall effect observed clinically or in vitro. The last few years have seen a rapid development in small scale, high through put in vitro screening methods for biological activities relevant to particular disease state. These methods have been applied both to drug discovery and ethnopharmacological studies. Because of polyvalency of extract, it is better to perform many assays covering different aspects, in order to cover a wide range of possible activities that could explain the traditional usage of the drug.

1.4 HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY (HPTLC)
As HPTLC has been used in the present investigation for separation and quantitative estimation of active constitution i.e. sarsasapogenin, a brief introduction, principle,
technique and application in the natural product separation, identification and quantitative estimation is reported. Thin layer chromatography (TLC) is the oldest form of planer chromatography. With advanced electronics and technology, additional quantitativeness was introduced both in material used for the technique of separation as well as the equipment used for detection and data acquisition. HPTLC is a planer chromatography, where separation of sample components is achieved on high performance layers with detection and data acquisition using an advanced workstation. It is a valuable quality assessment tool for the evaluation of botanical materials. It represents qualitative/quantitative determination of various components present in extract/formulation irrespective whether or not their exact identity is known. It allows for the analysis of a broad number of compounds both efficiently and cost effectively. These high performance layers are pre-coated plates with a sorbent of particle size 5-7 µ and a layer thickness of 150-200 µ. The reduction in the thickness of the layers and the particle size results in increasing the plate efficiency as well as nature of separation. Separation on high performance thin layer plates gives sharper and more compact bands with shorter distance of migration.

1.4.1 Salient features of HPTLC

1. Simultaneous processing of sample and standard with better analytical precision and less accuracy need for internal standard.
2. Several analysts need work with a better resolution.
3. Lower analysis time is required and it is cost effectiveness.
4. Simple sample preparations handle samples of divergent nature.
5. No prior treatment for solvents like filtration and degassing.
6. Low mobile phase consumption per sample.
7. No interference from previous analysis, fresh stationary and mobile phase for each analysis without any contamination.
8. Visual detection possible with an open system.

Thus HPTLC provides a complete profile of the substances with accuracy and better precision.
REFERENCES


53. Rudakewich M., Ba F, and Benishin C.G. (2001). Neurotropic and neuroprotective actions of Ginsenosides Rb(1) and Rg(1), Planta Medica, 67, 533-537.


