CHAPTER 1

INTRODUCTION

1.1 MEDICINAL PLANTS

Plants have been an exemplary source of medicine for thousands of years (Roberts 1988). The World Health Organization estimates that up to 80% of people still rely mainly on traditional remedies such as herbs for their medicines. Plants are also the source of many modern medicines (Pezzuto 1996). It is estimated that approximately one quarter of prescribed drugs contain plant extracts or active ingredients obtained from plant materials. The most popular analgesic, aspirin, was originally derived from species of Salix and Spiraea. Some of the most valuable anticancer agents such as Paclitaxel (Taxus brevifolia) and Vinblastine (Catharanthus roseus) are derived solely from plant sources.

India is the largest producer of medicinal plants and is rightly called the "Botanical garden of the World" (Govind and Maduri 2006). Around 20,000 medicinal plants have been recorded (Ballabh and Chaurasia 2007) however traditional communities are using only 7,000 to 7,500 plants for curing different diseases (Perumal and Ignacimuthu 2000). These medicinal plants are listed in various indigenous systems such as Siddha, Ayurveda, Amchi, Unani and Allopathy (Rabe and Staden 1997). Plant secondary metabolites such as alkaloids, flavanoids, saponins, phenols, steroids and tannins are the primary active ingredients of ayurvedic drugs. The medicinal plants, besides having natural therapeutic values against various diseases, also
provide high quality food and raw materials for livelihood. Currently the pharmacologically active ingredients of many ayurvedic medicines are being identified and their usefulness as drugs was determined. Hence the traditional knowledge with its holistic and systematic approach supported by scientific documentation can serve as an innovative and powerful discovery engine for newer, safer and affordable medicines (Azaizeh et al 2003).

1.2 ANTIDIABETIC MEDICINAL PLANTS IN INDIA

The Indian system of traditional medicine (Ayurveda) provides a number of medicinal plants to treat diabetes in India (Varier 1995). Traditional knowledge and historic literatures on medicine play an important role in the discovery of novel leads from medicinal plant (Grover et al 2002). It has been estimated that the global burden of diabetes for 2011 would be 285 million which is projected to increase to 438 million in 2030 (WHO 2002). One of the major problems with this herbal formulation is that the active ingredients are not well defined. It is important to know the active component and their molecular interactions, which will help to analyse therapeutic efficacy of the product and also to standardize the drug. Efforts are now being made to scientifically investigate the mechanism of action of natural products (Manisha et al 2007).

There are many active hypoglycemic constituents isolated from the medicinal plants such as dipropyl disulphide oxide (Allium cepa), allicin (Allium sativum), lophenol (Aloe vera), andrographolide (Andrographis paniculata), β-sitosterol (Azadirachta indica), leucodelphinidine (Ficus bengalensis), gymnemic acid (IV) (Gymnema sylvestre), pterostillbene (Petrocarpus marsupium), swerchirin (Swertia chiratia) and trigonellin (Trigonella foenum). Though many of the plants are reputed in the indigenous system of medicine for their hypoglycaemic activities but lack scientific evidence proving their efficacy and safety (Grover et al 2002).
The traditional medicinal practice of Kolli hills of Namakkal district, Tamilnadu, India have revealed the use of Andrographis lineata for various ailments including diabetes, snakebites, cancer and inflammation (Balu and Alagesaboopathi 1995). Although the leaves of A. lineata were very effective in treating diabetes, there was no proper scientific documentation pertaining to the active principle for its antidiabetic effect. In this context an attempt was made to scientifically document the traditional claim in this endemic medicinal plant of South India.

1.3 ANDROGRAPHIS LINEATA WALL. EX. NEES: AN ENDEMIC MEDICINAL PLANT

Scientific classification

Kingdom : Plantae
Division : Magnoliophyta
Class : Magnoliopsida
Order : Lamiales
Family : Acanthaceae
Genus : Andrographis
Species : lineata

A.lineata belongs to the family Acanthaceae which is said to be a large tropical family of about 240 genera and over 220 species. It is endemic in distribution namely in the hilly regions of Asia, Africa, Brazil and Mexico (Lawrence 1969). The genus Andrographis comprised of annual herbs or small shrubs, including about 40 species distributed in the hilly habitats of tropical Asia (Anonymous 1948). About 18 species of Andrographis are reported to occur in India of which A.lineata is relatively unexplored (Lawrence 1969). It is an erect perennial herb which is 1.5-2.0 m tall, endemic to Deccan and Carnatic regions of South India (Gamble 1956). This species is herbaceous (Mathew 1983; Fyson 1915) which is known to occur in
the hills above 1440 m (Figure 1.1 a) (Mathew 1983) in moist or dry or rocky soils. It is called as “Periyanangai” local name in Tamil. The root stock is 80-100 cm. The stem and branches are four angled with thick leaves (Figure 1.1 b). Inflorescence are 10.0 to 15.0 cm long and panicles are glandular hairy. The corolla is white in color with purplish blotches. The capsules (seeds) are green in color of oblong shape with glandular hairs (Figure 1.1 c and d). The fruiting period occurs during August to November and the flowering period occurs from July to October.

![Figure 1.1 Habitat of Andrographis lineata Wall. ex. Nees.](image)

- a) Habitat (Kolli hills, Namakkal District)
- b) Habit of A. lineata
- c) Flowers and seeds of A. lineata
- d) A. lineata grown in green house

**Figure 1.1** Habitat of *Andrographis lineata* Wall. ex. Nees.
1.4 NEED FOR CONSERVATION OF MEDICINAL PLANTS

Most of the plants used in traditional medicines are collected from the wild and only a few have been domesticated (Lucy 1999). Hence, there is a real danger of genetic erosion, which in turn calls for the need for conservation by using propagation techniques. Micropropagation is an important tool in plant tissue culture which produces mass multiplication of “true to type” rare, endemic and endangered medicinal plants under disease free conditions which are independent of seasonal constraints (Chaturvedi 1979). Therefore, owing to its poor regeneration efficiency by conventional methods (seed germination or stem cutting) an attempt was made to micropropagate this potentially important endemic medicinal plant (A. lineata) through shoot tip and nodal explants.

1.5 CLONAL FIDELITY IN MICROPROPAGATED PLANTS

Extensive reports are available on the occurrence of tissue culture induced variation (Chen et al 1998) which warrants the need to verify clonal fidelity of micropropagated plant. Tissue culture induced variations can be determined at the morphological, cytological, biochemical and molecular levels with several techniques (Barazani et al 2002). Molecular markers suitable for generating DNA profiles have proved to be an effective tool in accessing the genetic stability of the regenerated plants (Bennici et al 2003). These markers are not influenced by environmental factors but can generate reliable and reproducible results. DNA based techniques which include restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP) and random amplified polymorphic DNA (RAPD) are used to study the genetic stability of the in vitro regenerated plants. Among these techniques, RAPD is the most preferred in the detection of genetic diversity since it has the advantage of being technically accurate, rapid and requires only small amount of DNA (Shiran et al 2007). In the present study,
the genetic fidelity of in vitro regenerated *A. lineata* was analysed using RAPD technique.

