

## CHAPTER 2

### LITERATURE REVIEW ---

#### 2.1 *Enicostemma hyssopifolium*

**2.1.1 Common names:** *Enicostemma littorale*, mamejav.

**2.1.2 Ethno-botanical information:** This plant has been used as folk medicine for the treatment of diabetes mellitus in Western and Southern India (Gupta et al., 1962). Various Ayurvedic formulations containing *E. hyssopifolium* one of the ingredients have been shown to produce antihyperglycemic activity. Hot aqueous extract of *E. hyssopifolium* is used by the tribal inhabitants of north Gujarat, for the treatment of diabetes, fever, stomach ache, dyspepsia and malaria in interior part of Gujarat. It is cited in ancient literature as an antimalarial, antipyretic and as a laxative (Vyas et al., 1979).

**2.1.3 Pharmacological studies:** The herb is reported for its hypoglycemic, antioxidant and hypolipidaemic potential in newly-diagnosed NIDDM patients (Vasu et al., 2003). It is also known for its anti-inflammatory (Sadique et al., 1987) and anticancer property (Kavimani and Manisenthilkumar, 2000). Recently aerial part of *Enicostemma littorale* was reported to show hypolipidaemic effect in *p*-dimethylaminobenzene (*p*-DAB) induced hepatotoxic animals (Gopal et al., 2004). Studies had confirmed the hypoglycemic potential in alloxan-induced diabetic rats (Vijayvargia et al, 2000, Maroo et al, 2002, 2003a, 2003b) and also as hypoglycemic, antioxidant and hypolipidaemic in newly-diagnosed NIDDM patients (Vasu et al, 2003). The antidiabetic effect was also reported by other workers (Murali et al, 2002,

Srinivasan et al, 2005, Upadhyay and Goyal, 2004). The herb is reported as an anti-inflammatory (Sadique et al, 1987) and anticancer agent (Kavimani and Manisenthal kumar, 2000) and also as a constituent in an antidiabetic herbomineral preparation (Babu and Prince, 2004).

**2.1.4 Chemical constituents:** The plant has been found to contain flavonoids like apigenin, genkwanin, isovitexin, swertisin, saponarin, 5-O-glucosylswertisin and 5-O-glucosylisowertisin (Ghosal, 1980), Monoterpene alkaloids viz., enicoflavin and gentiocrucine (Chaudhury et al, 1975, Retnam and DeBritt, 1988), catechins, saponins, steroids (Retnam and DeBritt, 1988) a triterpene sapogenin, betulin (Rai and Thakar, 1966, Desai et al, 1966). An alkaloid, gentianin (Govindachari et al, 1966) and a secoiridoid glycoside swertiamarin (Rai and Thakar, 1966, Desai et al, 1966), Phenolic acids like, vanillic acid, syringic acid, p-hydroxy benzoic acid, protocatechuic acid, p-coumaric acid and ferulic acid (Daniel and Sabnis, 1978) were also reported. Aminoacids like L-glutamic acid, tryptophane, alanine, serine, aspartic acid, L-proline, L-tyrosine, threonine, phenyl alanine, L-histidine monohydrochloride, methionine, iso leucine, L-arginine monohydrochloride, DOPA, L-Glycine, 2-amino butyric acid and valine (Retnam and DeBritt, 1988) were identified.

## 2.2 *Gymnema sylvestre*

**2.2.1 Common name:** Guduchi, gudmar, giloy, madhunashini, meshasringi.

**2.2.2 Ethno-botanical information:** Sushruta describes *Gymnema sylvestre*, as a destroyer of madhumeha (glycosuria) and other urinary disorders. On account of its property of abolishing the taste of sugar it has been given the name of gur-mar meaning sugar destroying and it is believed therefore that it might neutralize the excess of sugar present in the body as found in DM. The plant is also reported to be bitter, astringent, acrid, thermogenic, anti-inflammatory, anodyne, digestive, liver tonic emetic, diuretic, stomachic, stimulant, anthelmintics, laxative, cardi tonic, expectorant, antipyretic and uterine tonic. It is useful in dyspepsia, constipation, jaundice, haemorrhoids, renal and vesical calculi, cardiopathy, asthma, bronchitis, amenorrhoea, conjunctivitis and leucoderma (Nadkarni KM, 1993, Bhava Prakash Nighantu, 1998).

