2. REVIEW OF LITERATURE

2.1 ROLE OF MEDICINAL PLANTS IN HEALTH AND DISEASES

The literature survey shows that concerted efforts made in documenting the traditional medicinal knowledge along with systematically explored flora of the world during the last two decades have paved the path to acquire more knowledge regarding the use and efficacy of medicinal plants. Besides, the history of treatment of various kinds of diseases using medicinal plants goes back to the Vedic age (Mazid et al., 2012) with scanty scientific basis of functional mechanism.

In view of the onset of modern medicine, the traditional plant medicine has taken a back seat although they still play vital remedial roles more in developing countries than in the developed countries. The disadvantage of the use of modern drugs in view of their short and/or long term side effects has made the scientists to develop or design and formulate the newer drugs with negligible side effects for treatment of diseases from medicinal plants. This has accelerated the effort to understand the scientific basis underlying the effective control and treatment of the chronic diseases by the medicinal herbs. As a result of this, combined effect of the ethnochemical formulations of home remedies, Ayurveda, Unani and Siddha systems subsequently emerged out as a scientific basis in medicinal system.

With advanced scientific approach towards the plant based treatment and profound studies conducted on plants with ethnomedicinal correlations, prospective extensive utility of natural products have been established. This has created a new area in the field of medicine regarding the isolation and characterization of phytoconstituents. As a result of this investigation, a new field known as phytochemistry has emerged. It has been estimated that about 25% of modern drugs
directly or indirectly are being derived from the plant products. Scientific evidences suggest that the plant based phytochemicals provide a basis for the development of newer drugs by trial and error methods with traditional and molecular approaches over many years (Harvey, 1999). Certain alkaloids like reserpine, morphine, nicotine, caffeine, cocaine, vincristine and vinblastine obtained from plants like *Rauwolfia serpentina*, *Papaver somniferum*, *Nicotiana tabacum*, *Coffea arabica*, *Erythroxylum coca* etc., do possess some biological properties such as cardiac stimulation, vasodialation and muscle relaxation etc. They have, in fact, revolutionized the management of hypertension, heart ailments, respiratory disorder, digestive disorders, rheumatoid diseases and skin ailments (Maridass and De-Britto, 2008). A recent report by Hyman (2011), on the effects of glucomannan, a soluble fiber derived from the Asian potato like tuber (*Amorphophallus konjac*), shows that it has a biological property of reducing obesity. It has been observed that Konjac fiber or glucomannan was being used as medicine in the form of food because of its medicinal properties in the 6th century. On absorption, konjac fiber undergoes expansion in the stomach, small and large intestines wherein it absorbs and accelerates elimination of fat, reduces cholesterol, blunts absorption of sugar and facilitates weight loss. This observation establishes the fact that the value of traditional foods being considered to be therapeutic.

According to Hyman (2011), plant based food with medicinal values are the most promising medicine available to heal chronic diseases, that can cause an estimated over 50 million deaths and cost $47 trillion by the year 2030. It is well known that plants are the source of special ingredients called phytonutrients which are secondary metabolites that can be employed in preventing/treating diseases and also enhance health through improving the gene regulation and metabolism. The protective
mechanisms of polyphytonutrients against ageing, inflammation, hormone imbalance, toxicity etc are believed to be due to vast array of colors present in vegetables that account for nearly 25,000 beneficial chemicals. It is well established that eating varieties of fruits and vegetables is beneficial as they contain phytonutrients. Table.1 presents the phytonutrients of some vegetables and fruits with biological activity conferred due to their colours.
Table 1: Sources of Phytonutrients and their functions (Hyman, 2011)