**1.6 PHYTOCHEMICALS AS ANTIOXIDANTS**

Phytochemicals are chemical compounds formed during the plant normal metabolic processes. These chemicals are often referred to as “secondary metabolites” of which there are several classes including alkaloids, flavonoids, coumarins, glycosides, gums, polysaccharides, phenols, tannins, terpenes and terpenoids (Harborne 1973). They are present in a variety of plants and utilized as an important components of food (fruits, seeds, herbs and vegetables) in both human and animals (Okwu 2010). There are several standard methods used for the phytochemical screening of medicinal plants as described for alkaloids, steroids, phenols, flavonoids, saponins, glycosides and tannins (Mohammad et al 2011). Methods for quantitative analysis of phytochemicals such as phenolics, flavonoids, alkaloid, saponins and glycosides were also estimated by Edeoga et al (2005).

Antioxidants are secondary metabolites found to occur naturally in plants such as fruits and vegetables. An antioxidant can be defined in simple terms as a compound that inhibits or prevents oxidation of a susceptible substrate. Plants produce a very impressive array of antioxidant compounds that includes carotenoids, flavonoids, cinnamic acids, benzoic acids, folic acid, ascorbic acid, tocopherols and tocochromens to prevent oxidation of the susceptible substrate (Alam 2009). Common antioxidant includes vitamin A, vitamin C, vitamin E, carotenoids, lutein and β-carotene (Dahanukar et al 2000). These plant based dietary antioxidants are believed to have an important role in the maintenance of human health because our endogenous antioxidants provide insufficient protection against the constant and unavoidable challenge of reactive oxygen species (Fridovich 1998).
During metabolism and other activities beyond the antioxidant capacity of a biological system gives rise to oxidative stress (Erhola et al 1997). Oxidative stress plays a major role in diabetes (Sian et al 2003). Supplementation of dietary antioxidants reduces the risk of oxidative damage and thus arising a necessity to extract these antioxidants from the plant matrices (Urisni et al 1994).

DPPH assay (1, 1 diphenyl 2, picryl hydrazyl), nitric oxide scavenging activity, superoxide scavenging activity, lipid peroxidation assay, reducing power ability, FRAP method (Ferric Reducing Ability of Plasma), cytochrome C test and xanthin oxidase method are the various in vitro models which are used to evaluate the potency of the plant extract to scavenge free radicals (Wang and Curtis 2006). Hence, in our study phytochemical screening is carried out to confirm the presence and quantify the phytoconstituents in the leaves of A. lineata using various solvents based on polarity. The best solvent which extracted maximum phytochemicals was selected for further in vitro antioxidant studies to evaluate the ability of the extract to scavenge free radicals.

1.7 IN VITRO AND IN VIVO MODEL SYSTEMS FOR ANTIDIABETIC ACTIVITY

α-glucosidase inhibition assay is the widely used in vitro model to screen the antidiabetic efficacy of the plant extract. It works on the principle of preventing the increase of post prandial hyperglycemia. On the other hand in vitro models are also available in cell lines for screening antidiabetic activity are pancreatic (RINm5F, BRIN BD 11, HIT T15), adipocytes (3T3L1), hepatic (HEP G2, H 4 IIE), muscle (L6, C2C12, CaCO2). These cell lines are based on the mechanism of impairment of glucose uptake from small intestine, stimulation of insulin secretion, insulin mimetic and insulin
sensitization activity at target tissue such as liver, skeletal muscle or adipocyte and antagonism of glucagons activity (Patel and Mishra 2011).

Prior to the cell line study the plant extracts are subjected to cytotoxic studies (MTT assay) to determine the viability of the cells. This assay works with the principle of reduction of yellow color (MTT) to purple formazan by the enzyme reductase which is present in living cells (Freshney 2000). Based on the cytotoxic studies, the dosage levels of the plant extract to the cell lines were optimized. Hence, the potency of the A.lineata extract to inhibit the carbohydrate digesting enzymes (α-glucosidase) by preventing post prandial hyperglycemia was studied. Insulin mimicking and sensitization activities were also evaluated in the extract to study the synergistic effect in 3T3-L1 cell line.

Animal models of diabetes are greatly useful and have distinct advantage in biomedical studies because they offer promise of new insights into diabetic research (Srinivasan and Ramarao 2007). The currently available compounds to induce diabetes in rats are alloxan monohydrate, streptozotocin (STZ), nicotinamide, ferric nitrilotriacetate, ditizona and antiinsulin serum. The most usual substances to induce diabetes in the rat are alloxan and streptozotocin (Raju and Balaraman 2008).

STZ (2-deoxy-2 (3-(methyl nitrosureido)-D-glycopyranose)) is synthesized by Streptomyces achromogenes and it is well known for its selective β-cell cytotoxicity, less toxic than alloxan and allows the consistent maintenance of diabetes mellitus (Balamurugan et al 2003). In the present study, the ameliorative effect of the EtALL extract was analysed in STZ induced rats.
1.8 BIOACTIVITY GUIDED FRACTIONATION

Bioassay guided fractionation is a procedure whereby the extract is chromatographically fractionated and refractionated until a pure biologically active compound is isolated. Each fraction produced during the fractionation process is checked for its purity in the thin layer chromatography (TLC) and further evaluated in a bioassay system (*in vitro* model). *In vitro* bioassays can be divided into two groups, molecular assays and cellular assays (Rees et al 2001). Molecular assays look for activity using isolated systems such as enzymes, receptors and genes, while cellular assays use intact cells. Molecular assays usually deal with a single subcellular target, while cellular assays detect any substance inhibiting cell growth (Atta-ur-Rahman et al 2005).

Lipogenesis is the process by which simple sugars such as glucose are converted into fatty acids, which are subsequently esterified with glycerol to form the triacylglycerols and are packed as very low density lipoproteins which are secreted from the liver (Gregoire et al 1998). The active fraction which exhibits the highest percentage of lipogenesis activity were selected and then subjected to spectral analysis to determine its structure and composition. The three main structure determining techniques used are mass spectrometry (MS), nuclear magnetic resonance spectroscopy (NMR) and infrared spectroscopy (IR). These methods have seen vast improvements in sensitivity and technological advances over the past ten years and when taken in tandem they represent very powerful means of solving unknown molecular structures (Mendonca-Filho 2006). High-resolution mass spectrometry in most instances, provide the molecular mass to such a precision that the molecular formula can also be determined. The exact number of hydrogen and carbon atoms may be verified from one dimensional (1D NMR and $^1$H-$^1$H
COSY) data, which gives information about connectivity’s between magnetic nuclei in the sample, elucidating the full structure. IR gives the details of the possible functional groups present in the bioactive compound. Therefore, in the present study the bioactive principle for antidiabetic effect in the leaves of *A. lineata* was isolated using bioassay guided fractionation technique and the structure elucidation of the bioactive compound was carried out using spectral analysis.

### 1.9 GENE EXPRESSION

Transcriptional profiling is a tool that provides unique data about disease mechanisms, regulatory pathways, and gene function (Welsh et al 2001). This technology not only allows comparison of gene profiles in normal and pathological tissues but also helps to establish interrelationships among genes (Yokomori et al 1999).

