# CHAPTER 2

# REVIEW OF LITERATURE

### *2.1. Significance and scope of the research area*

Medicinal plants play an important role in the management of diabetes mellitus especially in developing countries where resources are meagre. In a study conducted by Chan *et al.* (2012), it was reported that Asian and African continents have 56% and 17% share of the worldwide distribution of therapeutic herbal plants respectively. He further reported that India and China are the leading countries in Asia in plant research and there has been an increase in research on antidiabetic plants since 1995, in these regions. In India, base on ethnobotanical information, Patil *et al.* (2011) reported that about 800 plants may possess antidiabetic potential out of which few are experimentally proved.

The alarming increase in prevalence of diabetes and rate of mortality due to diabetes was reported by many studies (Naidu, 2003; Shaw *et al.,* 2010; Monesi *et al.,* 2012; Kaveeshwar and Cornwall, 2014; Bharati *et al.,* 2011). The high cost and poor availability of current therapies in rural parts of India necessitates the need of indigenous, inexpensive botanical sources of antidiabetic crude or purified drugs. India is a biodiversity centre with over 45.000 plant species, most with therapeutic properties. Research in antidiabetic plants is well suited and has a better scope in India due to rich traditional knowledge and plant sources (Naidu, 2003). *Cassia alata* Linn. is considered as a herbal source for the treatment of diabetes in North East India. The significance of the present research is to validate the antihyperglycemic property of this plant as a part of management of diabetes in developing countries like India.

### *2.3. Theory and practical base literature reviews relevant to the research*

The following is a comprehensive and contextualized literature review that describes theory base and published research articles relevant to the present work, categorized under common topics.

#### *2.3.1. Techniques of plant analysis*

The principles of plant analysis were developed since 1800s, beginning in Europe (Kalra, 1998). The importance of finding the correlation between the phytoconstituents and the bioactivity of plant was described by many researchers (Owolabi *et al.,* 2010; Singh *et al.,* 2014; Yadav *et al.,* 2014; Ragasa *et al.,* 2015), as

it is desirable to know for the synthesis of compounds with specific activities to treat various ailments.

*2.3.1.1. Botanical identification of the plant*

Botanical identity of the plant sample studied must be authenticated by an acknowledged authority or institution and provide a voucher number. The identity of the plant material should either be beyond question. For these reason, it is now a common practice in phytochemical research to deposit a voucher specimen of a plant examined in a recognized herbarium so that future reference can be made to the plant studied if this becomes necessary (Harborne, 1998).

*2.3.1.2. Plant extracts preparation techniques*

The basic concept of sample preparation method is to convert a real matrix into a sample in a format that is suitable for analysis. Grinding of plant materials facilitates subsequent extraction procedures by rendering the sample more homogeneous, increasing the surface area, and facilitating the penetration of solvents into cells (Harborne, 1998). The qualitative and quantitative studies of bioactive compounds from plant materials mostly rely on the selection of proper extraction method (Smith, 2003: Sasidharan *et al.,* 2011). Numerous authors have followed solid-liquid extraction for the extraction of solid samples, which is one of the oldest ways of solid sample pretreatment (Selvamani *et al.,* 2008; Mishra *et al.,* 2011; Karan *et al.,* 2013; Petchi *et al.,* 2013). Typical solid-liquid solvent extraction processes for herbal materials involve drying and grinding of the plant material, choosing a suitable extraction solvent and extraction procedure (Kashani *et al.,* 2012).

*2.3.1.3. Choice of suitable extraction solvent and procedure*

The theory of extraction of desired bioactive compounds depend on the choice of the extraction solvent as the solubility of the compounds varies base on the range of polarity of solvents used (Kashani *et al.,* 2012). In support of the above theory Gupta *et al.,* (2012) and Azman *et al.,* (2013) also have described the effect of solvent on extract preparation and bioactivity. Although water is generally used as an extractant in many traditional protocols, researchers now followed modern methods of extraction using organic solvents of varying polarities base on the compound(s) of interest.

If the polarity or the solubility of the compound(s) of interest is known, information such as the one in the table below can be used to select a suitable extractant solvent or a mixture of two or more solvents of different polarity. A summarized form of polarity and chemical profiles of some of the common extraction solvents are shown in Table.2.1.

Table.2.1: Polarity and chemical profiles of most of the common extraction solvents. (Adapted from Kashani *et al.,* 2012).

| References | Extracted chemical profile | Solvent | Polarity |
|---|---|---|---|
| Ayaffor *et al.,* (1994), Cowan, (1999) | Fatty acids, waxes , terpenoids | n- Hexane | Low |
| Perett *et al.,* (1995), Cowan, (1999), Bruneton, (1999) | Fatty acids, waxes , terpenoids C | Chloroform | |
| Bruneton, (1999), Scalbert *et al.,* (2005) | Less polar and polar flavonoids, tannins, terpenoids | Dichlorome thane | Medium |
| Bruneton (1999), Scalbert *et al.,* (2005) | Less polar and polar flavonoids, tannins, terpenoids | Ethyl-acetate | |
| Eloff, (1998), Bruneton, (1999), Scalbert *et al.,* (2005) | Less polar and polar flavonoids, tannins, terpenoids, glycosides | Acetone | |
| Cowan, (1999), Bruneton, (1999) | Polar flavonoids, tannins , glycosides (saponins) | Ethanol H | High |
| Bruneton, (1999), Scalbert *et al.,* (2005) | Carbohydrates, lecithin, amino acids, polypeptides, phenolic acids, phenylpropanoids, polar flavonoids, glycosides and alkaloids | Methanol | |
| Kaul *et al.,* (1985), Jones & Kinghorn, (2005) | Carbohydrates, lecithin, amino acids, polypeptides, phenolic acids, phenylpropanoids, polar flavonoids, glycosides and alkaloids | Water | |
| Bruneton, (1999) | Alkaloids | Aqueous acid or base | |

If the polarity of the compounds of interest is not known, the powdered plant material can be extracted simultaneously with a mixture of different proportions of two or more solvents of different polarity. Alternatively, the powdered plant material can be extracted sequentially with solvent of different polarity in what is known as a sequential extraction procedure (Bruneton, 1999). In sequential solvent extraction, the herbal material is extracted with a series of solvents of different polarity (Starmans and Nijhuis, 1996). The usual way is to start with a non-polar solvent and exhaustively extract the herbal material followed by a series of more polar solvents until several extracts are obtained of increasing solute polarity (Okwu, 2001). Many modern researchers opted sequential extraction procedure (Gokce and Haznedaroglu, 2008; Subash-Babu *et al,* 2008; Stankovic, 2011; Sudha *et al.,* 2011; Gahlaut and Chhillar, 2013; Algariri *et al.,* 2014; Saravanan and Parimelazhagan, 2014).

*2.3.1.4. Methods of separation of phytochemicals*

Separation techniques or procedures mainly depend mainly on the physical characteristics of the compounds. Any slight differences in any of their physical properties are exploited in the separation techniques. The separation and purification of the plant constituents is mainly carried out using one or other, or a combination of chromatographic techniques: Paper Chromatography (PC), Thin Layer Chromatography (TLC), Column Chromatography (CC), High Performance Liquid Chromatography (HPLC), Gas Liquid Chromatography (GLC), and Optimum Performnce Leminar Chromatography (OPLC) (Raaman, 2006). Chromatography is a technique used to separate molecules based on their size, shape or charge (Heftmann, 1992).

High Performance Liquid Chromatography separates compounds on the basis of their interaction with the solid particles of a tightly packed column and the solvent of the mobile phase (Katz, 1995). It provides a number of highly selective variants to resolve almost every type of separation problem: on the basis of this, HPLC and related techniques can be regarded as the most important analytical method in contemporary pharmaceutical analysis (Maria, 2011). However volatile compounds cannot be separated by HPLC. Gas Chromatography-Mass Spectrometry (GC-MS) is a hyphenated analytical technique that combines the separation properties of a-liquid chromatography with the detection feature of mass spectrometry to identify different substances within the test sample. GC is used to separate the volatile and thermally

stable substitutes in a sample whereas GC-MS fragments the analyte to be identified on the basis of its mass (Chauhan *et al.*, 2014).

GC-MS analysis of *Cinnamomum tamala* oil (CTO) was performed by Kumar *et al.* (2012) as a part of screening of antidiabetic, antioxidant and hypolipidemic potential of CTO. By analyzing the GC-MS chromatogram of CTO, the authors identified and quantified 31 components which accounted for 99.99% of the total oil. The main volatile components of CTO identified were cinnamaldehyde (44.898%), Trans cinnamyl acetate (25.327%), Ascabin (15.249%), Hydro cinnamyl acetate (3.384%), Beta-caryophyllene (2.669%) and it comprised of 91.527% of the oil (Kumar *et al.*, 2012).

Atangwho *et al.* (2013) subjected the chloroform extract of a potent antidiabetic plant *Vermonia amygdalina* Del. to GC-MS analysis which revealed approximately 15 compounds and four of which were identified to be fatty acids (2 saturated and 2 polyunsaturated fatty acids). They identified the constituents by matching the spectra with those found in the NIST 02 library, and computed the percentages with the total ion chromatogram. The authors pointed out that the combined amount of the polyunsaturated/essential fatty acids, linoleic acid and α-linolenic acid, was higher (4.72% and 10.8%, respectively) than the saturated fatty acids, palmitic acid and myristic acid (8.56% and 1.92%, respectively). The authors further underlined the nutritional and pharmacological importance of the plant by comparing their analyzed fatty acids with previously identified fatty acids in similar studies (Atangwho *et al.*, 2013).

The effect of solvent polarity in bioactive extraction contents of the plant *Securiqera securidaca* was evaluated via GC-MS analysis. They investigated the antidiabetic and antilipidemic activities of different extracts in STZ induced diabetic rats. They reported the best and significant result shown by the carbon tetrachloride extract of *Securiqera securidaca* when compared to other extracts. The authors further argued that the better significant activity of carbon tetrachloride extract is due to its more sterols and fatty acids content as revealed by GC-MS analysis (Ahmadi *et al.*, 2015).

Identification of bioactive compounds from the whole plant ethanolic extract of *Evolvulus alsinoides* by GC-MS was carried out by Gomathi *et al.* (2015). The author revealed the presence of various compounds like piperine, octodeconoic acids, hexadecanoic acid and squalene in the ethanolic extract of *Evolvulus alsinoides*.

Authors further argued that the plant might probably have chemopreventive, anticancer, anti-microbial activity, antioxidant and antidiabetic activity due to the presence of above said secondary metabolites in the ethanolic extract. Additionally, authors suggested that esters present can be used as a flavouring agent in food industries and thus have experimentally proved the traditional use of *Evolvulus alsinoides* in various disorders (Gomathi *et al.*, 2015).