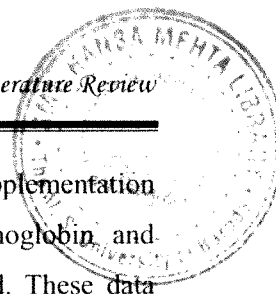
**2.2.2 Pharmacological studies:** The drug influenced the disturbed carbohydrate metabolism in hyperglycaemic animals by limiting the carbohydrate turnover and thus

inhibiting the vicious cycle of hyperglycaemia (Gupta and Seth, 1962). Studies reports that antidiabetic activity of *Gymnema sylvestre* is comparable with other conventional indigenous oral antidiabetic drugs like *Coccinia indica*, *Pterocarpus marsupium*, *Momordia charantia* (Gupta SS, 1963). Crude leaf powder showed improvement in abnormal accumulation of lipids, glycogen and protein depletion in the liver, kidney and muscle in alloxan induced diabetic rabbits. *Gymnema sylvestre* is reported not only to produce blood glucose homeostasis but also increases the activities of the enzymes affording the utilization of glucose by insulin dependent pathways; it controll phosphorylase levels, gluconeogenic enzymes and sorbitol dehydrogenase. The uptake and incorporation of glucose into the glycogen and protein are increased in the liver, kidney and muscle in *Gymnema sylvestre* administered diabetic animal when compared to the untreated diabetic animals. (Shanmugasundaram et al., 1983).

It has been found that glucose receptor interacted with the leaf extracts of *Gymnema sylvestre* and purified Gymnemic acid causing inhibition of GIP release which was similar in specificity to the active glucose transport system (Fushiki et al., 1992). Two water soluble fraction of *Gymnema sylvestre* extract GS<sub>3</sub> and GS<sub>4</sub> doubled the islet number and beta cell number in pancrease of streptozotocin induced diabetic rats. GS<sub>3</sub> and GS<sub>4</sub> appeared to bring about blood glucose homeostasis through increased serum insulin levels provided by repair/regeneration of the endocrine pancreas (Shanmugasundaram et al., 1990a).

Leaf extract (25-100 mg/kg) reported to reduced the elevated serum triglyceride (TG), total cholesterol (TC), very low density lipoprotein(VLDL) and low density lipoprotein(LDL)-cholesterol in experimentally induced hyperlipidaemic rats. Antiantherosclerotic potential is found to be almost similar to that of a standard lipid lowering agent clifibrate (Bishayee and Chatterjee , 1994).

**2.2.3 Clinical trials:** In a clinical trial conducted on 27 patients with insulin-dependent Diabetes mellitus (IDDM) on insulin therapy, a water soluble extract (GS<sub>4</sub>) of the leaves of *Gymnema sylvestre* (400 mg/day) brought down the insulin requirements along with fasting blood glucose, glycosylated haemoglobin (HBA<sub>1c</sub>) and glycosylated plasma protein levels, while serum lipids returned to near normal level. GS<sub>4</sub> appeared to enhance endogenous insulin, possibly by regeneration/revitalization of the residual beta cells in insulin-dependent Diabetes mellitus (Shanmugasundaram et al., 1990b). Further clinical studies were conducted to test the effectiveness of GS<sub>4</sub> in controlling hyperglycaemia in 22 patients with Type2



Diabetes on conventional oral anti-hyperglycaemic agents. GS4 (400mg/day) supplementation found to show significant reduction in blood glucose, glycosylated haemoglobin and glycosylated plasma proteins and conventional drug dosage could be reduced. These data suggested the beta cells regeneration/repair in Type 2 diabetic patients on GS4 supplementation, which is further, supported by the appearance of raised insulin levels in the serum of patients after GS4 administration (Baskaran et al., 1990). When hypoglycaemic activity of *Gymnema sylvestre* was evaluated in ten normal and six diabetic patients. Aqueous decoction of the leaves (2 gm thrice daily for a 10 days) brought about a significant reduction in the fasting blood sugar levels in normal and diabetic patients which suggested a definite hypoglycaemic activity (Khare et al., 1983). Hypoglycaemic effect of *Gymnema sylvestre* was also studied in 16 normal 43 mild diabetic patients. A leaf powder 10 gm/day for 7 days results in hypoglycaemic effect comparable to tolbutamide. Serum triacylglycerol, free fatty acids and cholesterol levels in normal subjects were unaffected where as in diabetic patients it was significantly decreased (Balasubramaniam et al., 1992).