<table>
<thead>
<tr>
<th>Colour</th>
<th>Fruits/Vegetables</th>
<th>Phytonutrient</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red</td>
<td>tomatoes, watermelon, pink grapes</td>
<td>lycopene</td>
<td>Protect tissues from free radical damage, prostate cancer, heart and lung diseases.</td>
</tr>
<tr>
<td>Yellow/Green</td>
<td>spinach greens, collard greens, mustard greens, turnip greens, yellow corn, green peas, avocado, honeydew melon</td>
<td>Lutein, zeaxanthin</td>
<td>Reduce the risk of cataract and age-related muscular degeneration. Lutein may reduce atherosclerosis.</td>
</tr>
<tr>
<td>Orange</td>
<td>carrots, mangoes, apricots, pumpkin, sweet potatoes</td>
<td>$\alpha$-carotene, $\beta$-carotene</td>
<td>Protects the skin against free radical damage and helps repair damaged DNA and also against cancer, $\beta$-carotene good for night vision.</td>
</tr>
<tr>
<td>Orange/Yellow</td>
<td>pineapple, oranges, peaches, papayas</td>
<td>$\beta$-cryptoxanthin</td>
<td>Helps to prevent heart diseases. Protective fat in the skin of orange has been found to kill cancer cells in humans and animals as well.</td>
</tr>
<tr>
<td>White/Green</td>
<td>Celery, garlic, onions, pears, white wine</td>
<td>Allicin, Quercetin, Kaempferol</td>
<td>Anti-tumor and antioxidant properties.</td>
</tr>
<tr>
<td>Red/Purple</td>
<td>purple grapes, eggplant, red wine, grape juice, red apples, prunes, beet roots, strawberries, blue berries and black berries</td>
<td>Anthocyanins</td>
<td>Prevents blood clots, delay the aging of cells and onset of Alzheimer’s disease.</td>
</tr>
<tr>
<td>Green</td>
<td>broccoli, cauliflower, cabbage, chinese cabbage</td>
<td>sulforaphane, isocyanate and indoles</td>
<td>Help ward off cancer by inhibiting carcinogens, protects from harmful radiation (during cancer therapy).</td>
</tr>
</tbody>
</table>
2.2 DIABETES MELLITUS AND OXIDATIVE STRESS

Glucose metabolism plays a pivotal role in maintaining the physiological condition of the animal system. Carbohydrates are broken down by the digestive enzymes in the biological system releasing glucose molecules which stimulate the β-islets of pancreatic cells to secrete the hormone insulin. This key hormone regulates glucose metabolism by stimulating the glucose transporter (GLUT-4) in the target tissues like skeletal muscle (myocytes), liver (hepatocytes) and adipose tissues (adipocytes) for glucose uptake. Prolonged exposure of β-cells to elevated glucose levels (as a consequence of hyperglycemia referred to as glucose toxicity) which in turn leads to chronic oxidative stress resulting in generation of reactive oxygen species (ROS) (Tiwari and Rao, 2002). Both hyperglycemia and glucose toxicity hampers the β-cell function due to oxidative stress as there are low levels of antioxidant enzymes present in pancreas (Robertson, 2004). The diabetic consequences are shown in the form of flow chart (Fig.3).

Fig. 3 Diabetes and its consequences (Baquer et al., 2011).
Pathological changes associated with diabetes leads to risk factors such as obesity, high blood pressure, abnormal cholesterol level, high triglycerides, acceleration of the development of kidney failure, nerve damage, blindness, heart disease, stroke etc. Insulin resistance has been found to be associated with atherosclerosis (fat deposition in arteries) and cardiovascular diseases even before the diagnosis of diabetes (Vijayaraj et al., 2011).

It has been observed that debilitating effects of hyperglycemia are mediated and further complicated by oxidative stress (Singh et al., 2001). The reactive free radicals generated during hyperglycemia, modify DNA and cell membranes by attacking the cellular proteins, carbohydrates and lipids. The ROS also aggravates oxidative stress, producing toxic effects on the tissues and ultimately resulting in multi-organ damage (Flora et al., 2008). The free radicals are also responsible for chronic pathological conditions such as neurodegenerative diseases (like Parkinson’s and Alzheimer’s), inflammatory diseases, tissue injury leading to cardiovascular and cerebrovascular complications, pulmonary oxygen toxicity, respiratory distress syndrome (Elmastas et al., 2006).