*2.3.2. Antioxidant activity of an antidiabetic plant*

The roles of antioxidants are to neutralize the excess of free radicals, to protect the cells against their toxic effects and to contribute to disease prevention (Alam, 2013; Pham-Huy, 2008). In a diabetic patient auto-oxidation of glucose, shifts in redox balances, decreased tissue concentrations of low molecular weight antioxidants, such as reduced glutathione (GSH) and vitamin E, and impaired activities of antioxidant defence enzymes such as superoxide dismutase (SOD) and catalase (CAT) are the sources of generation of free radicals that causally linked to other metabolic abnormalities important to the development of diabetic complication (Matough, 2012).

Traditional medicinal practitioners may use antioxidant plants more to treat symptoms of diabetes than the disease itself. Oxidative stress is important in so many of the complications of diabetes that medicinal plants used for various symptoms and for the disease specifically can be expected to have antioxidant activity. Indeed, in a study of 35 medicinal species used for combination of diabetic symptoms, it was observed that the greater the number of symptoms a plant species is used for, the greater its antioxidant activity is. Antioxidants in medicinal plants and traditional diets may help control diabetic complications and possibly reduce the onset of diabetes (Soumyanath, 2006).

The above views are the foundation for the determination of antioxidant activity of antidiabetic plants as an important and unavoidable part of investigation of its antidiabetic activity. Various methods are used to investigate the antioxidant property of samples. Antioxidant activity should not be concluded based on a single antioxidant test model (Sudhakar and Singh, 2008). DPPH assay, Nitric oxide scavenging activity, Ferric reducing-antioxidant power (FRAP) assay, Reducing Power Assay and ABTS assay are the most commonly used method for the determination of antioxidant activity.

Two flavonoid compounds were isolated from an established antidiabetic plant *Bauhinia monandra* (Kurz) leaves and their antioxidative effects were determined using DPPH assay. Bioassay directed fractionation of the ethyl acetate soluble leaves extract led to isolation of two active compounds identified as: Quercetin-3-O-rutinoside (1) and Quercetin (2). The molecular structures elucidations of both compounds were carried out using spectroscopic studies (1H NMR, 13 C NMR and MS). These compounds are reported from the species for the first time. The authors have reported a higher antioxidant activity in compound 2 and a lower activity in compound 1 than the standard L-ascorbic acid (Aderogba *et al.,* 2006).

Seven flavonoids were isolated from the butanol fraction of the methanolic extract of the aerial parts of *Cynanchum acutum* L. (Asclepiadaceae). All the seven compounds were isolated for the first time from the genus *Cynanchum*. The compounds isolated were reported as quercetin-O-β-galacturonopyranoside (1), quercetin 7-O-β-glucopyranoside (2), tamarixin 3-O-β-galacturonopyranoside (3), kaempferol 3-O-β-galacturonopyranoside (4), 8-hydroxyquercetin 3-O--galactopyranoside (5), tamarixtin 3-O-α-rhamnopyranoside (6), and tamarixtin 7-O-α-ara-binopyranoside (7) on the basis of their chromatographic properties, chemical and spectroscopic data. Out of the seven the major three flavonoids isolates 1, 2, and 3 were reported to exhibit significant antioxidant and antidiabetic activities (Fawzya *et al.*, 2008).

The work on investigating the antioxidant and antidiabetic activity of hot water extract of *Helicteres isora* (L.) fruits was done by Suthar *et al.* (2009). The authors opted 1,1-diphenyl,2-picryl hydrazyl assay, ß-carotene-linoleate model and microsomal lipid peroxidation or thiobarbituric acid reactive species assays for determining antioxidant activity assay and illustrated maximum activity with $IC_{50}$ value 25.12±0.18 µg/ml for 1,1-diphenyl,2-picryl hydrazyl assay method, and low activity with $IC_{50}$ value 740.64±4.76µg/ml for microsomal lipid peroxidation assay. They stated that in the ß-carotene-linoleate model, the extract have exhibited 45.63% antioxidant activity. In the *in vitro* glucose uptake model of antidiabetic test the authors noted the significant ($P<0.05$) uptake of glucose by isolated rat hemi-diaphragm but less effective to that of the reference drug, metformin. Authors suggested further investigation in animal models and isolation of its active constituents (Suthar *et al.*, 2009).

Chakraborty and Das, (2010) evaluated the antidiabetic and antioxidant activities of *Cinnamomum tamala* leaf extracts in STZ induced diabetic rats. After treatment of the diabetic rats for 3 weeks with *C. tamala* aqueous extract (250mg/kg body weight dose) the authors deduced the results that specified marked decreased in the levels of fasting blood glucose and urine sugar, with a concomitant increased in body weight, a significant decreased in peroxidation products, viz., thiobarbituric acid reactive substances in treated rats. They pointed out that reduced glutathione and glycogen content, which had shown significant decreased following induction of diabetes, were increased in the hepatic tissue of STZ-diabetic rats treated with CTLEt. The authors further highlighted the antioxidant activity of the extract by revealing phenols, ascorbate and carotenoids present in the extract. They affirmed that CTLEt induced antihyperglycemic as well as antioxidant activities in STZ-diabetic rats (Chakraborty and Das, 2010).

Gupta, (2012) evaluated the antioxidant and antidiabetic activity of methanol extract of *Moringa oleifera* in experimental diabetes. The authors reported the reduction of progression of diabetes after the treatment of *Moringa oleifera* methanol extract (MOMtE) in diabetic mice. The authors further reported that MOMtE treatment increased antioxidant levels in pancreatic tissue, with concomitant decreases in levels of thiobarbituric acid-reactive substances. They assumed that MOMtE might be effective in preventing oxidative protein damage by decreasing oxidative stress which is thought to be involved in beta-cell damage in the diabetic condition. The authors concluded by stating that methanolic extract of pods from *M. oleifera* protects beta-cells against ROS-mediated damage by enhancing cellular antioxidant defences and minimizing hyperglycemia in STZ-induced diabetes (Gupta *et al.,* 2012).

Antioxidant and antidiabetic activity of Methanolic and aqueous extracts of *Acacia Arabica, Murraya koeingii, Catharanthus roseus* and *Rouwolfia serpentina* were examined by Aadil *et al.,* (2012). Antioxidant activities were evaluated by DPPH and $H_2O_2$ scavenging assay and putative antidiabetic activity by *in vitro* glucose diffusion and alpha-amylase inhibition assay. The authors reported the highest DPPH scavenging activity in methanol extract of *C. roseus* while the highest hydrogen peroxide scavenging activity was found in aqueous extract of *M. Koeingii*, however, in *in-vitro* antidiabetic test, highest α-amylase inhibition was proclaimed in

methanolic extract of *R. serpentina* and the highest diffusion rate of glucose was found in aqueous extract of *R. Serpentin* (Aadil *et al.,* 2012).

Teugwa, (2013) investigated the antioxidant and antidiabetic activity of two African medicinal plants *Picralima nitida* and *Sonchus oleraceus.* The authors reported higher polyphenol contents, free radical scavenging activity, significant blood sugar reduction capacity and the ability to reduce the levels of oxidative stress markers (MDA, $H_2O_2$ and catalase) in the methanol extract of *P. nitoda* leaves and the hydroethanol extract of *S. oleraceus.* They pointed out a significant correlation between the polyphenol content and both oxidative and hypoglycemic activity, suggesting polyphenols as the main determinant of antioxidant and hypoglycaemic effects (Teugwa *et al.,* 2013).

The effects of red ginseng extract (RGE) on adiposity index, some serum biochemical parameters and tissue antioxidant activity in obese diabetic rats were investigated by Shalaby (2013). The analyzed result was that the oral dosage of RGE to obese diabetic rats significantly ($P < 0.05$) reduced adiposity index; decreased serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (GGT) enzymes, total cholesterol (TC), triglycerides (TG), and low density lipoproteins (LDL-c) and improved atherogenic index, Blood glucose and leptin hormone decreased, but insulin increased by administration of RGE. The authors pointed out the increased activities of superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) antioxidant enzymes in kidneys tissues. The authors further stated that red ginseng extract produces antiobesity, antioxidant, and antidiabetic activities in obese diabetic rats (Shalaby *et al.,* 2013).

Four major alkaloids from *Catharanthus roseus* (L.) G. was established for its antioxidant and antidiabetic activities. Isolated and identified four alkaloids – vindoline I, vindoline II, vindoline III and vindoline IV – were from the dichloromethane extract (DE) of this plant's leaves. A relatively high glucose uptake in pancreatic β-TC6 or myoblast C2C12 cells also reported, in all the four isolates, with highest activity in case of III. Furthermore, a good protein tyrosine phosphatise-1B (PTP-1B) inhibition activity demonstrated by compounds II-IV was specified. Also reported the highest antioxidant potential of compound III in ORAC and DPPH assays and its ability to alleviate $H_2O_2$-induced oxidative damage in β-TC6 cells at 12.50 µg/ml and 24.0 µg/ml (Tiong *et al.,* 2013).

Anusooriya (2014) confirmed the hypoglycaemic activity of aqueous extract of *Passilora ligularis* after 30 days of treatment in STZ induced diabetic rats. The abilities of the extract to reversed the parameters like serum total albumin, globulin, albumin/globulin ratio, activities of hepatic and renal markers to near normal in extract and glibenclamide treated rats was pointed out. The authors tried to correlate the hypoglycemic activity and antioxidant activity of the extract. The extract (400mg/kg given orally for 30 days) showed significant elevation in enzymatic (SOD, catalase and Gpx), and nonenzymatic antioxidants (vitamin C, vitamin E, and reduced glutathione) and confirmed significant decrease in lipid peroxidation. It was verified that aqueous extract of *Passilora ligularis* fruit can decrease the blood glucose and reduce the oxidative stress by removing free radicals in diabetes (Anusooriya *et al.*, 2014).

Martha (2014) evaluated antidiabetic, antioxidant and antiglycating activities of the *Eysenhardtia polystachya.* The antioxidant capacities were evaluated by studying *in vitro* scavenging of DPPH and ABTS free radical, reactive species, chelating ability, ORAC, β-carotene-bleaching and lipid peroxidation, and antiglycation activities by haemoglobin, bovine serum albumin (BSA)-glucose, BSA-methylglyoxal and BSA-glucose assays. Levels of antioxidant parameters (SOD, Serum catalase, glutathione peroxidise) were determined in serum liver, pancreas and kidney. The authors attributed the antioxidant activity of the extract to the presence of phenolic and flavonoid compounds. Their study demonstrated the considerable antioxidant activity, reactive oxygen species (ROS) scavenging activity, anti-AGEs role, hepatoprotective roles and hypoglycaemic activities of the extracts. The authors presumably indicated that these effects might be mediated by interacting with multiple targets operating in diabetes mellitus (Martha *et al.*, 2014).