**2.2.4 Phytochemistry:** The major bioactive constituents of *Gymnema sylvestris* are a group of oleanane type triterpenoid saponins known as “gymnemic acids” (Kuzuko et al., 1989; Hong-Min et al., 1992) . The latter contain several acylated (tigloyl, methylbutyroyl etc.,) derivatives of deacylgymnemic acid (DAGA) which is 3-O-  $\beta$ - glucuronide of gymnemagenin (3 $\beta$ , 16 $\beta$ , 21 $\beta$ , 22 $\alpha$ , 23, 28-hexahydroxy-olean-12-ene) (Hong-Min et al., 1992). The individual gymnemic acids (saponins) include gymnemic acids I-VII, gymnemosides A-F, gymnemasaponins (Masayuki et al., 1997) etc.

**2.2.5 Toxicity:** The LD<sub>50</sub> of ethanol and water extract of *Gymnema* administered intraperitoneally in mice was found to be 375mg/kg (Bhakuni et al., 1971).

### 2.3 *Tinospora cordifolia*

**2.3.1 Common names:** Guduchi, Amrita, Gulvel, Gado, Galo.

**2.3.2 Ethno-botanical information:** The leaves are beaten with honey and applied to ulcers. Dried and powdered fruit, mixed with ghee or honey, is used as a tonic and also in the treatment of jaundice and rheumatism. The root is powerful emetic and used for visceral obstructions; its watery extract is used in leprosy. A decoction of the leaves is used for the treatment of gout, and young leaves, bruised in milk, are used as a liniment in erysipelas. The whole plant is used in scabies in swine. The vine is used as an appetizer and for internal

parasites in ruminants and for diarrhea in poultry. It is also used in stomach trouble. Stem, root and whole plant are used in sprain, abscess, tumour, wound, broken horn, cracked tail, anthrax, as a galactagogue and in the treatment of pneumonia, asthma, cough, swelling of lungs colic, constipation, tetanus, pox and compound fracture. The stem of *Tinospora cordifolia* is one of the constituents of several ayurvedic preparations used in general debility, dyspepsia, fever and urinary diseases. The stem is bitter, stomachic, diuretic (Nayampalli et al., 1988), stimulates bile secretion, causes constipation, allays thirst, burning sensation, vomiting, enriches the blood and cures jaundice. The extract of its stem is useful in skin diseases (Aiyer and Kolammal, 1963, Raghunathan and Mittara, 1982). The root and stem of *T. cordifolia* are prescribed in combination with other drugs as an antidote to snake bite and scorpion sting (Nadkarni and Nadkarni, 1976, Kirtikar and Basu, 1975, Zhao et al, 1991). Dry barks of *T. cordifolia* have anti-spasmodic, antipyretic (Ikram et al, 1987), anti-allergic (Jana et al, 1999), anti-inflammatory (Rai and Gupta, 1966, Pendse et al., 1977) and anti-leprotic (Asthana et al, 2001) properties. The aqueous extract of the stem antagonizes the effect of agonists such as 5-hydroxytryptamine, histamine, bradykinin and prostaglandins E1 and E2 on the rabbit smooth muscle, relaxes the intestinal, uterine smooth muscle and inhibits the constrictor response of histamine and acetylcholine on smooth muscle.

### 2.3.3 Pharmacological studies:

*T. cordifolia* is widely used in Indian *ayurvedic* medicine for treating diabetes mellitus (Stanely, 2001, Prince and Menon, 1999, Mathew and Kuttan 1997). *T. cordifolia* root extract caused a significant reduction in blood glucose and brain lipids in alloxan induced diabetic rats. Although its anti- hyperglycemic effect in different animal models was equivalent to only one unit/kg of insulin (Dhaliwal 1999). It is reported that the daily administration of either alcoholic or aqueous extract of *T. cordifolia* decreases the blood glucose level and increases glucose tolerance in rodents (Gupta et al., 1967, Grover et al., 2001). Aqueous extract also caused a reduction in blood sugar in alloxan induced hyperglycemia in rats and rabbits in the dose of 400 mg/kg. However, histological examination of pancreas has not revealed any evidence of regeneration of  $\beta$ -cells of islets of *Langerhans* and the possible mode of action of the plant is through glucose metabolism (Raghunathan and Sharma, 1969). The aqueous extract has also exhibited some inhibitory effect on adrenaline-induced hyperglycemia. Ethyl acetate extract of its roots has afforded a pyrrolidine derivative with hypoglycemic activity in rabbits (Mahajan and Jolly, 1985, Grover et al., 2000, Stanely et al., 2000). Another study has also revealed significant hypoglycemic effect of extract of leaves in

