CHAPTER – VIII

Summary & Highlights

Summary

Plants form one of the sources of drugs for the treatment of diabetes mellitus in Indian systems of medicine especially in rural areas. Out of many plants used thus, only a few have been evaluated for their efficacy as per modern standardization procedures. There is a widespread belief that the "Green medicine" is safe and more dependable than the synthetic drugs, many of which exert adverse side effect such as haematological, cardiovascular and gastrointestinal reactions. Diabetic coma and damage to kidney and liver are the side effects from the latter which can endanger the life of diabetic patients.

Because of the high reliability and effectiveness of the herbal medicines, the herbal industries are mushrooming every where. Since in the ayurvedic texts, which are the main basis of plant sources, the names of the plants are available in *Sanskrit,* there is a great confusion on the botanical identity of these plants. Therefore, the market is flooded with spurious drugs derived from many different sources. These drugs are available as raw drugs, powders or extracts and the latter two are often easily adulterated.

The identity of medicinal plants in powder or in extract form can be established only by detailed Pharamacognostic and Phytochemical methods. But the data on pharmacognosy and Phytochemistry as well as the biomarkers for individual plants are lacking for many plants. Therefore, the urgent need today is to provide such data which would help the manufacturer or the consumer to asses the purity of the medicinal preparations or raw materials. There are many antidiabetic herbs which had been reported to be pharmacologically active and still the search continues to find out an efficient herb which retards diabetic complications.

In the present project a detailed study of pharmacognosy, phytochemistry and pharmacology of the following plants were undertaken:

The plants selected and part used are:

1. *Cassia alata* Linn.

2. *Artocarpus heterophyllus* Lam.
3. *Costus Pictus.* D. Don.
4. *Bauhinia purpurea* Linn.

For all the above plants selected, the leaves were reported to posses potential antidiabetic properties and therefore, only the leaves were studied here. In addition to this, three herbal combinations of known antidiabetic plants in different ratios also were tested for their efficacy.

The combinations used were:

1. *Terminalia arjuna* Roxb **(Bark)**: *Gymnema sylvestre* Retz **(Leaf)**: *Syzygium cumini* L. Skeels **(Seed)** in the ratio 1:1:1.
2. *Syzygium cumini* (L.) Skeels **(Seed)**: *Trigonella foenum-graecum* L. **(Seed)**: *Phyllanthus emblica* L. **(Fruit)** in the ration 1:1:1.
3. *Aegle marmelos* (L.) Correa **(Leaf)**: *Catharanthus roseus* (L.) G. Don **(Leaf)**: *Syzygium cumini* (L.) Skeels **(Bark)** in the ratio 1:1:1.

Pharmacognostic studies included micromorphology, anatomy and powder study. Standard methods were followed in these studies. The diagrams of the stomata, trichomes, T. S. of the leaves were drawn using Camera Lucida at 400x magnification and the size of the crystals, trichomes etc. were measured using an ocular micrometer.

Micromorphological studies were conducted on the leaves and it was found that each plant possessed specific characteristic features useful in their identification. Types of stomata, trichomes, starch grains etc. are the specific features which help to identify the plants in their powder form.

Anatomical studies were conducted on the leaves and petiole of all the plants. The most distinctive characters found to be useful in identifying the plant are 1. the papillae (*C. alata*) 2. double layered palisade, trichomes containing simple starch grains, petiole containing three vascular bundles arranged two on the upper side and one on the lower side in a V shape (*B. purpurea*) 3. latex sac, double layered palisade (*A. heterophyllus*) 4. absence of palisade cells and vein fibres appearing as crystal pieces (*C. pictus*).

For phytochemical analysis various chromatographic methods such as PC, TLC and UV spectrophotometry have been used for the separation and identification of compounds.