Three important flavonoids, (1) 5-deoxyflavone (geraldone), (2) luteolin and (3) Isookanin were isolated from the methanol extract of stem bark of *Albizzia Lebbeck* Benth. and evaluated the efficacy of the isolated flavonoid on *in vitro* models (α-amylase and α-glucosidase enzymes inhibition assay) of type-II diabetes. Furthermore, the results of *in vitro* studies revealed to inhibit the α-glucosidase and α-amylase enzymes, correlating its capability to reduce plasma blood glucose. The isolated flavonoids were then structurally elucidated with the assist of 1H-NMR, 13 C-NMR, and MS. Molecular docking study was performed with GLIDE docking

software study radically corroborates the binding affinity and inhibition of α-glucosidase and α-amylase enzymes. The article demonstrates the anti-diabetic and antioxidant activity of the important isolated flavonoids with inhibition of α-glucosidase, α-amylase and DPPH which is further supported by molecular docking analysis (Ahmed *et al.,* 2014).

*2.3.3. In vitro models for assessing antidiabetic activity of a plant*

*In vitro* tests is based on a specific biological process relevant to the disease and its treatment and can play an important role in the evaluation of antidiabetic or other medicinal plants, as initial screening tools or as follow-up to human or animal studies (Soumyanath and Srijayanta, 2005). There are five different models for studying antidiabetic activity of plant extracts.

Models to study inhibition of carbohydrate-digesting enzymes – Alpha amylase and Alpha-glucosidase are the carbohydrate digesting enzymes involved in the breakdown of α-linkages of oligosaccharides and disaccharides into glucose (Rhabasa–Lhoret and Chiasson, 2004).

Models to study inhibition of intestinal glucose uptake – Can be arranged into two main groups: models prepared from whole small intestine and those prepared from isolated cells or cellular components (Wood and Lawrence, 1991).

Models to study insulin secretion from β-cells of the pancreas – A number of *in vitro* models have been developed for studying the pancreatic secretion of insulin. These include the perfused pancreas, intact isolated islets, purified β-cells, and insulin-secreting cell lines. Insulin released is measured by radioimmunoassay (using 125I-labeled insulin) or enzyme- linked immunoassay. The most widely used β-cell lines are RINm5F, HIT-T15, βTC, MIN6, INS-1, and BRIN-BD11 cells (Poitout *et al.,* 1996). Models based on insulin target tissue – Insulin target tissue includes muscles, adipocytes, liver and kidney. The uptake and utilization of glucose into the tissues is under influence of insulin. Abdominal muscle taken from mice has been used to study the effect of a number of plant extracts. Models based on study of interactions with the insulin receptor – An approach to find agents capable of interacting directly with the insulin receptor, thereby acting as an insulin-mimetic in a variety of biochemical cascades (Soumyanath and Srijayanta, 2005).

A synthesized imidazoline compound, S-22068, known for its antidiabetic effect *in vivo* was investigated for its insulin releasing capacity and the cellular mode of action. The authors used MIN6 cell line derived from *in vivo* immortalized insulin-secreting pancreatic β cells for the study. The results were elaborated by stating that S-22068 was able to release insulin from MIN6 cells in a dose dependant manner with half-maximal stimulation at 100 µM. Its efficacy (8 fold over the basal value), which did not differ whatever the glucose concentration, was intermediate between that of sulphonylurea and that of efaroxan. Similarly to sulphonylureas and classical imidazolines, S-22068 blocked $K_{ATP}$ channels and, in turn, opened nifedipine-sensitive voltage-dependent $Ca^{2+}$ channels, triggering $Ca^{2+}$ entry. Similarly to other imidazolines, S-22068 induced a closure of cloned $K_{ATP}$ channels injected to *Xenopus* oocytes by interacting with the pore-forming Kir6.2 moiety. S-22068 did not interact with the sulphonylurea binding site nor with the non-$I_1$ and non-$I_2$ imidazoline site evidenced in the β cells that is recognized by the imidazoline compounds efaroxan, phentolamine and RX821002. The authors concluded that S-22068 is a novel imidazoline compound which stimulates insulin release *via* interaction with an original site present on the Kir6.2 moiety of the β cell $K_{ATP}$ channels (Brigand *et al.*, 1999).

The evaluation of the antidiabetic potential of selected medicinal plant used to treat symptoms of diabetes was carried out through *in vitro* screening test. Through the ethnobotanical survey, the authors identified 9 species, and the crude extracts of this plants were screened for (i) potentiation of basal and insulin-stimulated glucose uptake by skeletal muscle cells (C2C12) and adipocytes (3T3-L1); (ii) potentiation of glucose-stimulated insulin secretion by pancreatic beta cells (betaTC); (iii) potentiation of adipogenesis in 3T3-L1 cells; (iv) protection against glucose toxicity and glucose deprivation in PC12-AC neuronal precursor cells; and (v) diphenylpicrylhydrazyl (DPPH) oxygen free radical scavenging. Four species were reported that it can induce basal glucose uptake in muscle cells or adipocytes, one species being as potent as metformin. The ability of the four species to accelerate adipogenesis was indicated with a potency roughly half that of rosiglitazone. Furthermore it was reported that five species protected PC12-AC cells against glucose toxicity and four protected against glucose deprivation. Antioxidant activity of the five species comparable to ascorbic acid was described; however no species that can

increase insulin secretion was reported. The authors revealed that *Gaultheria hispidula, Rhododendron tomentosum,* and *Vaccinium vitisidaea* exhibit a promising profile of antidiabetic potential and are good candidates for more depth evaluation (Harbilas *et al.,* 2009).

The effectiveness of *Sarcopoterium Spinosum* (L.) extract as an antidiabetic agent was ascertained through both *in vitro* and *in vivo* investigation. Authors determined the effect of the extract on insulin secretion test by using RIN-m pancreatic beta-cells and was reported that it can effectively induced insulin secretion. The extract was verified for its inhibitory potential on lipolysis in 3T3-L1 adipocytes. Also extract's ability to induce glucose uptake in 3T3-L1 adipocytes, in AML-12 hepatocytes and L6 myotubes was reported. Furthermore, the authors studied *in vivo* test using KK-A(y) mice, and IPGTT was also determined. The extract's preventive effect on the progression of diabetes was verified. In addition, catechin and epicatechin were detected in the extract using hyphenated LC-MS/MS. Based on the effect of the extracts on specific physiological functions, both *in vitro* and *in vivo* the authors claimed the antidiabetic activity of the extract (Smirin *et al.,* 2010).

The possible mechanisms of antidiabetic activity of methanol extract of aerial parts of *Phyllanthus niruri* were investigated. The authors studied the effect of the extract on glucose absorption and storage in diabetes to elucidate the mechanisms of blood lowering and glycaemia control in diabetes. In the study, effects of the extracts on the glucose mobilization and storage were assessed using the weight and glycogen content of the liver isolated from treated diabetic rats, while *in vitro* inhibition of α-amylase and α-glucosidase enzyme activities were used as indices of effect on glucose absorption. The results were documented that the extract lowered blood glucose, suppressed postprandial rise in blood glucose following a glucose meal, reduced haemoglobin glycation and increased absolute and relative weights as well as glycogen content of liver in diabetic rat. In *in vitro* test it was revealed that the extract inhibited α-amylase ($IC_{50}$: $2.15 \pm 0.1$ mg/mL) and α-glucosidase ($IC_{50}$: $0.2 \pm 0.02$ mg/mL) activities. Based on the findings the authors suggested that aerial parts of *P. niruri* may owe their blood glucose lowering properties to inhibition of glucose absorption and enhancement of glucose storage (Okoli *et al.,* 2011).

The pancreatic and extrapancreatic effects of crude aqueous extracts (AEs) of *Achillea santolina* L, *Eryngium creticum* Lam, and *Pistacia atlantica* Desf were

investigated. Bioassays of β-cell proliferation and insulin secretion as well as glucose diffusion were used in the study. Similar to L-alanine insulinotropic efficacy in MIN6 β-cell, glucose-stimulated insulin secretion was reported to be potentiated by AEs of *Eryngium creticum* (0.01 mg/ml) and *Pistacia atlantica* (0.01, 0.1 and 0.5 mg/ml). *Achillea santolina* was however reported to be ineffective. Comparable to glucagon-like peptid-1-enhanced β=cell proliferation in 2-day treatment wells, a dose dependent augmentation of bromodeoxyuridine incorporation was reported with the *Achillea santolina* AE (0.05-1mg/ml), and *Eryngium creticum* AE (0.1, 0.5 and 1mg/ml). *Pistacia atlantica* concentrations were indicated as lacked of pancreatic proliferative capacity. The reported findings signify the *in vitro* diverse therapeutic antidiabetes properties of the selected medicinal plants (Kasabri, 2012a).

Aqueous and methanol extracts of the bark of *Sclerocarya birrea* and *Ziziphus mucronata* were established for its *in vitro* antidiabetic properties. Both the extracts of the two plants were reported to have the ability to inhibit α-amylase and α-glucosidase activities. The inhibitory activities of both the extracts were confirmed to be dose dependent manner, with results being comparable to acarbose. Furthermore the authors evaluated the glucose uptake of the extracts in C2C12 myotubes, 3T3-L1 adipocytes and HepG2 hepatocarcinoma cells, and insulin secretion in RIN-m5F rat pancreatic beta-cells. The glucose uptake was reported to be significantly increased in C2C12, 3T3-L1 and HepG2 cells by the extracts; however, insulin secretion from RIN-5F cells was not altered. The authors also determined total polyphenolic content and antioxidant activity and have mentioned the strongest free radical scavenging capacity of methanol extracts of *S. birrea.* Based on the study the authors provided the evidence that the plants possess *in vitro* antidiabetic properties (Mousunho *et al.*, 2013).