normal and alloxan diabetic rabbits. However, the extract had no significant effect on total lipid levels in normal or treated rabbits (Wadood et al., 1992, Basnet et al., 1995). *T. cordifolia* is reported to benefit the immune system in a variety of ways (Kapil and Sharma, 1997, Nagarkatti et al., 1994, Rege et al., 1993). The alcoholic and aqueous extracts of *T. cordifolia* have been tested successfully for immuno-modulatory activity (Thatte and Dahanukar, 1989, Thatte et al., 1987, Rege et al., 1989, Manjrekar et al., 2000). *T. cordifolia* has significantly reduced the mortality from *E. coli* induced peritonitis in mice. In a clinical study, it has afforded protection in cholestatic patients against *E. coli* infection. It has been observed that it stimulates the macrophages as evidenced by an increase in the number and % phagocytosis of *S. aureus* by peritoneal macrophages in rats (Broker and Bhatt, 1953). The hepatoprotective action of *T. cordifolia* was reported in CCl<sub>4</sub> induced hepatopathy. Extract of *T. cordifolia* has also exhibited *in vitro* inactivating property against Hepatitis B and E surface antigen in 48-72 hrs (Mehrotra et al., 2000). The dried stem of *T. cordifolia* showed significant anti-inflammatory effect in both acute and subacute models of inflammation (Jana et al., 1999). In a clinical evaluation, a compound preparation 'Rumalaya' containing *T. cordifolia* was reported to significantly reduce the pain in patients suffering from rheumatoid arthritis. The aqueous extract of roots of *T. cordifolia* has shown the anti-oxidant action in alloxan induced diabetes rats (Stanely et al., 1999). Jagetia *et al* (1998) reported guduchi as an anti-neoplastic agent *in vitro* against HeLa cells. Ether extract of the stem distillate of aerial part of *T. cordifolia* has inhibited the *in vitro* growth of *Mycobacterium tuberculosis* at 1:50,000 dilution. Its ethanolic extract has exhibited significant antipyretic activity in experimental rats. 'Septilin' syrup, a compound preparation containing *T. cordifolia* (7.82% in 5 ml of syrup) was found to elicit good clinical response in children suffering from upper respiratory tract infection and chronic otitis media (Vedavathy and Rao, 1991).

**2.3.4 Chemical constituents:** Major constituents sesquiterpene tinocordifolin, sesquiterpene glucoside tinocordifolioside, tinosponone, tinocordioside, cordioside, furanoid diterpenes; a clerodane furano-diterpene viz. columbin, tinosporaside; an immunologically active arabinogalactan; two phytoecdysones viz., ecdysterone and makisterone and several glycosides isolated as polyacetates. Alkaloids viz., jatrorrhizine, palmatine, berberine, tembeterine; phenyl propene disaccharides cordifolioside A, choline, tinosporic acid, tinosporal, tinosporon, 20-hydroxyecdysone, palmatosides C, cordifolisides D and diterpenoid furanolactones.

**2.3.5 Clinical trials:** Studies on induced oedema and arthritis and on human arthritis proved the anti-inflammatory potency of the water extract of this plant. Phase I and Phase II of adjuvant induced arthritis were also inhibited. The anti-inflammatory activity of this plant resembles that of nonsteroidal anti-inflammatory agents. It also has weak antipyretic action and is a morphine potentiator.