2.3 PRESENT AND FUTURE PROSPECTUS IN THE TREATMENT OF DIABETES

Modern method of treatment

The therapeutic strategies for the treatment of type 2 diabetes mellitus (T2DM) include the stimulation of endogenous insulin secretion, enhancement of action of insulin at the target tissues and controlling the post prandial hyperglycemia (PPHG) by regulating the carbohydrate metabolizing enzymes like α-amylase and α-glucosidase, in order to have slow degradation of starch and oligosaccharides.
In such cases, the most commonly used drugs are insulin, sulfonylureas, biguanides, \( \alpha \)-glucosidase inhibitors and insulin mimetics. However, their utility is restricted owing to limited action and accompanying side effects like weight gain, hypoglycemia, cardiovascular defects and gastrointestinal disturbances (Tahrani et al., 2011). Also it may be noted that these pharmaceutical drugs fail to address the complications arising due to oxidative stress. In spite of the availability of numerous drugs for the treatment and management of T2DM, according to WHO the number of diabetic cases will be more than 390 million by the end of by 2025 all over the world (WHO Diabetes facts and figures, 1998).

**Traditional method of treatment**

This method of treatment of T2DM is different from the allopathic medicinal system. Here herbal medicines and alternative systems of medicines like Ayurveda, Siddha and Unani are in use to treat ‘Madhumeha’ (diabetes) since time immemorial in view of its effective management in treating and controlling T2DM and also its associated complications. According to ethnobotanical survey there are more than 1200 medicinal plants with hypoglycemic potential activity being used for the treatment and management of type 2 diabetes mellitus (Sudha et al., 2011). The ethnopharmacological and biochemical studies have confirmed the potential diverse activity of phytoconstituents such as polyphenols, flavonoids, tannins, saponins, alkaloids, steroids, polysaccharides, terpenoids, glycosides, amino acids, essential oils etc. These are isolated from many medicinal plants and thereby providing scientific evidence for their therapeutic usage as antidiabetic agent as has been mentioned in folklore medicine (Saxena and Vikram, 2004). Therefore, there is a curiosity and keen interest in the minds of researchers searching for new plants and herbs based on
pharmacological evidences having multiple beneficial health effects in rejuvenating pancreatic β-cells, improving insulin sensitivity, controlling PPHG and protecting the tissues from oxidative damage.

2.4 PHYTOMEDICINE FOR DIABETES IN ANCIENT AND MODERN WORLD

Natural compounds may be possible alternatives for the treatment of diabetes and its associated complications at the behest of failure in sustainable cure from the modern drugs. In view of the effectiveness, safety and low cost production of plant medicines, consumption of the source in the daily diet can potentially manage and may even reduce the risk of the disease. The medicinal plants have played a remarkable and characteristic role as therapeutic agents in the treatment of chronic pathologies that are linked to diabetes before the introduction of insulin in 1922 (Gray and Flatt, 1999). In this connection the plant sources (if taken in the form of diet) are considered to be very effective and safe because they potentially manage and control the risk factors associated with the diabetes.

Different parts of the plants grown in orchards and backyards are being used as pivotal agents in managing diabetes since ancient times as alternative medicines. Plants such as *Ocimum sanctum* (Tulasi), *Gymnema sylvestre* (Madhunasini), *Allium cepa* (Onion), *Allium sativum* (Garlic), *Embelica officinalis* (Gooseberry), *Aloe vera* (Indian aloe), *Aegle marmelos* (Bilwa), *Syzygium cumini* (Black plum), *Rauwolfia serpentina* (Sarpagandha), *Momordica charantia* (Bitter gourd), *Terminalia chebula* (Chebulic myrobalan), *Ficus bengalensis* (Banyan), *Coccinia indica* (Ivy gourd), *Withania somnifera* (Ashwagandha), *Centella asiatica* (Brahmi), *Asparagus racemosus* (Shatavari), *Tinospora cordifolia* (Amrithaballi), *Azadirachta indica*
(Neem), Glycorrhiza glabra (Jesthamadhu), Trigonella foenum-graecum (fenugreek), Curcuma longa (Turmeric), Mentha piperita (Peppermint), Syzygium aromaticum (Clove) are some frequently used plants, worldwide till date. (Bailey and Day, 1989; Jayapreethi, 2013).

As supplement to the above information, Chinese herbal medicine, the oldest known remedial system, containing a combination of non-toxic herbs (instead of one herb) with pronounced effect is being used in the treatment of diabetes. The plants used during Rigvedic times as mentioned in compendium of Charaka Samhita and Sushruta Samhita, with antidiabetic property are being used presently in our day to day life (Thatte and Dhanukar, 1989).