Carbohydrate metabolism and differentiation of 3T3-L1 adipocytes are associated with diabetes. Peroxisome proliferators activated receptor (PPAR-γ) and the CCAAT/enhancer binding protein family (C/EBP-α, β, and δ) are critical factors in 3T3-L1 preadipocyte differentiation (Po-Jung et al 2005). PPAR-γ is a member of the nuclear receptor super family of transcription factors and it is predominantly expressed in adipose tissue. C/EBP families are basic leucine zipper transcription factors (Wei et al 2005). C/EBP family and PPAR-γ are sequentially expressed during 3T3-L1 preadipocyte differentiation. The promoters of several adipogenic genes are regulated by these transcription factors during adipocyte differentiation. Hence, in the present study the isolated bioactive principle was evaluated for the expression level of PPAR-γ and C/EBP-α genes.
1.10 MOLECULAR DOCKING IN ANTIDIABETIC DRUG DEVELOPMENT

Plant based compounds now serve as the base for drug discovery. Computer-aided drug design (CADD) is an exciting and diverse discipline where various aspects of applied and basic research merge and stimulate each other (Brooijmans and Kuntz 2003). Drug discovery typically starts with an analysis of binding sites in target proteins or an identification of structural motifs common to active compounds. It ends with the generation of small molecule “leads” suitable to further chemical synthetic work (Becker et al 2006).

Molecular-docking methodologies ultimately seek to predict the best mode by which a given compound will fit into a binding site of a macromolecular target (Hongming et al 2006). The currently available docking software’s are GLIDE, GOLD, FLEX-X, and AUTODOCK. Among these software’s GOLD is highly regarded within the molecular modeling community for its accuracy and reliability. Hence, GOLD software was used to dock the isolated bioactive principle with the receptors (PPAR-γ and C/EBP-α).

There has been an extensive research focused on PPAR-γ belonging to nuclear receptor family and C/EBP-α CAAT enhancer binding proteins which are ligand-activated transcription factors. PPAR-γ and C/EBP-α is expressed most abundantly in adipose tissue and mediates the antidiabetic activity of the insulin-sensitizing drugs belonging to the thiazolidindione (Campbell 2005). This key transcriptional factor plays a pivotal role in regulating adipogenesis, insulin sensitivity and glucose homeostasis (Willson et al 2000). A drug molecule is triggered when the binding of small molecule to the receptor protein is perfectly done. Such protein-ligand interaction is comparable to the lock-and-key principle, in which the lock encodes the
protein and the key is ensembled with the ligand. The major driving force for binding appears to be hydrophobic interaction whose specificity is however controlled by hydrogen bonding interactions (Hugo 1998). Therefore, in the present study the binding efficiency is determined to evaluate the best fit molecule of the isolated bioactive compound to the receptors (PPAR-γ and C/EBP-α).

1.11 MOTIVATION OF THE PRESENT STUDY

*Andrographis lineata* is an erect perineal herb endemic to Deccan and Carnatic regions of South India. The leaves of *Andrographis lineata* have been reported for various ailments including diabetes, snakebites, cancer and inflammation. There are reports on insecticidal activity, antimicrobial, diuretic and hepatoprotective activity. Due to the wide range of medicinal applications, the plant is generally harvested from the wild, a process jeopardizing the natural biodiversity. The uncontrolled harvesting coupled with habitat destruction is the main cause of decline in the population of this plant and hence sustainable harvest of this plant from wild is no longer a viable option. Therefore, some other strategies should be devised to meet the requirement of this plant for traditional medicine, which is considered as an important mode of health care. *In vitro* conservation strategies have several benefits for the quick cultivation of medicinal plants, thereby providing a continuous supply of plant materials from elite germplasm lines, which can make significant contributions to the exploitation of therapeutic properties of these plant species and eliminate the need for harvest from the wild. Hence an attempt was made to formulate a protocol to ecorestore this valuable medicine plant for phytomedicine production.

Diabetes, particularly obesity and metabolic syndrome related type 2 diabetes, is a major health problem in the Western world, and it is becoming an increasing threat in developing countries as wealth accumulates and
lifestyles changes. It may be caused by metabolic syndrome, which is characterized by a reduced sensitivity to insulin signaling and a reduced efficiency of glucose transport, primarily in adipocytes and muscle cells, leading to hyperglycemia and hyperinsulinemia. Glitazones can enhance adipocyte differentiation to increase glucose uptake in cells to decrease blood glucose levels, the mechanism being widely used for the treatment. However, this could result in excessive accumulation of white adipose tissue. Recent studies showed that white fat tissue accumulation plays a crucial role in the development of obesity and type 2 diabetes.

Therefore, there is a contradiction in using glitazones for the treatment of obesity and T2D. Thus, a novel small active molecule with insulin-like actions, but having no effect on accumulation of white adipose tissue is desirable. Plants have been an exemplary source of medicine for thousands of years. The World Health Organization estimates that up to 80% of people still rely mainly on traditional remedies such as herbs for their medicines.

India is the largest producer of medicinal plants and is rightly called the "Botanical garden of the World". The traditional knowledge with its holistic and systematic approach supported by scientific documentation can serve as an innovative and powerful discovery engine for newer, safer and affordable medicines for their hypoglycaemic activities. There is a lack of scientific evidence proving their efficacy and safety.

One of the major problems with this herbal formulation is that the active ingredients are not well defined. It is important to know the active component and their molecular interactions, which will help to analyze therapeutic efficacy of the product and also to standardize the drug. Although the leaves of A. lineata were very effective in treating diabetes, there was no proper scientific documentation pertaining to the bioactive molecule for carbohydrate metabolism. Therefore the ethanolic crude extract of
*Andrographis lineata* was subjected to *in vitro* and *in vivo* study. Further the bioactive fraction with the highest lipogenic activity was identified and analysed for m-RNA expression (PPAR-γ and C/EBP-α). The *insilico* studies proved the identified bioactive molecule as the best fitted molecule for both the receptors with positive scores.

### 1.12 OBJECTIVES OF THE PRESENT WORK

- To optimize a reproducible micropropagation protocol and to confirm the genetic stability in the *in vitro* regenerants.
- Screening, isolation and characterization of active principle for antidiabetic activity.
- Evaluation of active principle by gene expression studies (PPAR-γ and C/EBP-α).
- To perform molecular docking studies in PPAR-γ and C/EBP-α for the identified active principle.
CHAPTER 2

REVIEW OF LITERATURE

2.1 SCIENTIFIC DOCUMENTATION OF MEDICINAL PLANTS

Nature always stands as a golden mark to exemplify the outstanding phenomena of symbiosis. Natural products from plants have been the basis of the treatment of human disease. Currently 80% of people in developing countries still depend on traditional medicine based largely on medicinal plants for their primary health care. India is a vast repository of medicinal plants that uses traditional medical treatments (Chopra et al 1956). The various indigenous systems such as Siddha, Ayurveda, Unani and Allopathy use several plant species to treat different ailments (Rabe and Staden 1997). The use of herbal medicine is becoming popular due to less toxicity and side effects than that of allopathic medicines (Agarwal 2005). The practices continue to till date because of its biomedical benefits as well as place in cultural beliefs in many parts of world and have made a great contribution towards maintaining human health (Sheetal and Singh 2008).

Tribal healers in most of the countries, use ethnomedical treatment to treat cut wounds, skin infections, swelling, aging, mental illness, cancer, asthma, diabetes, jaundice, scabies, eczema, venereal diseases, snakebites and gastric ulcer (Perumal and Ignacimuthu 1998). They keep no records and the information is mainly passed on verbally from generation to generation (Sofowara 1982). World Health Organization (WHO) has shown great interest
in documenting the use of medicinal plants used by tribals from different parts of the world (Kaido et al 1997).