Bhutkar and Bhise verified the antidiabetic potential of stem bark of *Albizzia lebbeck (A. lebbeck)* and seeds of *Mucuna pruriens (M. pruriens)* using various *in vitro* techniques. In the article the plant extracts were studied for their effects on glucose adsorbtion, diffusion amylolysis kinetics and glucose transport across yeast cells. The potential of the plant extracts to absorbed glucose in concentration dependent manner was reported. It was further detailed that no significant ($p \leq 0.05$) differences were observed between the adsorption capacities of *A. lebbeck* and *M. pruriens.* In the amylolysis kinetic experimental model the rate of glucose diffusion was reported to

increase with time from 30 to 120 min, and both the plant extracts demonstrated significant inhibitory effects on movement of glucose into external solutions across dialysis membrane as compared to control. A significantly higher ($p \leq 0.05$) retardation of glucose diffusion by *A. lebbeck* than *M. pruriens* was specified. *M. pruriens* was reported to exhibit significantly higher glucose uptake ability than the extracts of *A. lebbeck.* Additionally, the authors argued that the hypoglycaemic activity exhibited by the extracts was mediated by increasing glucose absorption, by decreasing glucose diffusion rate and at the cellular level by promoting glucose transport across the cell membrane (Bhutkar and Bhise, 2013).

The α-glucosidase inhibition, an antioxidant and antibacterial activity of *Polygonum senegalensis* leaves and *Pseudocedrela kotschyi* root was established. Hydro-alcoholic (50%) extracts were analyzed for their phytochemical content and examined for their inhibition potency on α-glucosidase. Further the assessment of antioxidant activity was achieved through DPPH, ORAC, FRAP and DCFH-DA (call based) assay. The authors finally evaluated the antibacterial activity using four Gram-positive Cocci (*Bacillus subtilis, Clostridium difficile, Enterococcus faecalis, Staphylococcus aureus),* three Gram-negative bacilli (*Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia),* and the yeast *Candida albicans.* The study reported a significant α-glucosidase inhibitory and antioxidant activities of the extracts. The results were further detailed stating that *Polygonum senegalensis* leaf extracts were the most active in *in vitro* assay with an IC50=1.5µg/ml for α-glucosidase inhibition and an IC50=6.8µg/ml for DPPH scavenging, -4.5 µmol Fe II/g of dry matter -9366 µmol Trolox/g DW – for FRAP and ORAC values, respectively and IC50 = 2.3 µgGA/ml for DCFH-DA assay. Considering the above results the authors concluded that the semi alcoholic extract of the two studied plants possess α-glucosidase inhibitory activity, antioxidant potency, and low antibacterial effect (Bothon *et al.,* 2013).

The antidiabetic activity of the long been used folk medicine *Awertia kouitchensis* was reaffirmed via *in vitro* antidiabetic activity test model like; test for its inhibitory activity on α-amylase and α-glucosidase, and insulin secretion test in NIT-1 cell line. A remarkable potential of the *Awertia kouitchensis* extract to inhibit free radicals, and α-amylase and α-glucosidase was described. Also the extract's ability to stimulate insulin secretion *in vitro* was also well indicated. Based on the *in vivo* test results the

authors highlighted the antihyperglycemic activity, antioxidant capacity, antihyperlipidemic activity of the plant (Wan *et al.*, 2013).

The antidiabetic activity of the leaves, stem bark and roots of West Africa traditional plant *Anthocleista djalonensis* and *Anthocleista vogelii* was revalidated through *in vitro* α-amylase inhibitory assay and *in vivo* investigations. The authors partitioned the crude methanol extract into hexane and ethyl acetate fractions which were tested *in vitro* and *in vivo.* The leaves and stem bark crude methanol extract of *Anthocleista djalonensis* was reported to give comparable α-amylase inhibition of 73.66% and 72.90% respectively which were higher than the 39.93% and 22.90% of the same plant parts given by the *Anthocleista vogelii.* The authors recorded a significant peak blood glucose reduction by the crude stem bark extract of *Anthocleista djalonensis* which was higher than the leaves or roots. The stem bark ethyl acetate fraction of *Anthocleista djalonensis* also was recorded to have the ability to reduce blood glucose *in vivo.* The article furthermore outlined the α-amylase inhibitory activity and antidiabetic activity of *Anthocleista djalonensis in vivo* suggesting that they might contain active principles for the management of the diabetes (Olubomehin *et al.*, 2013).

The eight linear furanocoumarins, pabulenol, oxypeucedanin methanolate, oxypeucedanin hydrate and 3-O-glucopyranosyl-β-sitosterol, and the crude extract were investigated for antidiabetic activity and were confirmed to have hypoglycaemic, hypolipidemic, and antioxidant effect as well as ameliorating kidney function. The authors performed preliminary *in vitro* evaluation of the antidiabetic activity of the extract and isolates using α-amylase, α-glucosidase, and α-galactosidase. The *in vivo* activity was investigated by measuring some oxidative stress markers. The blood glucose level, liver function enzymes, total protein, lipid, and cholesterol levels were reported to have significantly normalized by extract treatment. Further revealed the significantly ameliorated antioxidant markers, glucolytic and gluconeogenic enzymes. The elevated level of kidney biomarkers in the treated diabetic groups was also described. The highest potent inhibiting power of the imperatorin and 5-methoxypsoralen was highlighted (Shalaby *et al.*, 2014).

Antidiabetic activity of extracts of purchased plants, *Senna Alexandria* Mill. (Fabaceae), *Cymbopogon citrates* Stapf. (Poaceae), *Cucurbita pepo* L. (Cucuribitaceae), *Nuxia floribunda* Benth. (Stilbaceae), *Hypoxis hemerocallidea*

Fisch. and Mey (Hypoxidaceae), and *Cinnamomum cassia* Blume (Lauraceae) were evaluated and established for their *in vitro* antidiabetic activity using *in vitro* α-amylase and α-glucosidase inhibitory test as well as Islets of Langerhans excretory activity. The findings were detailed indicating the highest activity shown by the hexane extract of *S. alexandrina* (EC50=0.083 mg/ml), ethyl acetate extract of *H. hemerocallidea* (EC50=0.29mg/ml), and methanol extracts of *Cymbopogon citrates* (EC50 = 0.31 mg/ml) and *Cinnamomum cassia* (EC50=0.50=0.12 mg/ml). A good α-glucosidase inhibitory activity (>50%) was reported with the exception of some methanol (*Cinnamomum cassia, N. floribunda, Cymbopogon citrates*) and acetone extracts (*Cucurbita pepo and N. floribunda*). Only the *H. hemerocallidea* acetone extract was reported to have an insulin stimulatory effect (2.5 U/ml at 8 µg/ml) (Boaduo *et al.,* 2014).

The antioxidant and antidiabetic activity of biomodified alkali extracted from a deciduous plant *Acacia nilotica* have evaluated *in vitro.* The authors subjected the extracted alkali lignin to microbial biotransformation by ligninolytic fungus *Aspergillus flavus* and *Emericella nidulans* under varying concentration of carbon to nitrogen sources and further compared the structural feature of the modified lignin samples by FTIR, functional group analysis and (13)C solid state NMR. Modifications in all lignin samples were reported to have correlation with their antioxidant scavenging activity and reducing power. The antidiabetic properties were evaluated in terms of *in vitro* glucose movement inhibition and α-amylase inhibition assay. The ability of the modified samples to increase glucose binding efficiency were revealed as demonstrated by the decreased glucose diffusion (55.5-76.3%) and 1.16-1.18-fold enhanced α-amylase inhibition in comparison to their control samples. The authors concluded that the structure and functional modifications in lignin significantly affects its bioefficacy in term of antioxidant and antidiabetic activities (Barapatre *et al.,* 2015).

The antidiabetic, insulin secretogogue activities and the mechanisms involved in it, of an edible fruit from traditional medicinal plant *Capparis zeylanica* (CZ) are well elaborated in a study. Oral administration of CZ extract (CZME) (200mg/kg body weight) for 28 days was reported to be significantly effective in reducing the blood glucose levels by 35.53% and enhanced circulating insulin levels by 81.82% than the diabetic control rats. The insulin secretagogue activity mechanisms of the extract were

evaluated by using mouse insulinoma beta cell line (MIN 6). The study reported the ability of the extract to stimulate insulin release in a dependent manner of glucose concentration (3-16.7mM) and extract dose (5-500µg/ml). The insulin releasing effect of the extract was further studied deeply and was revealed that the insulin releasing effect of the extract was significantly enhanced by 3-isobutyl-1-methyl xanthine, glibenclamide, elevated extracellular calcium and $K^+$ depolarized media. The insulin released was reported to be significantly reduced in calcium blocking conditions (by nifedipine and EGTA), in the presence of potassium channel opener (diazoxide). With good investigation and better reasoning the authors argued that antidiabetic activity if CZME might be a result of its stimulating effect on insulin release from pancreatic beta cells via KATP channel dependent and independent ways (Balekari *et al.,* 2015).

The antidiabetic activity of *Gymnema sylvestre* (Roxb.), which is believed to work by regeneration of pancreatic beta cells, was reaffirmed. The authors investigated the effects of *G. Sylvestre* leaves crude aqueous extracts (AEs), on the pancreatic β-cell MIN6 proliferation and insulin secretion. The results were analyzed and reported that *G. Sylvestre* AE concentrations (0.01 and 0.1 mg/ml) induced MIN6 monolayers expansion by respective 130.3% and 127.4% ($p<0.001$ vs. Spontaneous control) comparable to GLP-1 (500nM) pancreatic proliferative capacity. Like L-alanine (10nM) insulinotropic efficacy and without exerting cytotoxicity, glucose-stimulated insulin secretion was revealed to be potentiated by *G. Sylvestre* AEs (5.10 and 25 mg/ml) (711.0%, 843.0% and 906.5%, respectively, $p<0.001$ vs. basal control). The potent plants' insulin secretory bioactivities were abolished in the depleted $Ca^{2+}$ conditions ($p<0.001$), similar to or list at antilipolytic efficacy, pancreatic lipase IC50 value for *G. Sylvestre* AEs was 106.3±7.2 µg.ml. Unlike acarbose (100µg/ml) dual inhibition of α-amylase/α-glucosidase, *G. Sylvestre* AE was reported to be inactive at used doses. Dissimilar to guar gum (50mg/ml) diffusional hindrance in a simple dialysis model, *G. Sylvestre* AEs (10. 25 and 50 mg/ml) proved inactive. It was concluded in the study that *G. Sylvestre* AEs augmented β-cell expansion and potentiated glucose-evoked $Ca^{2+}$ regulated insulin secretion; combined with impressive antilipolytic activity (Kasabri *et al.,* 2015).

*2.3.4. Animal models in diabetes research*

"An animal model for biomedical research is one in which normative biology or behaviour can be studied, or in which a spontaneous or induced pathological process

can be investigated, and in which the phenomenon in one or more respects resembles the same phenomenon in humans or other species of animals" (Antonios *et al.*, 2009). Rodents such as rat, mouse, hamster, guinea pigs and the rabbits are suitable models for the study of diabetes (Tripathi and Verma, 2014).