## 2.4 *Eclipta alba*

**2.4.1 Common names:** Bringaraj, bringraj, eclipta.

**2.4.2 Ethno-botanical information:** *Eclipta alba* is mainly used in hair oils, but it has been considered to have use in hepatotoxicity. In hair oils, it may be used along with *Centella asiatica* (Brahmi) and *Phyllanthus emblica* (Amla). It is used to prevent habitual abortion and miscarriage and also in cases of post-delivery uterine pain. A decoction of leaves is used in uterine hemorrhage. The juice of the plant with honey is given to infants with castor oil for expulsion of worms. For the relief in hemorrhoids, fumigation with *Eclipta alba* is considered beneficial. The paste prepared by mincing fresh plants has an anti-inflammatory effect and may be applied to insect bites, stings, swellings and other skin diseases. In Unani system, the juice of *Eclipta alba* is used in "Hab Miskeen Nawaz" alongwith aconite, *Croton tiglium*, "triphala", *Piper nigrum*, *Piper longum*, *Zingiber officinale*, and minerals like mercury, sulphur, arsenic, borax etc. for various types of pains in the body. It is also a constituent of 'Roghan Amla Khas' for applying on hair, and of "Ma'jun Murrawah-ul-arwah" (<http://www.banlab.com>). Dried aerial parts are used as purgative, emetic, cholagogue in Arabic Countries, against snakebites in China and Brazil, for asthma in Thailand, for headaches, it is ground in sesame oil and applied to the forehead in India, for common cold in Panama. Entire plant is used for tuberculosis and as haemostatic in China, for inflammation taken with black pepper and raw sugar in India (Jain et al., 1994), to treat wounds (caused by walking barefooted during rain) in Nepal (Manandhar, 1998), to treat vesicles on the skin, plant is crushed and soaked for an hour in water. Extract is applied to affected area in Somalia, to treat leprosy, plant is crushed and mixed with oil, and mixture is applied to skin and to treat diabetes mellitus in Taiwan. Leaves are used to treat epilepsy in India, leaves are pounded with garlic and pepper if the patient is unconscious the extract is dropped into the nostril, to treat stomach cancer mixed with *Ageratum conyzoides*, *Spilanthes acmella*, *Vernonia conyzoides* and jat, taken after meals in morning and evening in Indonesia and as an antiasthmatic, in colds, coughs, elephantiasis, hepatitis, splenitis,

vertigo in Peru. Roots are used for insanity, four to five pills made from the root paste are given twice a day for seven days in India (Jain et al., 1994), for women after childbirth in Malaysia, for jaundice, root plus seed of *Ricinus communis* are ground and paste is applied to eyes in India (Hemadri & Rao, 1984 ).

**2.4.3 Pharmacological studies:** The ether extract of the dried aerial parts showed antivenom effect, when administered 0.5 mg I.P. in mouse. The methanol extract (80%) of the dried aerial parts shows antihepatotoxic activity (Kim and Park, 1994). The hydroalcoholic extract of the dried leaf reported analgesic activity, when administered intragastrically in mouse (100 mg/kg). The chloroform and methanol extract of the dried leaf (1 gm/ml) showed antibacterial activity against *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* (Naovi et al., 1991; Phadke and Kulkarni, 1989; Farouk et al., 1983). Various biological activities have been reported in the literature for extracts of *E. prostrata* like hepatoprotective (Chandra et al., 1987; Singh et al., 1993; Sharma et al., 1991; Saxena et al., 1993); antiviral (Kusumoto et al., 1995; Jayaram et al., 1997; Zheng et al., 1988); antirheumatic (Dabral and Sharma, 1983); molluscicidal (Mendes et al., 1984); antimalarial and antifertility (Misra et al., 1991).

**2.4.4 Chemical constituents:** Ecliptal ( $\alpha$ -terthienyl aldehyde),  $\alpha$ -terthienyl-methanol, sixteen polyacetylenic thiophenes, 5'-seneciyoxyoxymethylene-2-(4-isovaleroxybut-3-ynyl) dithiophene, 5'-tigloyloxyoxymethylene -2-(4-isovaleroxybut-3-ynyl) dithiophene. The dried leaves of *Eclipta alba* have been reported to contain wedelolactone, a complex coumarin and its derivatives dimethylewedelolactone-7-glucoside and nor-wedelolactone. The roots contain polyacetylene substituted thiophenes and leaves have been reported to contain 2.2:5.2:5-terthienylmethanol; alkaloids, 20-epi-3-dehydroxy-3-oxo-5, 6-dihydro-4, 5-dehydroverazine, ecliptalbine, 4 $\beta$ -hydroxyverazine. The aerial parts of the plant have been reported to contain phytosterol,  $\beta$ -amyrin in the n-hexane extract and luteolin-7-glucoside,  $\beta$ -glucoside of phytosterol, a glucoside of a tritepenic acid and wedelolactone in polar solvent extract. Hentriacontanol and heptacosanol are reported in the roots. The polypeptides isolated from the plant yield cystine, glutamic acid, phenylalanine, tyrosine and methionine on hydrolysis. In addition, the aerial parts contain apigenin, cinnaroside, and sulfur compounds like diethyl-(2,2')-5-hydroxy-methyl-5'-(butyl-3-en-11-ynyl) derivatives of angelic acid, butyric acid, senecic and tiglic acids.