Systematic scientific studies carried out so far have provided the data in support of the plant’s tremendous recuperation effect on diabetic experimental animals at lesser dose when compared to standard drugs like glibenclamide, acarbose etc., The crude extract of many medicinal plants have drastic improvement or complete recovery from various conditions such as hyperglycemia, pancreatic β-cell destruction, plasma insulin decrease, decreased levels of antioxidant enzymes etc., as indicated by the diabetic experimental animals. Moreover, the activities of the crude plant extract are taken as manifestation of a sum total of different ingredients of complex mixture. The mechanism of action of synergism is suggested to have acted upon the diverse diseased conditions (Noor et al., 2013). The synergistic mechanism of action of plant components restoring the blood glucose levels is schematically represented in Fig.4.
The data obtained by scientific studies on Indian figs/Opuntia ficus, Mango/Mangifera indica, Pigeon pea/ Cajanus cajan, Vinca rosea/Catharanthus roseus, Ginger/Zingiber officinalis, Custard apple/ Annona squamosa, Mustard/Brassica juncea, Cinnamon/Cinnamomi cassia, Common sage/ Salvia officinalis, Bilwa/Aegle marmelosce, Tea/Camellia sinensis, Coconut/Cocos nucifera, Guaval/Psidium guajava have revealed an exceptional hyperglycemic control as well as radical scavenging effect and supports the conventional employment of commonly used and habitually available plants in herbal medicine as remedial agents for the treatment and control of diabetes (Kavishankar et al., 2011). Most of the studies carried out so far have indicated that the antidiabetic activities present in the plants are due to various types of biochemical ingredients which represent an innate antioxidant nature of the plants.
A review of literature by Bnouham et al., (2006), on the hypoglycemic studies of medicinal plants reported in Medline from 1990-2000, covered nearly 176 species belonging to 84 families. The study included several ethno potential medicinal plants with traditional back ground that demonstrated antidiabetic activity in vivo. The hyperglycemic model mostly used was streptozotocin and alloxan induced mice or rats and as nearly as 63 species from different families were shown to be more potent than the rest. Mechanism of action proposed for their antidiabetic property included insulin like activity, increased insulin sensitivity and synthesis, protection and revitalization of pancreatic β-cells by the plant extracts. The purported action of some plants might also have been due to improved glucose homeostasis either by increased peripheral utilization of glucose or increased glycogen synthesis by the hepatic cells and/or decreased glycogenolysis, retarded glucose absorption in the intestine and reduction of the glutathione effect, an indication of antioxidant interference. The review also indicates Trigonella foenum graecum, Momordica charantia, Ficus bengalensis, Polygala senega, Gymnema sylvestre and Opuntia streptacantha as some commonly explored species.

The combinatorial therapy of different medicinal plant extracts as a strategy to overcome the complications linked to diabetes has been the field of interest to some researchers. Proponents of herbal medicine also used combinations of such plants very often rather than single because they believed that dozens of biologically active compounds in plants work together to produce enhanced effect (Chang et al., 2013).

Triphala, an equi-proportional fruit mixture of three medicinal plants Emblica officinalis, Terminalia chebula and Terminalia bellerica, has shown to decrease the blood glucose level, inhibit lipid peroxide formation and also to scavenge superoxide
and hydroxyl radicals in a high fat and alloxan induced diabetic rats (Sabu and Kuttan, 2002; Prativadibhayankaram et al., 2008). In vitro screening of Triphala for radio protective effect alongside antioxidant activity correlated the significant property to the polyphenolic content of the ayurvedic formulation, especially gallic acid (Naik et al., 2006). Scientific studies carried out in the past two decades have validated many of the ethnomedicinal claims and studies have shown Triphala to possess free radical scavenging, antioxidant, anti-inflammatory, antipyretic, analgesic, antibacterial, antimutagenic, wound healing, anticariogenic, antistress, hypoglycemic, anticancer, chemoprotective, radioprotective and chemopreventive effects.