*2.3.4.1. The ethics of animal research*

There are national and international laws which govern the use of animals in research, all of which are based on the principles of the 4Rs – replacement, reduction and refinement and the rehabilitation of the use of animals in research: Replacement- replacement with alternatives like *in vitro* methods, Reduction- by following methods that can reduce number of animals, Refinement- example reducing pain, Rehabilitation- care of animals post experimentation. It is mandatory that all institutions involved in animal research develop and abide by the ethical review processes which promote good animal welfare practices by ensuring that the use of animals at the designated establishment is justified. Each institute involved in animal research should have an ethics committee for monitoring research activities on the animals (Mandal and Parija, 2013).

*2.3.4.2. OECD guidelines for toxicity testing (OECD Test Guidelines 420, 423, 425)*

OECD Test Guidelines is a world-wide recognized standard reference tool for chemical testing or toxicity testing in animals. The guidelines includes the collection of methods used to assess the hazards of chemicals and of chemical preparations, covering tests for physical and chemical properties, effects on human health and wildlife, and accumulation and degradation in the environment. There are three alternative tests for acute oral toxicity, Test Guidelines 420, 423 and 425 (OECD, 2001).

*2.3.4.3. Strain, age and sex of mice*

Many studies have demonstrated the strain-dependent differences in metabolic phenotypes in both wild-type and genetically modified mice (Coleman,1992; Colombo *et al.,* 2003; Kulkarni *et al.,* 2003; Goren *et al.,* 2004). Because of these factors, authors should list the source as well as the strain of animals used for experiments and/or breeding in methods sections. Metabolic experiments should be carried out on age-matched mice. As sex can also influence the phenotype resulting from genetic mutations, it is recommended that studies be conducted on mice from the

same sex. If mice from both sexes are used, investigators should attempt to include equal numbers of mice from each sex and make comparisons within same-sex groups, if sexual dimorphisms exist (Ayala *et al.*, 2010; Macotela *et al.*, 2009).

*2.3.4.4. Factors to consider during metabolic test*

It will not be possible for all metabolic tests to be performed in the exact same manner across different laboratories, as the degree of technical expertise and the availability of equipment and reagents will never be consistent from one laboratory to another. However, certain parameters can be standardized, regardless of where tests are conducted. Guidelines must also be established for the description of methods and presentation of results from metabolic tests. Standardisation of methodologies required basic considerations and ideas like choosing the appropriate test, performing the test(s), and describing methods and results, factors that are inherent to the mouse model(s) being tested, as well as factors related to how tests are conducted (Ayala *et al.*, 2010).

Ambient lighting and time of day: Because mice are nocturnal, many investigators house their mice in rooms in which the light-dark cycle is altered to fit the needs of the experiment (e.g. a reversed light-dark cycle). This enables metabolic tests to be conducted during hours that are convenient for the investigators (daytime) and that also occur during the metabolically active period for mice (dark cycle). Over a 24-hour period, mice, similarly to humans, experience variations in glucose and metabolic hormones, and these can affect interpretation of results. It is therefore important for all experiments in a given study to be carried out at the same time of the day (or night) (Ayala, 2010; Rudic *et al.*, 2004).

Fasting before treatment or chemical induction: In a typical metabolic study, mice are fasted for either 14–18 hours (overnight fast) or for 5–6 hours (morning fast). Overnight fasting provokes a catabolic state in mice, as they primarily consume at night. This metabolic stress is compounded by the fact that mice are typically housed at around 23°C, well below their thermo-neutral temperature of 30°C. Prolonged fasting of mice at sub thermo-neutral temperatures can result in torpor, characterized by a decrease in the metabolic rate (Geiser, 2004; Swoap *et al.*, 2006; Ayala *et al.*, 2006). Overnight fasting nearly depletes liver glycogen stores in the mouse. This has the advantage of reducing variability in baseline blood glucose. However, mice have a unique metabolic response to prolonged fasting that contrasts with the response seen

in humans. Specifically, a prolonged fast impairs insulin-stimulated glucose utilization in humans, but enhances it in normal mice as well as in some strains of transgenic mice. Therefore, overnight fasting is useful for studies where the focus is on glucose utilization (e.g. effects on muscle uptake of glucose). Otherwise, a 5- to 6-hour fast is sufficient to assess insulin action within a more physiological context (Ayala *et al.*, 2006; Heijboer *et al.*, 2005).

Anesthesia: To minimize stress, some investigators perform tests on anesthetized mice. Anesthesia affects heart rate and blood flow and induces hyperglycemia in mice. Therefore, assessment of glucose metabolism in anesthetized mice yields results that are not physiological. Because there are procedural means to minimize stress other than anesthesia, tests of glucose metabolism should not be performed in anesthetized mice and should instead be performed in conscious mice (Ayala *et al.*, 2012).

Blood sampling: The choice of which method to use for blood acquisition depends on a variety of factors, including the skill set available to the investigator, the nature of the test being conducted and the blood volume requirement. It is important that blood sample collection from experimental animals should be least stressful because stress will affect the outcome of the study. Some commonly used blood sample collection methods are-Tail prick method and Tail tip method (for less volume of blood) and Cardiac puncture (for large volume of blood) (Parasuraman *et al.*, 2010).

*2.3.5. Induction of diabetes*

At present time the best and quickest way to induce diabetes is with chemicals, viruses and genetically diabetic animals. And among above said means chemicals are widely used (Tripathi and Verma, 2012). Some of the most commonly used models of type 1 diabetes are outlined in Table.2.2. Chemical induction of diabetes is the most widely used model in the field of diabetic research.

Chemical induction of diabetes [Streptozotocin (STZ) and alloxan]: Diabetes is usually induced around 5–7 days prior to the start of the experiment to ensure stable hyperglycaemia. Two main compounds are used to induce diabetes: streptozotocin (STZ) or alloxan. Due to their similarity in structure to glucose, glucose can compete with alloxan and STZ, and thus, fasting animals tend to be more susceptible. Both alloxan and STZ are relatively unstable, and the solutions should ideally be made immediately prior to injection (King. 2012).

Table.2.2: Summary of rodent models of type 1 diabetes (Adapted from King, 2012).

| Induction mechanism | Model | Main features | Possible uses |
|---|---|---|---|
| Chemical Induction | High dose streptozotocin | Simple model of hyperglycaemia. | New formulations of insulin |
| | Alloxan | | Transplantation models. |
| | Multiple low dose streptozotocin | Model of induced insulitis. | Treatments that may prevent beta cell death |
| Spontaneous autoimmune | NOD mice | Beta cell destruction due to an autoimmune process. | Understanding genetics of type 1 diabetes. |
| | BB rats | | Understanding mechanism of type 1 diabetes. |
| | LEW.1AR1/-iddm rats | | Treatments that may prevent beta cell death. Treatments that may manipulate autoimmune process. |
| Genetically induced | AKITA mice | Beta cell destruction due to ER stress. Insulin dependent. | New formulations of insulin. Transplantation models. Treatments to prevent ER stress. (could also be used in type 2 diabetes research). |
| Virally-induced | Coxsackie B virus Encephalomyocarditis virus Kilham rat virus LCMV under insulin promoter | Beta cell destruction induced by viral infection of beta cells | Establish potential role of viruses in the development of type 1 diabetes. |

Chemically induced diabetes is appropriate to use when testing drugs or therapies where the main mechanism of action is lowering blood glucose in a non-beta-cell-dependent manner; for example to test new formulations of insulin (Jederstrom *et al.*, 2005; Sheshala *et al.*, 2009; King, 2012). At the end of the experiment, any 'cured' animal should be nephrectomized of its graft bearing kidney and reversal to hyperglycaemia observed to rule out regeneration of the endogenous beta cells (Baeyens *et al.*, 2005; Rackham *et al.*, 2011). In addition, the endogenous pancreas can be removed for histological examination for insulin-positive cells or for insulin

content to be measured, although it should be noted that anatomical presence of beta cells is not necessarily correlated to beta cell function (Kargar and Ktorza, 2008).

STZ is the preferred chemical in induction of diabetes due to its following advantages over alloxan: sustained hyperglycemia and the development of well-characterized diabetic complications with a lower incidence of ketosis and mortality (Poretsky, 2010); at concentrations sufficiently high enough to induce the diabetic like state, the toxic effects on the liver and kidneys are diminished; greater specificity (Charles and Howard, 1972).

Multiple low dose injection of streptozotocin: The frequently used single intravenous dose in adult rats to induce IDDM is between 40 and 60 mg/kg b.w., but higher doses are also used. STZ is also efficacious after intraperitoneal administration of a similar or higher dose, but single dose below 40 mg/kg b.w. may be ineffective. For instance, when 50 mg/kg b.w. STZ is injected intravenously to feed rats, blood glucose (determined 2 weeks after treatment) can reach about 15mM (Szkudelski, 2001). Multiple small injections of streptozotocin produce a delayed, progressive increase in plasma glucose in mice within 5-6 days after the injections, in association with pronounced insulitis and less change of death of mice (Rossini *et al.,* 1977). Five daily injections of either 40 or 60 mg/kg STZ induce bone pathologies similar to spontaneously diabetic mouse and rat models and to human T1 diabetic bone pathology. In some models, especially rats, a single dose of STZ is effective at inducing T1 diabetes. In mice, however, multiple low doses (40 mg/kg) are the most effective at maintaining mouse viability and inducing pancreatic dysfunction in part through immune destruction (Motyl and McCabe, 2009).

*2.3.6. In vivo metabolic test*

A test widely used for glucose tolerance classification is the oral glucose tolerance test (OGTT). The OGTT, which for its simplicity, would be a method suitable for large studies, provides information on insulin secretion and action but does not directly yield a measure of insulin sensitivity (Mari *et al.,* 2001). The main reason for performing it is to diagnose impaired glucose tolerance (IGT) or diabetes by virtue of the 2-h value. Both of these are risk factors for cardiovascular disease (CVD) and IGT predicts the development of diabetes (Davidson, 2002). Researchers performed OGTT to check the effect of the antidiabetic plant extracts on glucose tolerance in glucose

induced normal or diabetic mice or both (Sachdewa *et al.,* 2001; Tatar *et al.,* 2012; Kar *et al.,* 1999; Chaturvedi *et al.,* 2004).

