Chapter VI

RESULTS AND DISCUSSIONS
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The present work is a comprehensive study of various aspects of the medicinally important plant, *Costus pictus* D. Don.

The study of *Costus pictus* involved the investigations of following aspects. The observations recorded during the study and the resultant conclusions are summarized as under.

Micropropagation:

Micropropagation of *Costus pictus* D. Don is a maiden effort and is being reported for the first time.

- Dormancy of Axial Bud was successfully broken to develop Shoots. The best response for simultaneous Shoot and Root initiation was observed with MS medium fortified with PGR BAP and NAA in the ratio 1.0:1.0.
- Subcultures were established in the same combination of PGR.
- Predominant Shoot and scanty Root initiation was best observed with PGR BAP and NAA with the ratios 3.0: 1.0 and 4.0:1.0 (Table 2.2). Prominent Roots with not so well developed Shoots were best observed for BAP to NAA Ratio 1.0: 3.0 and BAP to 2, 4- D Ratio 0.5:3.0 (Figures 2.4a to 2.4i).
- The average time for bud break was 3 to 4 days and first leaf developed within 6 days.
- Sub culturing was necessary within 20 days. Transfer on fresh medium was done aseptically and contamination free plantlets were developed.
- Multiple Shoots proliferated best in BAP to NAA Ratios 4.0: 0.0 and 4.0:1.0 (Figures 2.3a and 2.3b). Shoots were separated and sub cultured on same medium. Rate of survival after subculturing was about 95%.
• The plantlets were successfully transferred to field after hardening for 10
days. Every twig was shown to develop into about 100 plantlets within a span
of 2 months.

• Callus initiation from leaves of Monocots is reported to be not possible. In the
current study, a large number of permutations and combinations of many
Auxins and Cytokinins (some of which not used normally) of varying
strengths were tried in a systematic manner (Table 2.4). All possible parts of
the plant ranging from Inflorescence to Roots and Rhizomes, even immature
and mature leaves, were inoculated as Explants for Callus initiation. Negative
response was reported in all the Callus initiation experiments.

Isolation and Study of Phytoconstituents:

Extraction of Phytoconstituents from Powder of dried leaves was carried out
successfully by employing Soxhlet Extraction procedure and Methanol was solvent
of choice. Methanolic Extract was subjected to Chemical analysis and its toxicity and
Efficacy as possessing anti-diabetic agent were tested further.

Fingerprinting of Phytoconstituents present in the leaves Costus pictus D. Don is a
maiden effort reported in the present study.

• Mobile Phases were developed for Alkaloids, Flavonoids, Saponins and
Triterpenoids in order to cause good separation of Phytoconstituents of that
class, present in Methanolic Extract of leaves of the plant.

• Fingerprints with respect to Alkaloids, Flavonoids, Saponins and
Triterpenoids were successfully recorded by employing HPTLC technique.
Parameters like chamber saturation time, amount of extract loaded and size of
plate were optimized.

• As per reported studies, only a few members of Zingiberaceae family have
Alkaloid contents and that too in less numbers. Analysis of fingerprints
obtained using two Mobile Phases, A1 and A2, for Alkaloids confirmed the
fact that even *Costus pictus* D. Don, a member of Zingiberaceae family, has only a few Alkaloids present.

- **Presence of Flavonoids was confirmed as the Fingerprints clearly brought out good separation of fractions of Flavonoids.**
- Presence of Saponins was also confirmed with Fingerprinting technique.
- **Separation of Triterpenoid fractions was tried using three Mobile Phases, T1, T2 and T3. It was observed that Mobile Phase T2, (Toluene: Ethyl acetate: Methanol (7: 1: 0.5)) produced the best separation and it was further observed that Triterpenoid fractions were largest in number.**
- Chromatograms were recorded under visible light as well as under UV 254 nm, 366 nm and 580 nm, both before and after derivatization.
- From Chromatograms so recorded, useful information regarding \( R_F \) values of separated constituents and their relative concentration was obtained.
- \( R_F \) can be used as supplementary information while establishing the identity of a new compound.

Triterpenoids are known as anti-oxidant agents responsible for improvement in general health and some Triterpenoids are reported to possess anti-diabetic and Hepatoprotective ability also. As the focus of the present study was on Diabetes during the course of the present study, Triterpenoids were studied in greater details.