Ayurvedic preparation Dihar, main ingredients being leaves of Gymnema sylvestre, Azadirachta indica, fruits of Momordica charantia, seeds of Syzigium cumini, roots of Tinospora cordifolia, Curcuma longa, fruits of Embelica officinalis and Enicostemma littorale given orally to diabetic rats for a period of 6 weeks restored the elevated blood sugar levels to normal range. The antioxidant potential of the formulation was clearly indicated by a significant decline in lipid peroxidation level and increased catalase, superoxide dismutase and reduced glutathione levels in diabetic rats. Reinstatement of serum creatinine and urea levels did prove the antihyperlipidemic action of Dihar (Patel et al., 2009).

Several potential hypoglycemic agents have been isolated from medicinal plants possessing antidiabetic property (Conman et al., 2012). These biomolecules with a variety of intricate structures belonging to different classes of metabolites, exerts unique hypoglycemic action like stimulating insulin secretion, sensitizing pancreas to secrete insulin, improve glucose tolerance, carbohydrate metabolizing enzyme inhibiting activity, effect on glucose transporter etc. Previous investigations
have revealed the efficacy of the bioactive drugs obtained from medicinal plants, to be more potent than the known oral hypoglycemic agents like tolbutamide, daonil and chlorpropamide (Bnouham et al., 2006).

Glycosides and saponins from bitter apple and bitter cucumber, tannins from banana, betel and Cassia, volatile oil from holy basil and tulsi, withanolides from winter cherry, stevioside, a glycoside from honey grass, mucilage from desert Indian wheat and sand plantain, alkaloid from ivy gourd, triterpene oligoglycosides from common horse chestnut, kolaviron from bitter kola are some of the bioactive components isolated that has shown to possess hypoglycemic function (Garg and Garg, 2008).

\textit{In vitro} and \textit{in vivo} studies on the flavonoid fractions of plants Artocarpus heterophyllus, Artocarpus altilis and Piper betel have indicated strong antioxidant and antidiabetic potential (Sindhu, 2013). The fractions of these plants have reported to contain phenolics and flavonoids quercetin, rutin, kaempferol, morin, morusin, cyclomorusin and catechin. Amelioration of type 2 diabetes mellitus by modulation of insulin receptor and leptin levels by the isolated compounds such as 2,5 dihydroxy-6,7-dimethoxy flavones and 3,7,3’,5’-tetramethoxy-4-hydroxy flavones from Anethum graveolens, rutin and quercetin-3-O-\alpha-L-rhamnopyranoside from Triticum aestivum, indicated the protective action of the flavonoids on DNA against reactive oxygen species (Nithyakala, 2013).

Many traditional plants containing active principles that are isolated have paved way for the synthesis of new drugs with multi targeted approach towards treatment of the diseases. Galegine, isolated from Gallega officinalis, widely used plant for treating T2DM since ancient times, has been a baseline prototype for the
development of one of the extensively used drug Metformin (Goldstein and Wieland, 2008).

A study on medicinal plants with potential antidiabetic activity supports the comprehensive approach for Diabetic management to take an account of dietary management and physical exercise in hand with natural plant products. A number of reviews on the medicinal plants screened for the hypoglycemic potential not only provides great insight into the phytochemicals and their mode of action in combating Diabetes but also mention the need to harvest and harness the herbs with multiple therapeutic beneficial effects (Garg and Garg, 2008).

2.5 ANALYTICAL TECHNIQUES USED IN CHARACTERISATION OF PHYTOCONSTITUENTS

Modern analytical techniques have been used in characterization of active pharmacological ingredients obtained from the medicinal plants and in this aspect the new techniques lends a promising hand in the field of phytochemistry.