In a study, 30 hypoglycaemic herbs were selected from indigenous folk medicines, Ayurvedic, Unani and Siddha systems of medicines. The authors performed oral glucose tolerance tests (GTT) on 16 h fasted albino rats using 1.5 g glucose/kg body weight fed orally and by feeding single dose of the plant sample to study the effect of the same on GTT. For the organic part of the concerned herbal sample, dose selected was 250 mg dried ethanolic extract of the sample per kg body weight and for inorganic 90 mg pure ash/kg bodyweight, suspended in 2% gelatin in warm water as vehicle solution. It was indicated that the herbal samples under study (organic or inorganic parts), which showed definite lower peak blood glucose values 1 h after glucose load, also have given lower values almost always at the end of 3 h readings than corresponding fasting or 2 h readings indicating more pronounced hypoglycemic activities of the concerned samples. It was also reported that inorganic parts (consisting of mineral elements) of some plant samples, sometimes showed more pronounced action of glucose tolerance factor than their corresponding organic parts, these are, *Cinnamoumum tamala* (leaves), *Eugenia jambolana* (seed), *Moringa oleifera* (stembark), *Mucuna prurita* (seed), *Momordica charantia* (fruit), *Tinospora cordifolia* (stem), *Trigonella foenum-graecum* (seed), etc. (Kar *et al.,* 1999).

In a study, various insulin sensitivity indices derived from the OGTT with whole-body insulin sensitivity measured by the euglycemic insulin clamp technique were compared. 153 subjects (66 men and 87 women, aged 18–71 years, BMI 20–65 kg/m2) with varying degrees of glucose tolerance (62 subjects with normal glucose tolerance, 31 subjects with impaired glucose tolerance, and 60 subjects with type 2 diabetes) were studied. After a 10-h overnight fast, all subjects underwent, a 75-g OGTT and a euglycaemic insulin clamp, performed with the infusion of [3-3H] glucose. The authors compared the indices of insulin sensitivity derived from OGTT data and the euglycaemic insulin clamp by correlation analysis. Further it was indicated in the article that the mean plasma glucose concentration divided by the mean plasma insulin concentration during the OGTT displayed no correlation with the rate of whole-body glucose disposal during the euglycaemic insulin clamp (r = 20.02, NS). From the OGTT, they developed an index of whole-body insulin sensitivity (10,000/square root of [fasting glucose 3 fasting insulin] 3[mean glucose 3mean

insulin during OGTT]), which is highly correlated (r = 0.73, P<0.0001) with the rate of whole-body glucose disposal during the euglycaemic insulin clamp. They have derived a novel estimate of insulin sensitivity that is simple to calculate and provides a reasonable approximation of whole-body insulin sensitivity from the OGTT (Matsuda *et al.,* 1999).

The optimal conditions under which to assess glucose tolerance in chow- and high-fat-fed C57BL/6J mice were determined. Authors fed the mice either chow or high-fat diet for 8 wk. Variables tested in the study were fasting duration (0-, 3-, 6-, and 24-h and overnight fasting), route of administration (intra-peritoneal vs. oral) load of glucose given (2, 1, or 0.5 g/kg and fixed 50-mg dose), and state of consciousness. Basal glucose concentrations were reported to be increased in high-fat- compared with chow-fed mice following 6 hour fasting (9.1+/- 0.3 vs. 7.9+/-0.4 mmol/l P=0.01). Glucose tolerance was most different and therefore significant (P = 0.001) in high-fat-fed mice after 6 hour fasting (1,973+/-96 vs. 1,248+/-83 mmol.l (-1). 120 min (-1). The difference in glucose tolerance was greater following an OGTT (142%), in contrast to an IPGTT, with a 127% difference between high fat and chow. Also reported that administering 2 g/kg of glucose resulted in a greater level of significance (P=0.0008), in glucose intolerance in high-fat-compared with chow-fed mice. A fixed dose of 50 mg glucose regardless of body weight was enough to show glucose intolerance in high-fat- vs. chow-fed mice. Finally reported that high-fat-fed mice showed glucose intolerance compared with their chow-fed counterparts whether they were tested under conscious or anesthetized conditions. The authors thus conclude that 2 g/kg glucose administered orally following 6h of fasting is best to assess glucose tolerance in mice under these conditions (Andrikopoulos *et al.,* 2008).

Aslam *et al.* (2009) investigated the hypoglycaemic effects of petroleum ether, chloroform and ethyl acetate fractions isolated from ethanolic extracts of *Coccinia cordifolia* and *Catharanthus roseus* on normal control and orally glucose-induced hyperglycaemic rats. Single doses (150 mg/kg) of different fractions of *C. cordifolia* and *C. roseus* extracts were intraperitoneally administered. The serum blood glucose level was found recorded at time 0, 30, 60, 90, 150 and 270 minutes. The interpreted result of the study was that in the orally glucose induced hyperglycemic rats, chloroform-coccinia (CHCl3-CC) fraction showed maximum reduction of blood glucose level by 21.94% on 60 minute of the experiment. On the other hand

maximum reduction ($p<0.05$) of 17.92% was reported for petroleum ether-catharanthus (PET-CR) on 30 minute of the experiment. It was further interpreted that the CHCl3-CC fraction is relatively more potent than other fractions of *C. cordifolia* and PET-CR better than other fractions of *Catharanthus*. Phytochemical screening test results reported that chloroform fraction of *C. cordifolia* contain saponins and flavonoids compounds, which are known to be hypoglycaemic and petroleum ether fraction of *C. roseus* contains tannins, flavonoids and alkaloid compounds which produced varying degree of blood sugar reduction. On the pharmacological point of view the authors declared *C. cordifolia* and *C. roseus* as a valuable plant, which can be useful, at least as an adjunct, in the therapy of diabetes (Islam *et al.*, 2009).

Sharma *et al.* (2012) investigate the antidiabetic and antioxidant potential of the powdered corm of *Stephania hernandifolia.* The investigation was in normal and Streptozotocin (STZ)-induced diabetic rats, using oral administration of ethanol and an aqueous extract (400 mg/kg body weight) of *Stephania hernandifolia* corm. After the oral administration of water and ethanol extracts at doses of 400 mg/kg body weight, blood glucose levels were monitored at specific intervals and it was reported that they were significant lowered. Glibenclamide was used as a standard drug at a dose of 0.25 mg/kg. The experimental data detailed that both extracts has significant antihyperglycemic and antioxidant activity in Streptozotocin-induced rats compared to the standard drug (Sharma *et al.*, 2010).

The antidiabetic effect of *Nigella sativa* seed ethanol extract (NSE) was evaluated in Meriones shawi after development of diabetes. The authors divided Meriones shawi randomly into four groups: normal control, diabetic control, diabetic treated with NSE (2 g eq plant/kg) or with metformin (300 mg/kg) positive control, both administered by daily intragastric gavage for 4 weeks. Glycaemia and body weight were evaluated weekly. At study's end, they performed an Oral Glucose Tolerance Test (OGTT) to estimate insulin sensitivity. Plasma lipid profile, insulin, leptin, and adiponectin levels were assessed. ACC phosphorylation and Glut4 protein content were determined in liver and skeletal muscle. NSE animals confirmed a progressive normalization of glycaemia, albeit slower than that of metformin controls. Moreover, they additionally claimed that NSE increased insulinemia and HDL-cholesterol, compared to diabetic controls. Leptin and adiponectin were reported to be unchanged. It was further reported that NSE treatment decreased OGTT and tended to decrease liver and muscle

triglyceride content. NSE stimulated muscle and liver ACC phosphorylation and increased muscle Glut4. More significantly, their data suggested that *in vivo* treatment with NSE exerts an insulin-sensitizing action by enhancing ACC phosphorylation, a major component of the insulin-independent AMPK signaling pathway, and by enhancing muscle Glut4 expression (Benhaddou-Andaloussi *et al.,* 2011).

*Elaeodendron glaucum* Pers. (Family/ Genus: Celastraceae; Hindi Jamrassi, bakra; ED) is a medium sized tree which have hypoglycemic properties. Total phenolic content of methanolic extract of ED (MED) was standardized and antidiabetic potential of the plant was affirmed. Inbreed adult male Charles-Foster (CF) albino rats were used in the experiment for hypoglycemic activity in oral glucose tolerance test (OGTT) and normoglycemic rats, and antidiabetic activity in alloxan induced rats. Preliminary phytochemical screening revealed that MED showed positive response to alkaloids, saponins and triterpenes, tannins, flavonoids, carbohydrates and sterols. Total phenolic content (285.2 mg/g) determined in the MED was reported. Results detailed showed that the continuous post-treatment for 21 days with the MED showed potential hypoglycemic activity in OGTT and normoglycemic rats and antidiabetic activity in alloxan induced rat models. Further, the authors suggested the need for isolation and establishment of exact mechanism of action of specific compound from MED (Lanjhiyana *et al*., 2011).

The antidiabetic potential of *Sorbus decora* was validated. The authors, in the investigation, used *in vivo* models of insulin resistance and diabetes, notably the streptozotocin Type 1 diabetic rat (STZ), the genetic KK-$A^y$ Type 2 diabetic mouse and the rat rendered insulin resistant with 10% glucose water consumption for 6 weeks. *Sorbus decora* ethanolic crude extract (SDEE) was administered orally (200 mg /kg) and compared to metformin (150 or 500 mg/kg). The results were detailed by stating that the intragastric (i.g.) gavage of SDEE transiently decreased glycemia in STZ rats in a bi-phasic manner but the effect was cumulative over several days. In mice KK-$A^y$, SDEE incorporated in food (0.12%), decreased glycemia by 15% within 1 week as compared to vehicle controls. In pre-diabetic insulin-resistant rats, SDEE fed daily by i.g. gavage for 2 weeks was reported to significantly decrease hyperglycemia and hyperinsulinemia, without affecting sugar water intake. Using the HOMA insulin resistance parameter, the effect of SDEE was equivalent to that of metformin. The authors concluded the article thereby revealing both anti-

hyperglycemic and insulin-sensitizing activity *in vivo* of ethanolic crude extract of *S. decora* (Vianna *et al.*, 2011).

Ramakrishna *et al.* (2011) evaluated antidiabetic activity of ethanolic extract of *Triumfetta Pilosa* Roth was evaluated for *in-vivo* hypoglycemic activity using Streptozotocin induced diabetic rats. Ethanol extract had shown significant protection and lowered the blood glucose levels when compared to normal in glucose tolerance test. In the acute toxicity study, the authors couldn't notice behavioural changes seen up to 4hrs and no mortality was observed up to the end of 48hrs even at the maximum tested dose level of 2000 mg/kg per oral (it is considered as LD50). Thus, 1/10th of LD50 is taken as the effective dose (100mg/kg and 200 mg/kg b.w) for the study. Total cholesterol levels, total triglyceride levels, serum urea, serum creatinine, serum insulin levels, HDL, LDL & VLDL levels were estimated after 21days of treatment and was reported that the treatment has led to significant decrease in serum urea, serum creatinine, Serum Total Cholesterol, Serum Total Triglycerides, LDL and VLDL levels, while it increased HDL levels in diabetic rats. Reported degeneration's in proximal tubular epithelial cells in the cortex of kidneys, hemorrhage in the interstitial area and periglomerular lympolytic infiltration and hyalinization of the arterioles after induction of diabetes and was reduced after feeding with ethanolic extract of *Triumfetta Pilosa* (Ramakrishna *et al.*, 2011).