In the phytochemical analysis, the chemicals identified from the extract and found to be useful in identifying the specific plant in its extract form are as follows:
In *Cassia alata* the flavonoids present were kaempferol, 3', 4'- OMe - Quercetin and 7, 3', 4'- tri - OMe-Quercetin. The phenolic acids present were vanillic, syringic, 2-hydroxy benzoic, melilotic, *o*-Coumaric and *p*-Coumaric acids.

In *Artocarpus heterophyllus*, flavonoids such as 4'- OMe Apigenin, 3', 4'- di - OMe - luteolin were found and phenolic acid such as vanillic and syringic acid were detected.

Bauhinia purpurea contained flavonls such as 3'- OMe-Quercetin, 4'- OMe-apigenin and phenolic acids such as vanillic, syringic and sinapic acids were found.

In *Costus pictus*, kaempferol, 3', 4'- di - OMe-quercetin and 4'-OMe-kaempferol were the flavonoids which were located out and phenolic acids such as, gentisic, 2, 5 – dihydroxy benzoic acid, *o*-coumaric, vanillic, syringic, melilotic, α-resorcyclic, 3,5-dihydroxy benzoic acid, *p*-hydroxy benzoic acid, *cis* and *trans-p*-coumaric acid. In all these four plants alkaloids and glycoflavones were absent. Quinones and steroids were present uniformly.

Pharmacological studies were attempted to investigate the antidiabetic effect of the aqueous extract of all these four plants. In addition to this, three herbal formulations of known antidiabetic plants in different ratios also were tested for their efficacy.

The rats were made diabetic by single dose of intraperitoneal injection of alloxan as well as streptozotocin. The injected animals exhibited massive glycosuria and hyperglycemia within few days. Diabetes was confirmed by measuring the fasting blood glucose concentration 96 hours after injection. The rats with blood glucose level above 250 mg/dl were considered to be diabetic and were used in the experiments.

After the induction of diabetes, the rats were divided into groups of six animals each and treated orally using an intragastric syringe for different time intervals. After the treatment the rats were fasted for 12 hours. The rats were then sacrificed by cervical dislocation and the blood samples were collected in tubes containing anticoagulant.

Plasma samples were obtained by centrifugation and stored at -20^{0}C till the experimental analysis.

Weighed kidney, liver and brain samples were taken and homogenized on ice in the appropriate buffer (1/10 parts (W/V)). The homogenates were centrifuged at 10,000xg for 15 min at 4^{0}C to discard any cell debris. The supernatants were immediately used for enzyme assays.

Before treating the diabetic induced rats with the extract, a preliminary study was done to check the toxic effect of the herbal drugs in normal rats, and to standardize the optimum dose. The following table represents the graded doses and the period of treatment in normal rats in each plant extract:

SR.	PLANT	EXTRACT	GROUPS	DOSES	PERIOD OF TREATMENT
1.	*Cassia alata*	Alcoholic extract	Six	Control, 100, 200, 400, 600 and 1000	15 days
		Aqueous extract of dry leaf	Six	Control, 100, 200, 400, 600 and 1000	15 days
		Aqueous extract of fresh leaf	Six	Control, 100, 200, 400, 600 and 1000	15 days
2.	*Artocarpus heterophyllus*	Aqueous extract of fresh leaf	Seven	Control, 100, 200, 400, 600, 800 and 1000	30 days
3.	*Bauhinia purpurea*	Aqueous extract of fresh leaf	Six	Control, 100, 200, 400, 600, 800 and 1000	30 days
4.	*Costus pictus*	Aqueous extract of fresh leaf	Six	Control, 100, 200, 400, 600 and 1000	30 days
5.	Herbal formulation A	Aqueous extract	Six	Control, 100, 150, 300, 400, and 500	15 days
6.	Herbal formulation B	Aqueous extract	Six	Control, 100, 200, 300, 400 and 500	15 days
7.	Herbal formulation C	Aqueous extract	Six	Control, 100, 200, 300, 400 and 500	15 days