Many developing countries have intensified their efforts in documenting the ethnomedical data on medicinal plants. Research to find out scientific evidence for claims by tribal healers on Indian herbs has been intensified. So these local ethnomedical preparations should be scientifically evaluated and documented for the betterment of mankind (Manandhar 1987). In this context, the present investigation was carried out to scientifically document traditional claim of antidiabetic activity in *A. lineata*.

2.2 MICROPROPAGATION AND RAPD ANALYSIS

Micropropagation is a well established technique for culturing and studying the physiological behavior of isolated plant organs, tissues, cells, protoplasts and cell organelles under precisely controlled physical and chemical conditions (Kozai 1991). Most of the endemic, rare and endangered medicinal plants are raised through seeds which are highly heterozygous and show great variations in growth, habit and yield of phytochemicals. Hence, they may have to be discarded because of poor quality of products for their commercial release (Dandekar 2003). Likewise, majority of the plants are not amenable to vegetative propagation through cutting and grafting, thus limiting multiplication of desired cultivars. Moreover plants propagated by vegetative means may contain bacteria, fungi and viruses which may affect the quality (Yamuna et al 1995). Hence, micropropagation has emerged as a promising technique to obtain genetically pure elite populations under *in vitro* conditions independent of seasonal constraints.

Selection of suitable explants is an important aspect for establishing a successful regeneration system (Lorraine 1990). Plant regeneration from shoot tip and nodal explant has yielded encouraging results in medicinal
plants like Catharanthus roseus, Cinchona ledgeriana, Digitalis spp, Rehmannia glutinosa, Rauvolfia serpentine and Isoplexis canariensis (Roy et al 1994; Paek et al 1995 and Perez-Bermudez et al 2002). Prathanturarug et al (1996) reported the significant response of various cytokinins on the shoot tip than in the nodal explants of Andrographis paniculata. The first report of high frequency regeneration of Andrographis paniculata through nodal explants was developed by Purkayastha et al (2008). An efficient method for the large scale propagation of an endemic medicinal plant Andrographis neesiana through in vitro culture of nodal explants has been developed by Karuppusamy and Kalimuthu (2010).

Cytokinins in general favor in vitro shoot proliferation (Thrope 1993). Cytokinins naturally fall into the category of N\(^6\)-isopentyl adenine derivatives. Unlike the purine derivatives, there exist other synthetic diphenyl urea’s that are even more active than their counterparts especially purines (Thomas and Katterman 1986). Successful regeneration protocol of Adhatoda vasica (Sangeetha and Alak 2005), Andrographis paniculata (Purkayastha et al 2008), Andrographis echoides (Hemalatha and Vadivel 2010) and Gratophyllum pictum (Justin koilpillai and Wilson 2010) in MS medium containing BAP for shoot induction have already been reported.

AdS provides an available source of nitrogen to cell and can be taken up more rapidly than inorganic nitrogen (Thom et al 1981). The benefits of adenine are often only noticed when it is associated with ammonium nitrate or cytokinins such as BAP or KN (Van et al 2008). The strategy for using AdS as an adjuvant has also been adopted effectively for many other plant species such as Holarrhena antidysenterica (Raha and Roy 2001), Curcuma angustifolia (Shukla et al 2007) and Bacopa monnieri (Ramesh et al 2006). Thus, BAP when added along with AdS exhibited synergetic effect and improved the cell proliferation efficiency (Ramesh et al 2006).
The simulative effect of GA$_3$ on elongation of shoots is well known as it has been found to promote cell division and elongation in the apical zone of shoots (George et al 1993). Elongation of shoots has been successful with GA$_3$ in same family such as *Andrographis paniculata* (Purkayastha et al 2008), *Graptophyllum pictum* (Justin and Wilson 2010), *Andrographis echoides* (Hemalatha and Vadivel 2010) and *Andrographis neesiana* (Karuppusamy and Kalimuthu 2010).

Although the promotive effect of auxins was achieved in eliciting rooting response (D’Silva and D’Souza 1992) their type and level in the nutrient medium were found to vary from tissue to tissue and species to species (Rao and Padmaja 1996). Effective rooting of IBA was observed in *Adhatoda vasica* (Sangeetha and Alak 2005) *Adhatoda vasica* (Khalekuzzaman et al 2008), *Andrographis paniculata* (Purkayastha et al 2008), *Thunbergia grandiflora* (Husein 2008), *Beloperone plumbaginifolia* (Shameer et al 2009) and *Justica gendarussa* (Dennis and Hoshino 2010).

The genetic diversity is analysed by using morphological as well as genetic based tools like DNA techniques (Bennici et al 2003) and advanced molecular methods (Barazani et al 2002 and Shiran et al 2007). RAPD markers have been successfully applied to detect the genetic similarities or dissimilarities in various plants (Sikdar et al 2010). Varshney et al (2001) established the genetic fidelity of in vitro raised *Lilium bulbets* through RAPD markers. Intraspecific variation of twenty five accessions of *Andrographis paniculata* was determined for its morphological characters and molecular analysis using RAPD technique (Maison et al 2005).

Fredric et al (2006) studied the genomic variation of micropropagated *Robinia ambigua*. Molecular evaluation and micropropagation of field selected elites *Rosa damacena* was assessed by Kaur et al (2007).
In vitro clonal propagation of *Mucuna pruriens* var. *utilis* and its evaluation of genetic stability through RAPD markers were analysed by Sathyanarayana et al (2008). The genetic variability in micropropagated propagules of *Ananas comosus* was reported by Santos et al (2008). Assessment of genetic fidelity of micropropagated apple root stock plants EMLA 111 using RAPD markers was assessed by Gupta et al (2009). Soumen et al (2010) reported the genetic fidelity of the *in vitro* raised *Ocimum kilimandscharicum*. Leela et al (2011) studied the morphological, physio-chemical and RAPD regenerants of *Jatropha curcas*. Therefore, the present investigation was carried out to study the genetic stability of micropropagated plant by RAPD analysis.

2.3 SCREENING OF PHYTOCHEMICALS

Natural products and secondary metabolites formed by living systems, notably from plant origin, have shown great potential in treating human diseases such as cancer, coronary heart diseases, diabetes and infectious diseases (Lai et al 2010). The phytochemical screening is an important step in the chemical and pharmacological study of a medicinal plant. It may suggest possible pharmacological effects of its extracts or fractions in comparison of identified phytochemicals groups, highlighting a close relationship with its main therapeutic uses.

Phytochemical screening of leaf, stem and root of *A. paniculata* based on physiochemical standardization was reported by Dahanukar (2000). Gebrie et al (2005) reported the phytochemical screening and pharmacological evaluation for antifertility effect of methanolic leaf extract of *Rumex steudelii*. Phytochemical analysis of the methanolic leaf extract of *Oxalis corniculata* revealed the antibacterial effect was due to the presence of phenolic compounds (Ragavendra et al 2006). The antimicrobial and
antivenom activity was reported in the leaf and flower of *A. paniculata* and *A. lineata* which was due to the presence of terpenoids (Perumal and Ignacimuthu 1998).