The leaves of *Caylusea abyssinica* (fresen.), used for management of diabetes mellitus, has been scientifically validated. The work design of the study was that male Animals were randomly divided into five groups for each diabetic, normoglycemic and oral glucose tolerance test (OGTT) studies. Group 1 served as controls and administered 2% Tween-80 in distilled water, (TW80); Group 2 received 5 mg/kg glibenclamide (GL5); Groups 3, 4 and 5 were given 100 (CA100), 200 (CA200) and 300 (CA300) mg/kg, respectively, of the hydroalcoholic extract of *C. abyssinica*. The blood glucose level (BGL) was determined at different time points. The results were documented are, in normal mice, CA200 and GL5 induced hypoglycemia starting from the 2nd h but the hypoglycemic effect of CA300 was delayed and appeared at the 4th h ($p < 0.05$ in all cases), in diabetic mice, BGL was significantly reduced by CA100 ($p < 0.05$) and CA300 ($p < 0.01$) starting from the 3rd h, whereas CA200 ($p < 0.001$) and GL5 ($p < 0.05$) attained this effect as early as the 2nd h. In OGTT, TW80 ($p < 0.01$) and CA100 ($p < 0.01$) brought down BGL significantly at 120 min, while

CA200 ($p < 0.001$) and GL5 ($p < 0.001$) achieved this effect at 60 min indicating the oral glucose load improving activity of the extract. By contrast, CA300 was reported to have no effect on OGTT. Acute toxicity study revealed the safety of the extract even at a dose of 2000 mg/kg. Preliminary phytochemical study demonstrated the presence of various secondary metabolites, including, among others, saponins, flavonoids and alkaloids. The results indicated that *C. abyssinica* is endowed with antidiabetic and oral glucose tolerance improving actions, particularly at the dose of 200 mg/kg in experimental animals. Further the authors claimed that the plant extract might be related to the presence of secondary metabolites implicated in antidiabetic activities of plant extracts via different hepatic and extra-hepatic mechanisms and thus support the traditional use of the leaf extract for the management of diabetes mellitus (Tamiru *et al.*, 2012).

The efficacy of the leaf extracts of *Ocimum gratissimum* in type-2 model diabetic rats was established. The results recorded were that the extracts and reference hypoglycaemic drug (glibenclamide) showed hypoglycaemic effect in the OGT test in normal and n-STZ-diabetic rats. Their findings showed that aqueous extract of *O. gratissimum* leaf can significantly reduce postprandial hyperglycaemia in type-2 diabetic model rats, but without the risk of hypoglycemia. The authors further explored the useful of *O. gratissimum* in type-2 diabetes human subjects with insulin resistance who are prone to high postprandial glucose surge (Oguanobi *et al.*, 2012).

Antihyperglycemic and antioxidant potential of oil of seeds of *Brassica nigra* (BNO) was studied and documented. The study was carried out in streptozotocin-nicotinamide (STZ). BNO was orally administered to diabetic rats to study its effect in both acute and chronic antihyperglycemic study. The body weight, oral glucose tolerance test and biochemical parameters viz. glucose level, insulin level, liver glycogen content, glycosylated hemoglobin and antioxidant parameters were estimated for all treated groups and was found compared against diabetic control group. Administration of BNO at a dose 500 mg/kg and 1000 mg/kg body weight p.o. to STZ diabetic rats reported a reduction in blood glucose level from 335 mg/dl to 280 mg/dl at 4th h and from 330 mg/dl to 265 mg/dl respectively which was found significant ($p<0.01$) as compared with diabetic control. BNO (500 mg/kg and 1000 mg/kg) and glibenclamide (0.6 mg/kg) in respective groups of diabetic animals administered for 28 days reduced the blood glucose level in streptozotocin-

nicotinamide induced diabetic rats. Authors specified a significant increase in body weight, liver glycogen content, plasma insulin level and decrease in glycosylated hemoglobin in test groups as compared to control group. *In vivo* antioxidant studies on STZ-nicotinamide induced diabetic rat's revealed decreased malondialdehyde (MDA) and increased reduced glutathione (GSH) (Kumar *et al.*, 2013).

In an article various extracts of flowers of *Cassia fistula* Linn (Leguminosae) such as petroleum ether (60-80°), chloroform, acetone, ethanol, aqueous, and crude aqueous extracts and two fractions of ethanol extract were tested for antihyperglycemic activity in glucose-overloaded hyperglycemic rats. The effective antihyperglycemic extracts and fraction were tested for their hypoglycemic activity at two dose levels, 200 and 400 mg/kg, respectively. To confirm their utility in higher models, authors subjected fraction of *C. fistula* to antidiabetic study in an alloxan-induced diabetic model at two dose levels, 200 and 400 mg/kg, respectively. Biochemical parameters like glucose, urea, creatinine, serum cholesterol, serum triglyceride, high-density lipoprotein, low-density lipoprotein, hemoglobin, and glycosylated hemoglobin were assessed in experimental animals. The petroleum ether and ethanol extracts of *C. fistula* and the water-soluble fraction of ethanol extract were confirmed that it can exhibit significant antihyperglycemic activity. The result was further analyzed indicating that the extracts, at the given doses, did not produce hypoglycemia in fasted normal rats, and the fraction exhibited weak hypoglycemic effect after 2 h of the treatment. Treatment of diabetic rats with ethanol extract and water-soluble fraction of the plant restored the elevated biochemical parameters significantly ($P<0.05$) to the normal level. No activity was reported in the petroleum ether extract of the plant. More effective property of water-soluble fraction of ethanol extract was highlighted which is even comparable with that of the standard, glibenclamide (5 mg/kg) (Jarald *et al.*, 2013).

Mishra *et al.* (2013) conducted hypoglycaemic activity of methanol extract of *Scoparia dulcis* on both *in vitro* and *in vivo* models along with determination of total extractable polyphenol. Methanol extract of *Scoparia dulcis* contains 4.9% and water extract contains 3.2% of total extractable polyphenol. The antioxidant activity was reported as very promising result in both the tested methods that is 2,2-diphenyl-1-picrylhydrazyl and ferric ion reducing capacity. Authors suggest a direct correlation of antioxidant activity to the antidiabetic potential of drug. The two enzymes (amylase

and glycosidase) found in intestine are responsible for the increasing postprandial glucose in body. *In vitro* model was performed on these enzymes and the results indicated that methanol extract of *Scoparia dulcis* was effective to check the postprandial glucose level. *In vivo* hypoglycaemic activity of methanol extract of *Scoparia dulcis* was performed on streptozotocin-induced diabetes mellitus showed significant inhibition of blood glucose level as compared to control and similar to that of standard glibenclamide. The overall data potentiates the traditional value of *Scoparia dulcis* as an antidiabetic drug (Mishra *et al.*, 2013).

Aqueous extract of *Terminalia paniculata* bark (AETPB) was investigated for its antidiabetic activity; both *in vivo* and *in vitro.* Possible phytoconstituents responsible for the actions were characterized. *In vivo* study was found conducted in streptozotocin-nicotinamide (65mg/kg–110mg/kg; i.p.) induced diabetic rats. The mentioned article reported that oral treatment of AETPB using rat oral needle at 100 and 200mg/kg doses significantly ($p< 0.001$) decreased blood glucose and glycosylated haemoglobin levels in diabetic rats than diabetic control rats. AETPB-treated diabetic rats body weight, total protein, insulin, and haemoglobin levels were further reported to be increased significantly ($p< 0.001$) than diabetic control rats. A significant ($p< 0.001$) reduction of total cholesterol and triglycerides and increase in high-density lipoprotein levels were reported in type 2 diabetic rats after AETPB administration. HPLC analysis confirmed the presence of biomarkers gallic acid, ellagic acid, catechin, and epicatechin in AETPB. AETPB and gallic acid notably indicated significant ($p< 0.001$) enhancement of glucose uptake action in presence of insulin in muscle cells than vehicle control. Also, verified that AETPB inhibited pancreatic α-amylase and α-glucosidase enzymes. In conclusion, the authors assumed that the above actions might be responsible for the antidiabetic activity of AETPB due to presence of gallic acid and other biomarkers (Ramachandran *et al.*, 2013).

Mahendran *et al.* (2014) evaluated the antihypoglycemic, antihyperlipidemic and antioxidant effect of compounds 1, 2, 8-trihydroxy-6-methoxy xanthone (1) and 1, 2-dihydroxy-6-methoxyxanthone-8-O-β-d-xylopyranosyl (2) in streptozotocin (STZ) induced diabetic rats. Diabetes was induced in male Wistar rats by a single intraperitoneal injection of STZ (60 mg/kg b.w.). It was confirmed that the isolated compounds 1 and 2 at a dose of 50 mg/kg b.w., produced the maximum fall of 83% in the blood glucose level in the diabetic rats after 3h of the treatment. The

administration of 1 and 2 (50 mg/kgb.w.) daily for 28 days in STZ induced diabetic rats, was revealed to significantly decrease the glucose level, glycosylated hemoglobin, SGOT, SGPT, ALP serum urea and creatinine with significant rise in plasma insulin level. Test compounds 1 and 2 displayed antihyperlipidemic activities as evidenced by significant decreased in serum TC, TG, LDL-C, VLDL-C levels coupled together with elevation of HDL-C level in diabetic treated rats when compared to diabetic untreated rats, indicated the protective role against liver and kidney damage. Further the authors determined effect of the two compounds on histopathologal changes in pancreas, liver and kidney after treatment and was reported to be protective. Moreover, the molecular interaction study of the ligands 1, 2 and glibenclamide with various diabetes mellitus related protein targets like glucokinase (PDB ID: 1V4S), fructose-1, 6-bisphosphatase 1 (PDB ID: 2JJK) 11-β-hydroxysteroid dehydrogenase (PDB ID: 2BEL) and modeled protein sulfonylurea receptor 1 (SUR1) indicated that ligand 1 and 2 possess binding affinity with all protein targets except for 2BEL target protein for which ligand 1 has no interaction. The ligand pose with 2BEL and SUR1 protein target of ligand 2 gave the best binding conformation. Hence, the authors concluded that 1 and 2 can be considered for developing into a potent antidiabetic drug (Mahendran *et al.,* 2014).