The results of the preliminary study indicated that all the plants were safe, after the treatment and the rats did not show any toxic effects or mortality up to a dose of 1gm/kg body weight in individual plant extract and 500 mg/kg body weight in herbal formulation. Even at this high dose there were no gross behavioral changes. Body temperature, state of stool, body weight, water and food intake were also not influenced by the treatment. In all the extracts except *A. heterophyllus* peak hypoglycemia occurred 2 hours after the administration of the leaf extract in normal rats. In *A. heterophyllus* peak hypoglycemia occurred 3 hours after the drug administration and the maximum glucose tolerance was observe in 90 minutes in normal glucose loaded rats whereas, in all other plants maximum glucose tolerance was observed in 30 minutes after the drug administration in normal glucose loaded rats.

After the optimum dose determination, the diabetes induced rats were treated for a selected period of time with each extract to test the antioxidant potential, hypolipidemic activity, renal toxicity and the effect of the extract on carbohydrate metabolizing enzymes along with the capacity of the extract to lower the blood glucose level.

Biochemical estimation and haematological parameters such as, estimation of glucose, TBARS, GSH, vitamin C, vitamin E; assay of SOD, CAT, GPX; assay of hexokinase, glucose-6-phosphates and fructose-1, 6-bisphosphatase; determination of urea, uric acid, creatinine, blood urea nitrogen; determination of cholesterol, triglycerides, phospholipids, free fatty acids, HDL-C, LDL-C and VLDL-C were carried out in plasma as well as in different organs. In diabetes induced rats.

The following table represents the type of extract, optimum dose selected and the period of treatment in the diabetic induced rats in each extract:

SR. NO	PLANT	EXTRACTS	GROUPS	DOSES (MG / KG BODY WEIGHT)	PERIOD OF TREATMENT
1.		Aqueous extract of dry	Seven	400 and 600	45 days

	Cassia alata	leaf			
		Aqueous extract of fresh leaf	Five	400 and 600	45 days
2.	*Artocarpus heterophyllus*	Aqueous extract of fresh leaf	Five	400 and 600	60 days
3.	*Bauhinia purpurea*	Aqueous extract of fresh leaf	Five	200 and 400	60 days
4.	*Costus pictus*	Aqueous extract of fresh leaf	Five	200 and 400	60 days
5.	Herbal extract A	Aqueous extract	Five	100 and 150	30 days
6.	Herbal extract B	Aqueous extract	Five	100 and 200	40 days
7.	Herbal extract C	Aqueous extract	Five	200 and 300	40 days

In this study it was seen that all the plant extracts tested could significantly ($p<0.05$) reduce the blood glucose level and the maximum reduction was observed in *C. pictus* (57.09% and 57.2%) after 60 days of treatment but the effect was less than the standard drug glibenclamide. In the herbal formulation A showed maximum reduction (62.3% and 65.7%) and the effect was better than the standard drug glibenclamide.

The maximum reduction in the level of TBARS was observed in *C. pictus* extract (Plasma: 38.8% and 52.7; liver: 30.5% and 47.2%; kidney: 40.1% and 45.9%; brain: 36.6% and 57.7%) and in the herbal formulation maximum reduction was observed in formulation (A) (Plasma: 46.3% and 48.7%; liver: 38.2% and 42.1%; kidney: 37% and 41.4%; brain: 35.4% and 52%), in plasma, liver, kidney and brain of treated diabetic rats.