Mohanta et al (2007) reported the antimicrobial activity of *Semicarpus anacardium* was due to the presence of extractable compounds such as alkaloids, flavanoids and tannins. Himal et al (2008) reported the presence of higher content of flavanoids in the medicinal plants such as *Azadiracta indica, Colquhounia coccinea, Curcuma longa, Elsholtzia fructicosa, Eucalyptus globules, Ocimum santrum, Rhodendron setosum* and *Zanthoxylum aromatum*.

Preliminary phytochemical investigation showed the presence of triterpenoids which were responsible for the antioxidant effect in *Albizia chevalieri* (Aliyu et al 2009). Diterpenoids, flavanoids and polyphenols were found to be higher in the methanolic whole plant extract of *A. paniculata* (Wen-Wan and Bi-Fong 2010). Mandal et al (2010) studied the qualitative analysis of phytochemicals and confirmed the presence of carbohydrates in the methanolic extract of *Hygrophila spinosa*.

Alagesaboopathi (2011) reported the antifungal and antimicrobial activity in *A. alata and A. ovata* was due to the presence of high polyphenolic content. The existence of steroids, gums, mucilages and glycosides was reported in *A. affinis*.

Alagesaboopathi and Sivakumar (2011) showed the antimicrobial activity in the methanolic leaf and stem extract of *A. nessiana* which was found to posses phytochemicals such as tannins, flavanoids and saponins. In the present study, the phytochemical analysis was performed in the leaf extract of *A.lineata*. 
2.4 IN VITRO ANTIOXIDANT ACTIVITY

An antioxidant is a chemical that prevents the oxidation of other chemicals. They protect the key cell components by neutralizing the damaging effects of free radicals, which are natural byproducts of cell metabolism (Ames et al 1993). The antioxidant activity of Rubus parvifolius, Lindernia anagallis and Zanthoxylum nitidum were reported to exhibit free radical and superoxide anion scavenging activity in dose dependent manner equally with catechin (Li et al 2005).

Kiselova et al (2006) showed the correlation between the in vitro antioxidant activity and polyphenol content of aqueous extract of Alchemilla vulgaris, Mentha spicata and Fragaria vesca. In vitro studies of polyphenol compounds, total antioxidant capacity and other dietary indices in a mixture of plants (Prolipid) were investigated by Zenon et al (2007). Nooman et al (2008) reported that Eugenia caryophyllus, Piper cubeba, Zingiber officinale and Piper nigrum exhibited anticancer, hepatoprotective and antiviral activity which may be due to the antioxidant property of the plant extract.

Aliyu et al (2009) reported the IC$_{50}$ values of the methanoilc leaf extract of Albizia chevalieri which showed significant reducing power ability due to the hydrophilic nature of the compound present in the extract. Sheela et al (2009) reported the anti inflammatory activity may be due to the presence antioxidant compounds such as flavanoids and poyphenols. The phenolic content and the antioxidant activity of A. paniculata ethanolic fruit extract was found to be higher compared to the leaf and stem extract (Arash et al 2010).

Dhan et al (2011) observed significant antioxidant activity in the methanolic leaf extract of A. paniculata in hydroxyl scavenging activity and
FRAP method. In the present investigation, the in vitro antioxidant activity was carried out in the ethanolic leaf extract of *A. lineata*.

### 2.5 *IN VITRO ANTIDIABETIC ACTIVITY*

One of the therapeutic approach for treating diabetes is to decrease the post prandial hyperglycemia. This is performed by retarding the absorption of glucose through the inhibition of the carbohydrate hydrolyzing enzyme (α-glucosidase) in the digestive tract delaying carbohydrate digestion time, causing a reduction in the rate of glucose absorption and consequently blunting the postprandial plasma glucose rise (Chiasson et al 2004). Many α-glucosidase inhibitors such as flavonoids, alkaloids, terpenoids, anthocyanins, glycosides and phenolic compounds have been isolated from plants.

Ji-Youn et al (2004) demonstrated the α-glucosidase inhibitory effect in the aqueous extract of *Commelina communis*. Estrada-Soto et al (2007) showed the antidiabetic activity of methanolic extract of *Tournefortia haetwegiana* through α-glucosidase inhibition mechanism. In vitro experiments conducted by Rammohan et al (2008) suggested that inhibitory effect (α-glucosidase and α-amylase) and hypoglycemic effect in the ethanolic leaf extract of *A. paniculata* may be due to the presence of andrographolides.

Adipose tissue is considered as a key link between obesity and diabetes by promoting the development of lipotoxicity, i.e. cell damage as a consequence of elevated intracellular lipid concentrations and insulin resistance (Lelliott and Vidal-Puig 2004). Insulin resistance at the adipocyte levels contribute to hyperglycemia. However, adipocytes from different sites of the body may have different biological or pathological effects. Pathways related to insulin resistance may be studied in cell lines of adipocytes such as murine or mouse fibroblast 3T3-L1 cells (Karalee et al 2001) which are
employed as tools to evaluate the effects of natural products upon glucose uptake (insulin mimicking/sensitization) and lipogenesis.

The MTT assay is used to assess the viability and the proliferation of cells (Freshney 2000). It is also used to determine the cytotoxicity of the crude extracts obtained from medicinally important plants. The cytotoxic nature of *Vismia schultesii* (Ivana et al 2006), *Annona squasoma* (Beena and Remani 2008), *Embelia ribes* (Beena and Laskhmi 2010) and *Terminalia arjuna* (Alam et al 2011) were studied in the mouse fibroblast 3T3-L1 cell lines.

The insulin stimulated glucose uptake in adipose tissue is a critical factor for reducing post prandial blood glucose concentration. 3T3-L1 cells are an excellent experimental model to quickly screen the effects of crude drugs on glucose uptake activity (Liu et al 2001). The crude drug may act by mimicking insulin or either by stimulating insulin release or by potentiating insulin action or by reducing hepatic glucose production (Yu-Chiao et al 2003). For instance, there are reports on medicinal plants exhibiting insulin mimicking activities *Agaricus campestris* (Gray and Flatt 1998), *Vernonia amygdalina* (Atanbho et al 2010), poly herbal extract APK-004 (Padmanabha and Kaiser 2011) and *Curcuma longa* (Mohankumar and McFarlane 2011). Some medicinal plants were reported for insulin sensitization activity by Yu-Chiao et al (2003) in *Toona sinesis*. Guy et al (2007) in *Lagerstroemia speciosa*, Patrick et al (2008) in *Psidium guajava* and *Morinda citrifolia*, Padmanabha and Kaiser (2011) in *Eugenia jambolana* and Aruh and Issac (2011) in *Dennettia tripetala*. The α-glucosidase inhibition, insulin mimicking and sensitization activity of EtALL extract was demonstrated in current research work to prove the antidiabetic potential of *A. lineata*. 
2.6 **IN VIVO ANTIDIABETIC AND ANTIOXIDANT STUDIES**

Animal models of diabetes are therefore greatly useful and advantageous in biomedical studies because they offer promises of new insights into human diabetes. Most of the available models are based on rodents because of their small size, short generation interval, easy availability and economic considerations (Srinivasan and Ramarao 2007).