In the characterization of phytochemicals, the primary step is authentication. This is to be followed before adapting the procedure for the extraction of active components using suitable solvent(s). Various extraction methods are used such as maceration, infusion, digestion, decoction, soaking, percolation, hot continuous extraction (soxhlet), fermentation, counter current extraction, sonication (ultra sound extraction), super critical fluid extraction (SFE) and phytonics process. The other parameters are, selection of part of the plant, drying methods, use of suitable solvent, nature of the phytoingredients etc., in which one is interested. For example, for non polar compound extraction non polar solvents like hexane, petroleum ether etc., may be used whereas for polar molecules, polar solvents such as
ethyl acetate, methanol, aqueous methanol etc., are used. In case of thermolabile compounds, cold maceration, counter current extraction (CCE) or percolation methods can be adapted, whereas in the case of thermostable compounds, soxhlet extraction method is the best. In all these cases the time factor plays a very crucial role to ensure a complete extraction of the active phytoingredients. However, it can be noted that the concentration method is supposed to play a guaranteed role regarding the stability of the plant extracts. The solvent extracts are generally dried under pressure using rotary vacuum evaporator. However, recent technique such as lyophilization has become popular in view of obtaining stable compounds, although this method is very expensive (Handa et al., 2008).

Synergistic method of isolation and purification has become significant in pharmaceutical industry wherein chemists are using chromatographic techniques (based on the principle of separation) to get the required compounds in pure form. The phytoconstituents extracted from the medicinal plants are subjected to adsorption, affinity, partition and/or ion-exchange procedure from which the constituents are separated (Hans-Jorg and Stephan, 2011). For example thin layer chromatography (TLC) (an inexpensive method) is first performed to recognize the suitable solvent for column chromatography and also to know the number of compounds present in the fraction of the plant extract. From this method, identification and purity of the compound against the standard can be known. Nevertheless preparative TLC plates have been gaining importance in view of their utility in isolation of characteristic bioactive components present in the plant extracts.

High performance or High Pressure liquid chromatography (HPLC) is another versatile technique from which the qualitative and quantitative aspects of the bio-
components can be obtained. In this technique, the basic principle of separation is based on the different migratory rates of the phytoconstituents in a given mobile phase under thermodynamic condition. The separation of bio-active components can be done as quickly as possible since the procedure is accurate. However, the results obtained depend on the nature of mobile phase, flow rate, suitable detectors and columns used.

Liquid chromatography coupled with mass spectrometry (LC/MS) is another powerful technique for the analysis of the complex plant extracts and provides information on the nature of the constituents of the plant extracts. This method is, in fact, versatile in view of its applications. In modern analytical techniques, both HPLC and MS linked facility, facilitates rapid and accurate identification of chemical compounds present in the medicinal herbs even in the absence of pure standards (Ye et al., 2007).

In addition to the above techniques used for the identification and characterization, the most vulnerable technique being used in the field of phytochemistry is Fourier-transform infrared spectroscopy (FT-IR). This is also an important tool used for identification of the characteristic functional groups of compounds (Eberhardt et al., 2007). The FT-IR spectra of compounds are unique and are treated as molecular "fingerprints". The spectrum of an unknown compound can be identified on comparison with a library of known compounds (Hazra et al., 2007).

Nuclear magnetic resonance (NMR) spectroscopy is an analytical technique used in research for determining the content and purity of a sample as well as its molecular structure. NMR can quantitatively analyze mixtures containing known
compounds and for unknown compounds, NMR can either be used to match against spectral libraries or to infer the basic structure directly (Pellecchia et al., 2008).

2.6 CURRENT SCIENTIFIC STATUS OF *Cassia auriculata* L.

The Pharmacological evaluation studies of different parts of *Cassia auriculata* conducted till date has established scientific evidence for its ethnomedicinal usage. Investigation of antihyperglycemic, antipyretic, hypolipidemic and *in vitro* anticancerous activity of leaf extract, nephroprotective activity of root extract, antidiabetic, antioxidant, antipyretic and hepatoprotective action of flower extract has been carried out by several researchers. In all the above mentioned investigations, the focus was only on crude extracts.

Both methanolic and ethanolic extracts of flowers screened for antioxidant activity using 2,2'-azinobis-(3-ethylbenothiazoline-6-sulfonic acid (ABTS) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) methods showed potent radical scavenging activity and proposed the antioxidant action to be one of the mechanism by which the flowers exert the antihyperglycemic action (Kumaran and Karunakaran, 2007).