The changes observed in non-enzymatic antioxidants (GSH, vitamin C and vitamin E) in the diabetic condition was significantly ($p<0.05$) recovered by all plant extracts, the level of GSH and vitamin C was increased in plasma, liver, kidney and brain of diabetic rats. After the treatment this level was significantly recovered, in the individual plant extract

the highest percentage of recovery in GSH and vitamin C was observed in *C. alata* fresh leaf extract [GSH (Plasma: 35.7% and 38.4%; liver: 50% and 53.5%; kidney: 50% and 53.5%; brain: 41% and 43.1%); Vitamin C (Plasma: 46.6% and 50%; liver: 31.8% and 34.8%; kidney: 41.2% and 43.4%; and brain: 31% and 35.3%)] and in the herbal formulation maximum recovery in the activity of GSH was observed in formulation (A), [GSH (Plasma: 30.6% and 37.1; liver: 46.3% and 51.7%; kidney: 75.5% and 76.8%; brain: 58.2% and 59.7%)] and maximum recovery in the level of vitamin C was observed in extract (B) (Plasma: 56.3% and 58.8%; liver: 33.3% and 42.8%; kidney: 50% and 60%; brain: 36.2% and 42.1%).

The level of vitamin E during diabetic condition was increased in plasma and decreased in liver, kidney and brain. All the plant extracts treated significantly ($p<0.05$) recovered the level of vitamin E, maximum recovery in the level of vitamin E was observed in *B. purpurea* in the individual plant extract, (Plasma: 24.2% and 26.2%; liver: 24.9% and 29.89%; kidney: 45.2% and 50.3%; brain: 27.7% and 31.9%) and in the herbal formulation maximum recovery in the level of vitamin E was observed in formulation (B) (Plasma: 27% and 29.9%; liver: 30.9% and 36.3%; kidney: 54.6% and 54.1%; brain: 40.2% and 41.4%) in the treated diabetic rats.

After the treatment with the extract the level of SOD, CAT and GPX was decreased in liver and brain and in kidney the activity of GPX was increased and SOD and CAT was decreased during the diabetic condition. After the treatment this condition was recovered in all the plant extracts, the maximum improvement in the activity of antioxidant enzymes in liver, kidney and brain was observed in *C. alata* fresh leaf extract and herbal formulation (A). The percentage of recovery observed was, *C. alata* [SOD (Brain: 47% and 49.5%; liver: 58.6% and 58.6%; kidney: 54.5% and 55.7%); CAT (Brain: 38% and 43%; liver: 40.1% and 48.2%; kidney: 38.9% and 48.4%); GPX (Brain: 32.4% and 36%; liver: 40.5% and 46.7%; kidney: 36.1% and 43.1%)]. Herbal formulation extract (A) [SOD (Brain: 51.4% and 53%; liver: 61.9% and 63.2%; kidney: 58.5% and 59.3%); CAT (Brain: 50.65 and 54.2%; liver: 56.3% and 57.7%; kidney: 42.2% and 44%); GPX (Brain: 48.8% and 52.8%; liver: 43.2% and 50%; kidney: 48.7% and 52.5%)] in the treated diabetic rats.

Administration of the leaf extract could also influence the activity of carbohydrate metabolizing enzymes. The activity of hexokinase was increased and the activity of hepatic gluconeogenic enzymes, glucose-6-phosphatase and ftuctose-1, 6 – bisphosphatase were decreased significantly ($p<0.05$) after the treatment. In the individual plant extract the maximum percentages of activity was retained by *B. purpurea* and in the herbal formulation, formulation (A) showed maximum recovery in the activity carbohydrate metabolizing enzymes in treated rats. The percentage of recovery observed in *B. purpurea* was: (hexokinase: 47.4% and 47.4%; glucose-6-phosphatase: 38.7% and 41.9%; fructose-1, 6-bisphosphatase: 35.2% and 39.8%) and in formulation A was: (hexokinase: 45% and 45%; glucose-6-phosphatase: 48.4% and 51.6%; fructose-1, 6-bisphosphatase: 44.4% and 46.3%).