STZ was selected for induction of diabetes in rats since it is well known for its selective pancreatic β-cell cytotoxicity which has been extensively used to induce diabetes in animals; it is less toxic than alloxan and allows a consistent maintenance of diabetes (Raju and Balaraman 2008). A low dose of STZ (40mg/kg b.w.) has been used in this study to induce diabetes where half of the population of pancreatic β-cells are destroyed leaving behind residual β-cells which secrete insufficient insulin causing hyperglycemia (Eliza et al 2009). Over-production (excessive hepatic glycogenolysis and gluconeogenesis) and decreased utilization of glucose by the tissues are the fundamental basis of hyperglycemia in STZ induced diabetes (Patil et al 2011).

Acute toxicity studies are performed as preliminary pharmacological evaluation to fix the LD$_{50}$ value of the plant extract. The dosage level of *Flacourtia jangom* (Ajay and Jyoti 2010), *Cassia osscidentalis* (Emmanuel et al 2010) and *Citrus limetta* (Sripama et al 2011) were similar to EtALL extract dosage level performed in the present study.

Impaired oral glucose tolerance (IGT) is an indicative of a predisposition of an animal to diabetes condition. Agents that exhibit antihyperglycaemic effects are capable of bringing blood glucose concentration within normal limits will help to arrest the progression of impaired glucose tolerance to diabetes (Raju and Balaraman 2008). Many
researchers have reported the reduction in the blood glucose level in the OGTT analysis in *A. paniculata* (Rammohan et al 2008), *Coccinia cordifolia* and *Catharanthus roseus* (Islam et al 2009), *Telfaria occidentalis* (Olorunfemi et al 2010) and *Calotropis gigantean* (Nanu et al 2011).

STZ induced diabetes is characterized by a severe loss in body weight (Al-Shamaony et al 1994) and increased food intake (Szkudelski and Szkudeslka 2002). Body weight loss might be due to the result of protein wasting due to unavailability of carbohydrate as an energy source (Chen and Ianuzzo 1982). Body weight gains, decrease of food and water intake were observed in extract treated diabetic induced rats of *Trifolium sp.* (Maissa and Rawi 2007), *Costus speciosus* (Eliza et al 2009) and *Rhinacanthus nasutus* (Visweswara et al 2010).

There may be several causes for persistant hyperglycemia and the most important among them is the failure of blood sugar regulation and high plasma insulin level in diabetes (Bolkent et al 2000 and Graham et al 2001). The glucose lowering ability and insulin secretory activity was well correlated by Reyes et al (2006) in *Andrographis paniculata*, Eliza et al (2009) in *Costus specious*, Kondeti et al (2010) in *Petrocarpus santalinus* and Arokiaraj et al (2011) in *Hypericum perforatum*.

Increased non enzymatic glycosylation is also one of the possible mechanism linking vascular hyperglycemia and vascular complications of diabetes. During diabetes excess glucose present in the blood reacts with hemoglobin to form HbA₁C (Kondeti et al 2010). HbA₁C was found to increase in diabetic patients up to 16% (Koeing et al 1976) and hence, it is a reliable index of glycemic control in diabetes (Gabbay 1976) which reflects the average blood glucose concentration (Murray et al 2000). The extracts of *Gymnema sylvestre* (Shanmugasudaram et al 1990), *Tinospora cordifolia* (Rajalakshmi et al 2009), *Costus specious* (Eliza et al 2009) and *Zizyphus*
spins-christi (Michel et al 2011) were also found to possess properties that control glycemic index.

Glycogen is the primary intracellular storable form of glucose and its level in various tissues is a direct reflection of insulin activity as insulin promotes intracellular glycogen deposition by stimulating glycogen synthase and inhibiting glycogen phosphorylase (Golden et al 1979). The glycogen content of skeletal muscle and liver markedly decreased in diabetic rats (Welihinda and Karuanayake 1986) in proportion to insulin deficiency (Stalmans et al 1997). The extracts of Eugenia jambolana (Sharma et al 2003) and Costus specious (Eliza et al 2009) have also prevented the decrease of muscle and liver glycogen content in diabetic rats.

Hexokinase, glucose -6 - phosphatase and fructose -1, 6- phosphatase are rate limiting glycolytic enzymes that are severely impaired during diabetes condition. These enzymes play a very important role in the final step of glucogenolysis and gluconeogenesis (Hassan et al 2009). A decrease in the activity of hexokinase, glucose-6-phosphatase and fructose -1, 6- bisphosphatase has been shown to slow down the pentose phosphate pathway in diabetic conditions (Abdel-Rahim et al 1992).

There are previous reports of Gymnema montium (Ananthan et al 2003), Tinospora cordifolia (Rajalakshmi et al 2009) and Hypericum perforatum (Arokiyaraj et al 2011) which have shown the recovery of carbohydrate metabolizing enzymes closer to normal in diabetic treated rats. The most common lipid abnormalities in diabetes are hypertriglyceridaemia and hypercholesterolaemia (Khan et al 1995 and Mitra et al 1995). The development of hyperlipidemia in uncontrolled diabetes is a consequence of a number of metabolic abnormalities that occur sequentially (Lopez 2001). The antilipidemic activity was reported in Eugenia jambolina (Ravi et al 2005), Annona squamosa (Kaleem et al 2006) and Costus specious (Eliza et al 2009).
Renal diseases is one of the most common and severe complications of diabetes (Rhodes et al 2006). The diabetic hyperglycemia induces elevation of the serum levels of urea, creatinine and uric acid which are considered as significant markers of renal dysfunction (Almdal and Vilstrup 1987). The extracts of *Vinca rosea* (Ghosh and Surawanshi 2001), *Costus specious* (Daisy et al 2008) and *Elephantopus scaber* (Daisy et al 2009) were also reported to control these renal dysfunction markers.


The increased level of lipid peroxidation could be associated to increase in free radicals generation in diabetes caused primarily due to high blood glucose levels, which upon autoxidation generates free radicals and secondarily due to the effects like STZ (Ivorra et al 1989). Previous studies have reported the increased lipid peroxidation in the liver and kidney tissues of diabetic rats (El-Missiry and El-Gindy 2000). The treated lipid peroxide mediated tissue damage and plasma in diabetic rats were reported by Leelavinothan and Muniappan (2004) in *Scoparia dulcis*, Kaleem et al (2006) in *Annona squamosa*, Daisy et al (2008) in *Costus specious* and Purnima et al (2010) in *Mimus elengi*.

Superoxide dismutase (SOD) and Catalase (CAT) have been postulated as one of the most important enzyme in the enzymatic antioxidant
defence system. Since it catalyses the dismutation of super oxide radicals to produce $\text{H}_2\text{O}_2$ and molecular oxygen which have diminishing toxic effects caused by these radicals (Baynes 1995 and Yan and Harding 1999). The restoration of normal levels of these enzymatic antioxidants was reported in *Annona squamosa* (Kaleem et al 2006), *Petrocarpus marsupium* (Maruthupandian and Mohan 2011) and *Mimus elengi* (Purnima et al 2010).

GPx plays a primary role in minimizing oxidative damage. GSH metabolizing enzymes, glutathione peroxidase and Glutathione-S-transferase work in concert with glutathione in the decomposition of $\text{H}_2\text{O}_2$ and other organic hydro peroxides to non-toxic products, at the expense of reduced glutathione. As enzymatic antioxidants are saturated by excessive levels of free radicals, the presence of non-enzymatic antioxidants is presumably essential for the removal of these radicals (Allen 1991). The earlier reports of Leelavinothan and Muniappan (2004) in *Scoparia dulcis*, Palani et al (2010) in *Chloroxylon swietenia* and Kannampalli et al (2010) in *Cassia fistula* showed the curative effect in GPx and GSH levels in the diabetic treated rats.