In a study, the effects of plant (*Costus igneus*) derived diosgenin on cardiovascular risk, insulin secretion, and pancreatic composition was investigated through electron microscopical studies of normal and diabetic rats. Diosgenin at a dose of 5 or 10 mg/kg per body weight (bw) was orally administered as a single dose per day to diabetic induced rats for a period of 30 days. Authors recorded the effect of diosgenin on blood glucose, HbA1c, PT, APTT, Oxy-LDL, serum lipid profile, electron microscopical studies of pancreas, antioxidant enzymes (in liver, kidney, pancreas) and hepatoprotective enzymes in plasma and liver both in normal and diabetic rats. The results indicated that fasting blood glucose, PT, APTT, Oxy-LDL, TC, TG, LDL, ALT, AST, ALP, glucose-6-phosphatase, fructose-1,6-bisphosphatase and LPO levels were significantly ($p < 0.05$) increased, whereas HDL, SOD, CAT, GSH and the glycolytic enzyme glucokinase levels were significantly ($p < 0.05$) decreased in the diabetes induced rats and these levels were significantly ($p < 0.05$) reversed back to normal in diabetes induced rats after 30 days of treatment

with diosgenin. Through electron microscopical studies of the pancreas the article further reported that the number of beta cells and insulin granules were increased in streptozotocin (STZ) induced diabetic rats after 30 days of treatment with diosgenin. Describing their data in detail the authors assured that diosgenin has potential effects on cardiovascular risk, insulin secretion and beta cell regeneration in STZ induced diabetic rats (Kalailingama *et al.,* 2014).

*2.3.7. Molecular characterization of medicinal plants*

Characterization of plants with the use of morphological and molecular markers is an ideal approach for the conservation of plant genetic resources and genetic improvement, for the identification of distinct populations or genotypes for conservation, optimum sites for germplasm collection, and ongoing changes in the pattern of diversity over time. Additionally, morphological and molecular markers are useful for the evaluation and utilization of genetic resources, the study of diversity of pre-breeding and breeding germplasm, and for the protection of the breeder's intellectual property rights (Semagn *et al.,* 2006). ISSR, RAPD, and AFLP are ideal as markers for population studies because of their abundance and high degree of polymorphism between individuals within a population of closely related genotypes (Semagn *et al.,* 2006; Hokanson *et al.,* 1998). Sequencing based molecular techniques provide better resolution at intra-genus and above level, while frequency data from markers such as random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and microsatellites provide the means to classify individuals into nominal genotypic categories and are mostly suitable for intra-species genotypic variation (Arif *et al.,* 2010a). The development and use of molecular markers for the detection and exploitation of DNA polymorphism is one of the most significant developments in the field of molecular genetics. Molecular markers should not be considered as normal genes, as they usually do not have any biological effect, and instead can be thought of as constant landmarks in the genome. They are identifiable DNA sequences, found at specific locations of the genome, and transmitted by the standard laws of inheritance from one generation to the next (Semagn *et al.,* 2006). There are several techniques for developing these markers, including the traditional approach using bacterial colonies containing microsatellite inserts or newer methods involving next-generation sequencing. Regardless of the initial approach, putative primers must eventually be designed and

tested. One of the most costly steps in this process is the fluorescent labelling of individual microsatellite primers, with costs varying depending upon the dye used (e.g., 6-FAM, VIC, NED) and the manufacturer (Culley *et al.*, 2013). Among the polymerase chain reaction (PCR)-based molecular techniques, random amplified polymorphic DNA (RAPD) is convenient in performance and does not require any information about the DNA sequence to be amplified. Due to its procedural simplicity, the use of RAPD as molecular markers for taxonomic and systematic analyses of plants as well as in plant breeding and the study of genetic relationships have considerably increased (Arif *et al.*, 2010a).

RAPD method was utilized for the genetic fingerprinting of 11 plant species of desert origin (seven with known medicinal value), *Andrachne telephioides, Zilla spinosa, Caylusea hexagyna, Achillea fragrantissima, Lycium shawii, Moricandia sinaica, Rumex vesicarius, Bassia eriophora, Zygophyllum propinquum subsp migahidii, Withania somnifera,* and *Sonchus oleraceus* which were collected from various areas of Saudi Arabia. The five primers used were able to amplify the DNA from all the plant species. The amplified products of the RAPD profiles ranged from 307 to 1772 bp. Authors observed a total of 164 bands for 11 plant species, using five primers. The number of well-defined and major bands for a single plant species for a single primer ranged from 1 to 10. The highest pair-wise similarities (0.32) were observed between *A. fragrantissima* and *L. shawii*, when five primers were combined. The lowest similarities (0) were observed between *A. telephioides* and *Z. spinosa; Z. spinosa* and *B. eriophora*; *B. eriophora* and *Z. propinquum*. The RAPD method successfully discriminates among all the plant species, therefore providing an easy and rapid tool for identification, conservation and sustainable use of these plants (Arif *et al.*, 2010b).

RAPD, ISSR and SSR primers were used to assess genetic diversity and phylogenetic relationships among 28 species of *Cassia* (2n = 16, 26, 28). RAPD, ISSR and SSR primers revealed 36.12, 42.7 and 54.4% polymorphism, respectively. The Dendograms based on RAPD, ISSR, and SSR data precisely organized 28 species of *Cassia* into different clusters. SSR primer could distinguish all species analyzed within the genus. Polymorphic index varied from 0.1 to 0.5 for both SSR and RAPD markers; primer index values were substantially higher for RAPD primers (0.35 – 4.65) than for SSR primers (0.35 – 1.73). They identified possible accessions with the help of RAPD, ISSR and SSR markers. Dendograms constructed from RAPD, ISSR

and SSR data revealed DNA marker-based genetic identification in Cassia. Four groups of *Cassia* that were resolved corresponded to species grouped earlier taxonomically. *Cassia mimosoides* with a different genomic set up showed close relation to *C. javanica. Cassia siamea* and *C. spectabilis*; *C. grandis* and *C. nodosa* in a core group with close morphological similarities. *Cassia artemisioides* and *C. covesii*, both showing morphologically drought tolerance characters are closely placed, indicating that they are the wild progenitors of these species (Mohanty *et al.,* 2010).

Generic relationships were examined among twenty-four species belonging to genus *Cassia L., Senna Mill.* and *Chamaecrista Moench* using RAPD marker. Total 80 primers were screened, 514 amplification products obtained with 38 informative primers, of which 514 were polymorphic. Authors observed high degree of polymorphism (100%) among them. UPGMA cluster analysis of genetic similarity indices grouped all the species into three major clusters. Cluster I included four species of *Cassia L.*, Cluster II included eighteen species of *Senna Mill.* and Cluster III included two species of *C. Moench*. Highest similarity (0.9%) was shown between *Cassia fistula* L. and *Cassia fistula* with nodded filaments and least (0.001%) between *Cassia fistula L.* and *Senna splendida*. The Polymorphic information contents (PIC) of the twenty-four species with RAPD marker varied from 0.08 to 0.49 with an average of 0.005. Finally proved the statement of Irwin and Barneby, they divided the genus *Cassia L.* into three subgenera; *Cassia L.*, *Senna* Mill. and *C. Moench* on the basis of morphological characters. Based on the results authors suggested that RAPD marker is a sensitive, precise and efficient tool for genomic analysis of *Cassia L*. that may be useful in future studies by assigning new unclassified germplasm to specific taxonomic groups and reclassify previously classified species and genera (Tripathy and Goswami, 2011).

Random amplified polymorphic DNA (RAPD), Inter simple sequence repeat (ISSR) and Amplified fragment length polymorphism (AFLP) markers were used to verify the segregation of the genus *Cassia* L. *senso lato* into three distinct genera namely *Chamaecrista* Moench., *Senna* P. Mill. and *Cassia* L. *sensostricto.* Eighteen representatives of the three taxa were characterized using the molecular markers. 25 RAPD, six ISSR primers and six AFLP primer combinations resulted in the amplification of 612, 115 and 622 bands (loci) respectively. Most of the loci are

reported to be polymorphic, showing high degrees of genetic diversity among the different taxa studied. The dendrogram constructed on the basis of the RAPD, ISSR and AFLP data using SHAN clustering, divided *Cassia* L. *senso lato.* into three different clusters as *Chamaecrista* Moench. *Senna* P. Mill. and *Cassia* L. senso stricto High bootstrap value revealed that all the clusters were stable and robust. It was reported from the present investigation that these genera have their identity at molecular level, which supports the elevation of the genus *Cassia* L. *senso lato* to the level of subtribe *Cassiinae* and segregation into three distinct genera instead of intrageneric categories (Acharya *et al.,* 2011).

DNA fingerprinting of six medicinally important *Senna* species was done so as to aid easy recognition of the Senna species for pharmacognostic researches. The species studied were *S. alata, S. obtusifolia, S. siamea, S. hirsuta, S. occidentalis* and *S. polyphylla*. Random amplified polymorphic DNA (RAPD) was used to develop markers for authentication of *Senna* species. The generated dendogram from RAPD analysis indicated that all the species are at least 62% similar. *S. alata* and *S hirsuta* were 95% genetically identical and far from other accessions in terms of similarity. This is common in speciation. The RAPD analysis led to a clear distinction between *S. alata* and *S. hirsuta* and other species. The study has shown that RAPD marker technique is very useful for genetic variability and species relationship in the genus, *Senna* (Jimoh *et al.,* 2013).

To assess the morphological and molecular genetic diversity in 18 *C. forskohlii* genotypes collected from different places of central India, RAPD, ISSR, and AFLP marker systems were employed. Eleven RAPD, ten ISSRs and eight AFLP primers were reported to produce 101, 80, and 483 fragments, respectively. Among the three marker system used in this study, RAPD and ISSR showed 61.39 and 68.75% polymorphism, respectively, while eight AFLP primer combinations produced 70.81% polymorphism. UPGMA cluster analysis method group genotypes in two clusters with all marker systems separately and after combined analysis. Authors indicated that both morphological and molecular factors are effective in observing variations. Their results also indicate that the RAPD, ISSR, and AFLP approaches, along with pharmaceutically important morphological trait analysis, seemed to be best-suited for assessing the genetic relationships among distinct *C. Forskohlii* genotypes with high accuracy (Tripathi *et al.,* 2013).

Randomly Amplified Polymorphic DNA (RAPD) markers are used to study genetic variation of some edible *Cassia* species. The four informative primers were used to evaluate degree of polymorphism. The primers produced multiple band profiles and bands were found in the size range of 900kb to 1250kb. Of the four plants used, though three are of *Cassia tora* type and one is *Cassia sophera*, they exhibited phylogenetic differences as revealed by the dendrogram. Intraspecific genetic variability and physical distances were exhibited by three plants of *Cassia tora* collected from different localities which might be useful in proper identification of the plants (Rao and Suresh, 2015).