The water soluble ethanol extract of flowers in alloxan induced diabetic rats, remarkably increased the plasma insulin levels and decreased the blood glucose levels indicating the protection rendered by the extracts towards β-cells and enhance transport of blood glucose to peripheral tissues. Hepatic toxicity studies based on the levels of marker enzymes alanine transaminase (ALT), aspartate transaminase (AST), acid phosphatase (ACP) and alkaline phosphatase (ALP), also presented restorative effect of the flower extract on the damaged liver cells. Presence of number of secondary metabolites which differ in their chemical and pharmacological effects
suggested that synergistic effect of many hypoglycemic agents could have resulted in the antidiabetic approach (Hakkim et al., 2007).

The hypolipidemic activities of the aqueous extract of *Cassia* flowers in streptozotocin induced diabetic condition suggest the beneficial effect against atherosclerosis. The ability of the extract to regulate cholesterol, free fatty acids, triglycerides and phospholipids level indicated antihyperlipidemic effect of the flower constituents. The down regulation of lipogenesis may cause resistance to tissue damage and diabetes (Latha and Pari, 2003). Similar kind of observation in alloxan treated experimental animals supplemented with flower and leaf extracts also suggest the reduced effect on hyperglycemia associated hyperlipidemia (Devi et al., 2006; Pari and Latha, 2002).

The leaf extract treatment for hyperglycemia induced oxidative stress, resulted in significant fall of the fasting blood glucose (FBG) level and also decreased thiobarbituric acid reactive substances (TBARS) which is an indicative of the antioxidant nature of the extract. Significant increase in the erythrocyte reduced glutathione level elevated the antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT). Toxicity assessment at a dose level 1000 and 2000 mg kg\(^{-1}\) body weight ingested in healthy rats for 21 days produced no adverse effect on general behavior and survived the test period. Since there were no signs of toxic symptoms like restlessness, respiratory distress, diarrhea, convulsions and coma, the safety evaluation was established (Gupta et al., 2009).

A potent antidiabetic activity was observed in STZ induced diabetic rats on supplementing different solvent extracts from stem bark of *C.auriculata*. Methanolic extract was found to express more potent hypoglycemic effect compared to hexane,
ethyl acetate and aqueous extracts and the histological observations of β-islets of pancreas supported the recuperation effect of different solvent extracts from the bark (Daisy and Jeeva Kani, 2012).

Safety pharmacological and drug interaction studies of hydro alcoholic and supercritical fluid extract of *C.auriculata* seeds did mention the need for pharmacokinetic herb-drug interaction studies as an evidence for safety. Both the extracts did not show any adverse effects and found to be pharmacologically safe. Although pharmacokinetic evaluation of traditional hydro-alcoholic extract was found to be safe, technology based supercritical extract caused significant reduction in absorption of metformin (Puranik et al., 2010).

An investigation focused on the serum insulin augmentation, antihyperglycemic and antihyperlipidemic activities of a combined aqueous extract of *C.auriculata* and *A.marmelos* on streptozotocin induced diabetic rats resulted in a significant recovery in serum insulin levels, hyperglycemia and hyperlipidemic conditions. The restored insulin level by the combined plant extracts was evidenced by the histological studies of endocrinal pancreatic β-cells (Sivaraj et al., 2009).

Polyherbal formulation of different parts of the plant with other medicinal plants such as Dianex (Mutalik et al., 2005), Diamed (Pari et al., 2001), Hyponidd (Babu and Prince, 2004) and Diasulin (Saravanan and Pari, 2005) have also revealed antihyperglycemic and antilipidemic effects in experimental animals. Concurrent ingestion of herbal tea prepared from flowers with carbamazepine in wistar rats enhanced the serum levels of ophylline by 32.5%, indicating the increased bioavailability of the drug for effective management of diabetes influenced by the flower components (Thabrew et al., 2004).
Myristyl alcohol, β-sitosterol β-D-glucoside, quercetin 3-O-glucoside, rutin and di-(2-ethyl –hexylphthalate are some of the compounds isolated from leaves (Nageswara Rao et al., 2000). O-β-D-xylopyranoside has been isolated from stem bark of the plant (Sanghi et al., 2000), Polysaccharides, dimeric procyanidins (Kumaran and Karunakaran, 2007), flavonoids from roots (Rai and Dasaundhi, 1999) and anthracene derivatives have also been isolated from the plant. Colorimetric estimation of chrysophanol, emodin and physcione in free and combined form analyzed has shown 0.53% abundance in bark, flower, leaf, pericarp, seed and stem wood (Khan et al., 2005).