The major nitrogenous waste products, such as urea, uric acid and creatinine used to gets accumulated in the blood during diabetic condition, because the kidney fails to filter out this nitrogenous waste due to increased blood glucose level or due to kidney failure. In this study, these levels were decreased significantly ($p<0.05$) after the treatment in all the studied plant extract. Maximum reduction was observed in *C. alata* fresh leaf extract (urea; 40% and 48.4%, uric acid, 55.6% and 62.4%; creatinine; 72.1% and 79%) and in the herbal formulation (A) (urea; 60.7% and 63.9%, uric acid; 77.9% and 80.1%; creatinine; 79.1% and 81.4%).

Administration of all the extracts significantly reduced the lipid levels with an increase in the HDL-C. Maximum effect was exhibited by *C. alata* fresh leaf extract and herbal formulation (A). *C. alata* (Cholesterol: 40.4% and 44.3; free fatty acid: 20.2% and 26.9%; phospholipids: 18.6% and 28.6%; triglycerides: 43.9% and 52%; LDL: 53.3% and 58.6%; VLDL: 43.8% and 52.1% with an increase in HDL: 56.6% and 59%), herbal formulation (A) (Cholesterol: 68.8% and 68.4%; free fatty acid: 48.4% and 52.9%; phospholipids: 36.2% and 38.5%; triglycerides; 34.7% of & 44.8% with an increase in the HDL: 61.4% and 63.8%)

Thus, among the individual plants used *C. pictus* showed maximum hypoglycemic activity and *B. purpurea* was the most efficient in recovering the level of vitamin C, vitamin E and carbohydrate metabolizing enzymes. *C. alata* showed potential antioxidant activity and hypolipidemic effect; apart from this it also decreased the level of urea, uric

acid and creatinine in the treated rats, which clearly shows that the plant extract, protects the diabetic rats from alloxan induced renal damage. Though, the results observed in all these three plant extracts were significant, ($p<0.05$), these effects were less than the standard drug glibenclamide. The herbal formulation A was more efficient than the individual plant extracts and glibenclamide. The more efficacy of the formulation can be due to various properties of the different herbs. Ayurvedic remedies for diabetes are usually mixed formulations containing blood sugar lowering herbs in combination with immunomodulators, diuretics and detoxicants and in the present study it is clearly observed that herbal formulation was more effective than the single extract.

Highlights:

1. **Biomarkers (Pharmacognostic and Phytochemical) of different plants used are the following:**

Sr. No.	Name of the plant	Pharmacognostic and Phytochemical Biomarkers
1.	*C. alata*	➢ Lower surface of the epidermis were papillose towards the lamina, The papillae appeared as outgrowths, where as in surface view they gave the impression of rounded bodies. ➢ Compound starch grains and calcium oxalate crystals. ➢ Unicellular trichomes with swollen base. ➢ Flavonoids: Kaempferol, 3', 4'- OMe - Quercetin and 7, 3', 4'- tri OMe-Quercetin. ➢ Phenolic acids: 2-hydroxy benzoic, melilotic, *o*-Coumaric and *p*-Coumaric acids. ➢ Proanthocyanidins

2.	*B. purpurea*	➢ Multicellular multiseriate trichomes. ➢ Trichomes containing simple compound starch grains. ➢ The palisade was double layered. ➢ In T.S of the petiole, three vascular bundles were arranged two on the upper side and one on the lower side in a V shape. ➢ Flavonols: 3'-OMe-Quercetin, 4'-OMe-apigenin. ➢ Phenolic acids: Sinapic acids.
3.	*C. pictus*	➢ Palisade layer was absent. ➢ Simple unicellular, pointed non-glandular trichomes. ➢ The vein fibers are like shattered crystal pieces in structure present mostly in parenchyma cells. ➢ Flavonoids: Kaempferol, 3', 4'-di OMe-quercetin and 4'-OMe-Kaempferol. ➢ Phenolic acids: gentisic, 2, 5 – dihydroxy benzoic acid, *o*-Coumaric, melilotic, α-resorcyclic, 3,5-dihydroxy benzoic acid, *p*-hydroxy benzoic acid, *cis* and *trans* - *p*-coumaric acid.
4.	*A. heterophyllus*	➢ Latex ducts ➢ Double layered palisade