Biochemical parameters are sensitive index to changes due to xenobiotics and constitute important diagnostic tool in toxicological studies (Dorman 2000). Phosphatases are important and critical enzymes in biological processes, they are responsible for detoxification, metabolism and biosynthesis of energetic molecules for different essential functions (Bengt and Kent 1975). The reverting levels of these biochemical marker enzymes to normal were reported in El-Demerdash et al (2005) in *Allium sativum*, Daisy et al (2008) in *Costus specious* and Muhammad et al (2011) in *Digera muricata*. All these previous studies suggested us to focus on the evaluation of *in vivo* antidiabetic and antioxidant activity of EtALL extract.
2.7 BIOACTIVITY GUIDED FRACTION AND LIPOGENESIS

In the investigation of bioactive natural compounds, it is essential to perform biological tests to locate required activities. The systematic approach has been shown towards the discovery of drugs from medicinal plants which have been initiated using bioassay guided fractionation (Sener 1994).

The promising outcome of the *in vitro* and *in vivo* (antidiabetic and antioxidant) studies of EtALL extract further encouraged to probe the bioactive compounds present in it. Hence, an attempt was made to isolate the bioactive fractions using column and TLC technique.

Lipogenesis is the process by which simple sugars such as glucose are converted into fatty acids, which are subsequently esterified with glycerol to form the triacylglycerols that are packaged as very low density lipoproteins and secreted from the liver (Gregoire et al 1998). Adipocytes plays a common link between diabetes and obesity, which stores excess energy in the form of triglyceride and releases free fatty acids in response to energy requirements such as fasting. 3T3-L1 adipocytes cell line are used as *in vitro* models to evaluate the antidiabetic action of the drugs since adipocytes mimic fat cells and induce insulin resistance which are the major contributor of diabetes (Guilherme et al 2008).

Blumea balsamifera extract in 3T3-L1 preadipocytes and adipocytes was investigated by Hiroaki et al (2009).

Martineau et al (2010) showed the anti-adipogenic activity of Alnus incana and Populus balsamifera bark extracts. Kamalakannan and Prince (2006) examined the effect of methanolic extract of Caralluma fimbriata in the 3T3-L1 preadipocytes for cell division. The isolated bioactive compounds from Cinnamomum cassia (Baddireddi 2008) and Costus pictus (Shilpa et al 2009) in the methanolic extract of Costus pictus were also known to exhibit antiadipogenic activity. Since the results of in vivo studies of EtALL extract had good potential of antidiabetic activity an attempt was made to explore the bioactive principle responsible for antidiabetic activity.

2.8 ISOLATED COMPOUNDS OF ANDROGRAPHIS SPECIES

Phytochemistry is a distinct discipline in between organic chemistry and plant biochemistry. It deals with a variety of organic substances accumulated in plants. Hence the plants may be considered as a biosynthetic laboratory. Two new ent-labdane-type diterpenoids, wightional and wightiolide have been isolated from the leaves of A. wightiana (Balawanth et al 1996). Andrographolide (C_{20}H_{30}O_{5}) is the major diterpenoid in A. paniculata, which constitutes about 4% (whole plant), 0.8~1.2% (stem) and 0.5~6% (leaf) respectively (Burgos et al 1997). The successive extraction of the whole plant of A. echioides has led to the isolation of a new flavanone, dihydroechioidinin together with four known flavones, echiodinin, echiodin, skullcapflavone 2'-O-methyl ether, and skullcapflavone 2'-O-glucoside (Jayaprakasam et al 2001).

Two new acylated flavone glucosides, skullcapflavone I 2'-O-b-D- (30 -E - cinnamoyl) glucopyranoside and skullcapflavone I 2'-O-b-D- (20 - E - cinnamoyl) glucopyranoside, together with skullcapflavone I 2'-O-b-
D-glucopyranoside and andrographidine have been isolated from the whole plant of \textit{A. serpyllifolia} (Damu et al 1999). Two new 2′-oxygenated flavones, 5, 2′, 6′-trihydroxy-7-methoxyflavone and skullcapflavone I 2′-O-b-D-(40-E-cinnamyl) glucopyranoside, together with three known flavones, 7-O-methylwogonin, skullcapflavone I and skullcapflavone I 2′-O-b-D-glucopyranoside were isolated from the aerial parts extract of \textit{A. elongata} (Jayakrishna et al 2001).

Andrographolide was isolated from the aerial parts methanolic extract of \textit{A. affinis} (Reddy et al 2003). 5, 7, 2′, 3′-tetramethoxyflavanone (Koteswara et al 2004) was isolated from the methanolic leaf extract of \textit{A. alata}. 5, 7, 2′, 6′-oxygenated flavone glycosides along with the known 5, 2′, 6′-trihydroxy-7-methoxyflavone-2′-O-b-D-glucopyranoside (Biswanath et al 2006), andrographic acid (Li et al 2007) and two new ent-labdane diterpenoid glycosides were isolated from the aerial parts (Zhou et al 2008) of \textit{A. paniculata}.

Andrographolide has been reported to exhibit multiple pharmacological properties and is a potential chemotherapeutic agent. Andrographolide contains an \(\alpha\)-alkylidene, \(\gamma\)-butyrolactone moiety and three hydroxyls at C-3, C-19 and C-14 positions which are responsible for the cytotoxic activities against many cancer cell lines (Varma et al 2009). Using bioactivity-guided chromatographic separation, the anti-inflammatory compounds such as 14-deoxy-11, 12 - didehydroandrographolide, ergosterolperoxide, \(\beta\)-sitosterol, stigmasterol and 5 - hydroxy - 7, 8 - dimethoxyflavanone was reported from ethyl acetate whole plant extract of \textit{A. paniculata} (Wen-Wan et al 2010).

From ethyl acetate soluble fraction of the ethanol/methanol extract, 5 - hydroxy - 7, 8 - dimethoxyflavone, 5 - hydroxy - 7, 8, 2′, 5′ - tetramethoxyflavone, 5 - hydroxy - 7, 8, 2′, 3′ - tetramethoxyflavone,
5-hydroxy-7,8,2’-trimethoxyflavone, 7-O-methylwogonin and 2’-methyl ether were isolated from *A.paniculata* (Radika et al 2010). Phytochemical investigation of the ethanolic extract of the leaves of *A. paniculata* yielded one novel diterpene 13R, 14R, 3, 13, 14, 19-tetrahydroxy-ent-labda-8(17), 11-dien-16, 15-oxide 1 which has an uncommon cis-diol groups in the lactone moiety (Xu et al 2010).

### 2.9 ANTIDIABETIC BIOFLAVANOIDS OF PROMISE

Bio-flavonoids are well-known for their multi directional biological activities including antidiabetic efficacy (Brahmachari and Gorai 2006). The flavonoids can act as insulin secretagogues or insulin mimetics, probably by influencing the pleiotropic mechanisms to attenuate the diabetic complications. Bio-flavonoids are regarded as promising and significantly attractive natural substances to enrich the current therapeutic options for the treatment of diabetes.