The whole plant contains nicotine and stigmasterol. Seeds contains proteins (15.6%), fats (13.1%). The entire plant contains triterpenes: ecalbatin, echinocystic acid, oleanic acid, ursolic acid; flavone: luteolin.

**2.4.5 Clinical trials:** A clinical trial conducted on 50 children suffering from hepatitis. All patients were administered *Eclipta alba* powder with honey in doses of 50 mg/kg body wt. in three divided doses for a period of 1-5 weeks, revealed that 80% of patients recovered fully. A clinical trial conducted on 30 patients of viral hepatitis with a compound herbal preparation containing *Eclipta alba* as one of the ingredients showed an excellent in terms of clinical as well as biochemical parameters as compared to the placebo group (Ministry of Health and Family Welfare Government of India, 2001).

**2.4.6 Contraindications:** The American Herbal Products Association established that it can be safely consumed but it does not have to be taken by patients with acute or chronic diarrhea, or patients who have disorders of spleen. Its effects on lactating children are not documented, and thus it is not recommended to be used during breast-feeding.

## 2.5 Research Envisaged

The longstanding, successful use of herbal drug combinations in traditional medicine makes it necessary to find a rationale for the pharmacological and therapeutic superiority of many of them in comparison to isolated single constituents. Modern molecular-biological methods (including new genomic technologies) can enable us to understand the various synergistic mechanisms underlying these effects. Synergistic effects can be produced if the constituents of an extract affect different targets or interact with one another in order to improve the solubility and thereby enhance the bioavailability of one or several substances of an extract. The verification of real synergy effects can be achieved through detailed pharmacological investigations and by means of controlled clinical studies performed in comparison with synthetic reference drugs. All the new ongoing projects aim at the development of a new generation of phytopharmaceuticals which can be used alone or in combination with synthetic drugs or antibiotics. This new generation of phytopharmaceuticals could lend phytotherapy a new legitimacy and enable their use to treat diseases which have up till now been treated using synthetic drugs alone (Wagner and Ulrich-Merzenich 2009).

Synergy research in phytomedicine has established itself as a new activity in recent years to understand a comparative account of magnitude of efficacy of a marker as single constituent

9 vice a vice the extracts or the drug as a whole. It is believed that the effects of the mixture of bioactive constituents and their byproducts combined in plant extracts are responsible for the improved efficacy of many of the extracts thus a synergistic status is achieved. However the mechanism underlying this property is still unexplained. It would only be possible as an approach to reveal the mechanism. The standardized extract singular and multicomponent preparations are assessed both chemically as well as biologically using clinical applications also. These studies are expected to provide certain data for standardization of medicinal preparations as regard to their quality, safety and efficacy. ( 9

Indian market is flooded with many such preparations claimed to be effective as combinations in the treatment of AIDS and other infectious diseases, hypertension, numerous types of cancer and rheumatic diseases. The multi-drug concept in cancer therapy is recently designated in some reports as biomodulatory – metronomic chemotherapy. These practices remain the actual basis of therapy in Traditional Chinese and *Ayurvedic* medicine. The mono-extract preparations administered contain a majority of several bioactive constituents and therefore exhibit the synergistic effects. }

India is the country where herbs and *ayurvedic* preparations are popular as effective therapy. Many of these preparations still need to be explored for their rationality of combination of different extracts and/or components responsible for their efficacy. An immediate need is also felt in order to provide convincing justification for their efficacy. A dire need felt, to develop methods for evaluating the chemical and biological profile of such formulations so as to build up a confidence in the patient as well as prescriber. The evolution of various guidelines, regulatory measures and their implementation has been taken up by the workers in the field. In order to follow similarly such activities the present study was planned on following line.

- Selection of some formulations and identification of their components prescribed in traditional system for anti-diabetic activity.
- Development of methods for detection and estimation of chemical markers in the selected formulations.
- Bio activity determination of extracts and identification of biomarkers from bio guided fractionation.
- Development of analytical methods for quality control of these selected formulations.
- Assessment of efficacy of these formulations.