		➢ The spongy cells were of two types arranged in two regions, the cells of upper region was loosely packed while the lower layer contains closely packed cells. ➢ The pith region is abundantly filled with spharaphides. ➢ Simple compound starch grains. ➢ Flavonoids: 4'- OMe Apigenin, 3', 4'- di - OMe – luteolin. ➢ Phenolic acids: Vanillic and Syringic ➢ Proanthocyanidins

2. The optimum dose determination of individual plants and the herbal formulation was determined and the optimum doses are as follows:

- *C. alata* : 400 mg & 600 mg/kg body wt (45 days treatment)
- *B. purpurea* : 200 mg & 400 mg/kg body wt (60 days treatment)
- *C. pictus* : 200 mg & 400 mg/kg body wt (60 days treatment)
- *A. heterophyllus*: 400 & 600 mg/kg body wt (60 days treatment)
- Extract A : 100 & 150 mg/kg body wt (30 days treatment)
- Extract B : 100 & 200 mg/kg body wt (40 days treatment)
- Extract C : 200 & 300 mg/kg body wt (40 days treatment)

3. In all the extract studied except *A. heterophyllus* peak hypoglycemia occurred 2 hours after the drug administration in normal treated rats. In *A. heterophyllus* peak hypoglycemia occurred 3 hours after the drug administration.

4. Maximum glucose tolerance was observed in 30 min after the drug administration in normal glucose loaded rats in all plant extracts except *A. heterophyllus* extract, in this the maximum glucose tolerance was observed in 90 min after the drug administration.

5. The study indicated that all the plants were safe, after the treatment and the rats did not show any toxic effects or mortality up to a dose of 1 gm/kg body weight in individual plant extract and 500 mg/kg body weight in herbal formulation. Body temperature, state of stool, food and water in take were also not influenced by the treatment.

6. All the plant extract showed antidiabetic activity, antioxidant activity, hypolipidemic activity. Apart from this the plants could also recovered the diabetes induced changes observed in carbohydrate metabolizing enzymes and in the plasma urea, uric acid and creatinine, but the maximum recovery was observed in the following plants:

7. *C. pictus* extract showed maximum reduction in blood glucose concentration and TBARS in the treated diabetic rats. The effect was less than the standard drug glibenclamide.

8. *B. purpurea* recovered the level of vitamin C, vitamin E and the activity of carbohydrate metabolizing enzymes in the treated diabetic rats. The effect was less than the standard drug glibenclamide.

9. *C. alata* showed potential antioxidant activity, hypolipidemic effect; apart from this it also decreased the level of plasma urea, uric acid and creatinine in the treated rats. The effect was less than the standard drug glibenclamide.

10. Herbal formulation (A) [*Terminalia arjuna* Roxb **(Bark)**: *Gymnema sylvestre* Retz **(Leaf)**: *Syzygium cumini* L. Skeels **(Seed)** in the ratio 1:1:1.], a combination

of three herbs in different ratio showed an excellent result against all the extracts studied and standard drug glibenclamide, the extract could bring down all the parameters except vitamin C and vitamin E, almost to normal level with in 30 days of the treatment at a dose of 100 mg and 150 mg/kg body weight. The dose at 150 mg/kg body weight was better than 100 mg/kg body weight.

11. The level of vitamin C and vitamin E was maximum recovered by formulation (B), but this recovery was observed after 40 days of the treatment in 100 and 200mg/ kg body weight. But the formulation A could recover all other parameters with in 30 days of treatment in 100 and 150mg/kg body weight so the formulation (A) is considered to the best for the treatment of diabetes.

12. Thus, increased efficacy of the herbal formulation (A) against the individual plant extract and glibenclamide for diabetes may enhance therapeutic effect and shorten therapeutic course. This in turn, increases patient compliance and efficacy when compared to single therapies for various manifestations.