Andradeceetto and Wiedenfeld (2001) isolated Isoorientin from the water and butanolic extracts of *Cecropia obtusifolia* (Ceropiaceae), which was found to exhibit potent hypoglycemic activity. Chronic treatment with hesperidin and naringin was found to lower the blood glucose level of db/db mice compared with the control group (Matsuda et al 2002). Kawabata et al (2003) isolated five 6-hydroxy-flavonoids (α-glucosidase inhibitory activity) from the methanolic extract of *Origanum majorana*.

Haraguchi et al (2003) isolated C-glucosidic flavone derivative named as Isoaffineyin (5, 7, 4, 3’, 5’-pentahydroxyflavone - 6 - C-glucoside) from *Manikara indica* (Sapotaceae), this flavonoid candidate exerted promising inhibition against porcine aldose reductase activity.
Kim et al (2004) isolated a new flavonol glycoside along with the known flavonoid glycosides such as kaempferol 3-O-β-D-glucopyranoside (Astragalin) and Quercetin 3-O-β-D-glucopyranoside (Isoquercetin) from the leaves of *Eucommia ulmoides* (Eucommiaceae), these flavonoid constituents were found to be glycation inhibitors.

Kaempferol - 3, 7 - O - (α) - dirhamnopyranoside (Kaempferitrin) isolated from the n-butanol fraction of the leaves of *Bauhinia forficata* (Leguminosae), which exhibited significant hypoglycemic effect (De-Sousa et al 2004). Rutin orally administered to diabetic rats showed decreased in plasma glucose levels up to 60% when compared to the control group (Kamalkannan and Prince 2006).

Isoprenyl flavonoid isolated from the *Phyllostachys nigra* (Gramineae) showed inhibitory efficacy against advanced glycation end products (Jung et al 2007). Tabopda et al (2008) reported six unusual C-4′-prenylated flavonols, isolated from the roots of *Dorstenia psilurus* (Moraceae) which was found to exhibit glycosidase enzyme inhibitory activity against α-glucosidase, β-glucosidase and α-mannosidase.

Jang et al (2008) reported two flavan-3-ol derivatives from the roots of *Actinidia arguta* (Actinidiaceae) that were found to exhibit inhibitory activity in vitro on the formation of advanced glycation end products. Torres-Piedra et al (2010) performed a comparative study of flavonoid analogues (Quercetin) on streptozotocin-nicotinamide induced diabetic rats and proved as a potential antidiabetic agent acting via 11β-hydroxysteroid dehydrogenase type 1 inhibition.

The constituents swertiamarin and swertisin (C-glycosyl flavanoid) isolated from *Enicostemma hyssopifolium* (Gentianaceae) are
Mohammad et al (2011) isolated sinensitin from leaves of *Orthosiphon stamineus* Benth (Lamiaceae) by bioassay guided fractionation method and reported the antihyperglycemic effect. Hence, an attempt was made to isolate a bioactive compound which may be responsible for antidiabetic activity in this study.

### 2.10 GENE EXPRESSION STUDIES IN DIABETES

Gene expression profiling has been used to identify candidate genes for disease diagnosis and to characterize gene expression patterns associated with potential disease treatments including diabetes (Welsh et al 2001). Yokomori et al (1999) results suggested that in addition to cell type specific transcription factor, methylation of specific CpG sites and the methylation sensitive transcription factor contributes to GLUT4 gene regulation during 3T3-L1 differentiation.

Fred et al (2001) studied the expression of the gene encoding resistin, a low molecular weight protein secreted from adipose tissue and postulated to link obesity and diabetes in 3T3-L1 adipocytes. Ulf et al (2001) concluded that thiazolidinediones improve insulin sensitivity in human as well as in different animal models of insulin resistance. Dietary flavanoids namely catechin, quercitin and kaempferol were reported to down regulate the transcription factors PPAR-γ and C/EBP-α in dose dependent manner (Po - Jung et al 2005).

Wenbin et al (2007) reported that the most abundant occurence of ginsenoside in ginseng root promotes adipogenesis in cultured adipocytes by enhancing expression of C/EBP-α and PPAR-γ. The isoforms of
gugglsterones (cis-GS and trans-GS) were studied for its apoptosis, adipogenesis and lipolysis activity in 3T3-L1 cells. Hu and Davies (2009) reported higher expression of GATA-2 and GATA-3 (DNA binding proteins) in PPAR-γ and C/EBP-α by Berberine (an isoquinoline derivative alkaloid).

Corosilic acid isolated from methanolic leaf extract of *Eriobotrya japonica* (Wei and Guangyuan 2007) and tannic acid isolated from banana extract (Xueqing et al 2005) was reported for its significant glucose uptake and inhibition of adipocyte differentiation mechanisms without increasing adiposity. Eva et al (2011) investigated the adipocyte cell differentiation, lipolytic activity of differentiated cells, expression levels of genes involved in adipogenesis associated with insulin activity. Therefore, in this study the isolated bioactive compound exhibited significant lipogenesis activity which prompted to evaluate the expression level of PPAR-γ and C/EBP-α genes.

### 2.11 MOLECULAR DOCKING STUDIES IN DIABETES

*In silico* molecular docking is one of the most powerful techniques to discover novel ligands for receptors of known structure and thus play a key role in structure-based drug design (Brooijmans and Kuntz 2003). Investigators often use docking computer programs to find the binding affinity for molecules that fit a binding site on the receptor.

*In silico* modeling drug development program led to clinical trials of a novel, anti-anxiety agonist 5-HT1A (Becker et al 2006). It is estimated that docking programs currently dock 70 – 80% of ligands correctly (Congreve et al 2005). Uzma and Mohammad (2008) proposed a pharmacore model for virtual screening of GANC_HUMAN inhibitors for diabetes. *In silico* docking of ligand 3-hydroxyl methyl xylitol with target protein ZnT-8 (insulin secretory granule) was determined by Praveena and Ignacimuthu (2009) in diabetes condition which had high affinity between the target and
the ligand protein suggesting ZnT-8 as a good drug to control blood glucose level.


A series of Quinoline-3-carboxylic acids was identified (Protein Tyrosine Phosphatase 1-Beta) as potential antihyperglycemic agents using Glide software (Rohan et al 2011). Satya et al (2011) demonstrated protein ligand interaction analysis and in silico potential drug target identification in diabetes and nephropathy for brain-derived neurotrophic factor by using Hex protein ligand docking program by assuming the ligand as solid grids. The Lamarkin Genetic Algorithm protein docking program was used to analysis the ability of the secondary metabolites of Trigonella foenum-graecum to serve as antagonist to angiotensin converting enzyme a potent vasoconstrictor (Priya et al 2011).

Molecular docking studies of Diasy et al (2011) showed lupeol isolated from Elephantopus scaber inhibited the activity of autolysin by forming a strong interaction with the active site residues during urinary tract infection in diabetic patients. Kadiyala et al (2011) studied the interactions of andrographolide isolated from A. paniculata and A. lineata with venom toxins such as disintegrin, aggregetin, echicetin, irditoxin, haditoxin and autolysin.
Jhansi et al (2011) studied the hydrogen bonding interactions of oxidosqualene cyclase, a major enzyme in cholesterol biosynthesis, targeted for hypercholesterolemia. Hence in the present study, the gene expression studies of the isolated bioactive compound showed the down regulating mechanism of the genes (PPARγ and C/EBPα). Hence, molecular docking analyses were performed to study the binding affinity of the bioactive compound.