So far there has been only one report (Juan–Badaturuge et al., 2011) on activity guided isolation of antioxidant principles from whole plant. Fractionation of crude methanolic extract in solvents of ascending polarity (pertroleum ether, chloroform, ethyl acetate, n-butanol, water) and evaluation for antioxidant activity employing in vitro lipid peroxide and DPPH radical scavenging assays and study of reducing power showed ethyl acetate as the most active fraction followed by chloroform. Further bioassay guided (using DPPH assay) fractionation of ethyl acetate portion has yielded kaempferol, luteolin, quercetin and kaempferol-3-O-rutinoside. Kaempferol was also isolated from the second most active chloroform fraction. The isolated compounds thus validate the antioxidant as well as antidiabetic properties of the plant as these compounds have been previously known to cause hypoglycemic effect.

Flowers of C.auriculata exhibited potential inhibition expressing IC₅₀ value 0.023mg mL⁻¹ among Aegle marmelos, Aegle lanata, Aegle heterophyllus, Cassia auriculata, Limonia acidissima, Momordica charantia, Passiflora incarnate,
Passiflora embelica, Passiflora marsupium, Passiflora pinnata, Tinospora cordifolia, T. foenum that were examined for in vitro and in vivo α-glucosidase inhibitory action. The maltase inhibitory activity was competitive in nature and a single oral administration of the methanolic flower extract alleviated the blood glycemic response towards maltose ingestion thus indicating the PPHG retardation achieved by inhibitory effect (Abesundara et al., 2004).

Crysophanol isolated from Cassia auriculata leaves has been screened for antidiabetic, antioxidant, antipyretic, anti-inflammatory activities. Crysophanol treated STZ induced diabetic rats restored blood glucose level to normal condition and the mechanism of action was proposed to the restored architecture in liver and kidney cells compared to the inflammation observed in STZ induced diabetic rats. The potential of Chrysophanol as a hypoglycemic agent was supposed to be mediated through insulin secretory action (Rose Mary, 2013).

Therefore, it is predictable that C. auriculata has a widespread use not only in traditional Indian medicine but also in other parts of the world which makes it more interesting medicinal herb for further exploration to isolate novel antidiabetogenic agent with protective function on tissues/organs against reactive free radicals.

2.7 ORIGIN OF THE PROBLEM

Although the antidiabetic activity of the plant C. auriculata was reported as early as 1967 (Jain and Sharma, 1967), other related effects such as antilipidemic, anti-inflammatory and antioxidant properties are reported only in recent years.

- However, studies involving bioassay guided isolation of active components has not been completely exploited.
• So far, there are no reports on the isolation of active principle ingredients of the plant extract exhibiting both antidiabetic and antioxidant activity.

• It will be interesting to know the influence of the plant extract on hyperglycemia and oxidative stress by simultaneously performing the experiments *in vitro* and *in vivo* conditions which will enable to evaluate the antidiabetic and antioxidant properties of the plant extract.

Thus, experiments have been designed in order to study the above objectives with a view to investigate the effectiveness of antioxidant and the antidiabetic property of *C. auriculata* by evaluating the parameters such as reducing power, chelating ability, anti-lipid peroxidative activity, free radical scavenging capabilities and the regulatory effect on carbohydrate metabolizing enzymes like α-amylase and α-glucosidase under *in vitro* conditions. The effects pertaining to activities of stress releasing enzymes, lipid peroxidation, glutathione content, levels of fasting blood glucose, glycosylated hemoglobin and plasma insulin have been determined under *in vivo* conditions. Besides, phytochemical analysis and spectrophotometric quantification of total phenolics, flavonoids and tannin content of the plant extract, the analysis of bioactive principles such as mixed polyphenols, quercetin, rutin, ferulic acid and cinnamic acid using RP-HPLC and HPTLC technique were performed. Based on the inhibitory effects on carbohydrate metabolizing enzyme, α-glucosidase and α-amylase activity and DPPH radical scavenging potential along with reducing power, the active principles are isolated and characterized.