- **10 Triterpenoid fractions were successfully resolved using Mobile Phase Toluene: Ethyl acetate: Methanol (7: 1: 0.5) using HPTLC technique and \( R_F \) values were obtained.**
- Analytical and Preparative TLC plates and HPTLC technique were used to isolate the fractions. In order to collect sufficient quantity of individual fractions and to facilitate further spectroscopic studies, fractions were collected from multiple plates developed in exactly same manner.
- All the 10 isolated fractions were subjected to FTIR analysis.
- FTIR absorption spectra of 10 fractions were distinct from each other. Hence, it can be concluded the fractions collected were distinct.
• Functional groups present in the fractions and the bonds responsible for IR absorption were identified.

Use of PTLC plates for isolation of fractions was reported as a novel method.

**Acute Toxicity Studies:**

As the plant under study is a new entrant in the research field of herbal medicines in India, *Acute Toxicity studies were conducted* on Albino Swiss female mice for test materials before taking up Efficacy studies.

• Acute Toxicity studies were carried out by strictly adhering to the protocol approved by the Institutional Ethics Committee.

• Powder and Methanolic Extract of dried leaves of *Costus pictus* were the test substances

• One group of animals was labeled as Normal Control and was fed water as dose material

• Animals separated in six other groups were fed predetermined test materials and pre decided dose levels on the first day of the study.

• The study was designed to obtain maximum tolerable dose without toxic effects and it was found that Maximum Dose of 2000 mg/ kg body weight for both Powder and Methanolic Extract also did not produce any toxic effects.

• Records of consumption of Water and Food were maintained. Throughout the study period, there was negligible variance in Food and Water consumption within a group for all groups, as well as when compared with Normal Control group.

• Thus it can be safely concluded that thirst associated with Toxicity was not observed and the appetite remained unaffected.

• Records of Body weights were maintained.

• *The body weights of all animals remained* almost constant over the study period and this fact is reaffirmed by the standard deviation values from daily average weights.

• There were no signs of higher rate of urination in any of the groups.
- There were no signs of toxicity in any animal.
- There was no report of either aggression or morbidity. Similarly, there was no report of mortality.

At the end of the study period, all animals from all the Dose groups remained healthy, without any signs of Toxicity.

The present study reports Toxicity Study for Powder of dried leaves of *Costus pictus* as a maiden effort and it has paved the way to use Powder of dried leaves as a test material for Efficacy study.

**Efficacy Study for Anti-Diabetic Activity:**

Efficacy study was conducted on Albino Wistar Female rats strictly as per the protocol approved by the Institutional Ethics Committee.

- Powder and Crude Methanolic Extract of dried leaves of *Costus pictus*, were the test substances.
- For Efficacy study, the maximum doses chosen were 900 mg/kg body weight of animal for Powder and 450 mg/kg body weight of animal for Methanolic Extract of leaves of *Costus pictus*, which were much below the highest dose tested for Toxicity (2000 mg/kg body weight doses). In the process, remote possibility of Toxicity due to long usage was avoided.
- Dose dependence of the test substances was also studied.

Comparison of Body Weights, Food and Water consumption, Blood Glucose levels and 9 other Blood Serum parameters of Diabetic Animals from Test material Dose groups with those of Normal Control, Diabetic Control and Modern Medicine Control Groups, brought out the following results:

- Increased Blood Sugar levels were observed to reduce due to administration of test materials and tendency towards normalization of Blood Sugar levels.
was observed in all animals treated with test materials. It proves that the leaves of *Costus pictus* D. Don possess anti-diabetic activity.

- Decreasing Body Weights see a reversal and increasing trend is set in.
- Abnormally high Water consumption steadily reduced and moved towards normalcy. Thus indirect conclusion of reversal of Polyuria can be drawn.
- Food Consumption exhibited trends of normalcy.
- Serum parameters like Cholesterol, Triglycerides, Total Proteins, Albumin, Globulins and Urea showed tendency towards normalization. It indicated Hepatoprotective activity possessed by leaves of *Costus pictus* D.Don
- Analysis of Serum Parameters like ALT, AST and Amylase of Powder and Extract Dose groups did not produce any conclusive results.
- The effect of Modern Medicine Glibenclamide, on the normalization of all deranged Serum Parameters values was very similar to that of the earlier reported studies on Diabetic animals.
- Efficacy results are found to be Dose dependent. Medium and High doses of Powder and Methanolic Extract produced best results.
- In Powder Low dose group, mortality of 3 animals was reported. It may be concluded that Powder Low dose of 100 mg/ kg body weight may be slow in producing desired reduction in Hyperglycemia.
- Extract Low dose was found to be better than Powder Low dose in controlling and correcting the deranged parameters.
- Powder Medium and High doses produced comparable results with corresponding Medium and High doses of Methanolic Extract with respect to all the monitored parameters.
- Hence it may be concluded that Powder and Methanolic Extract display equal efficacy.

Therefore, it can be emphatically stated that leaves of *Costus pictus* D. Don, whether in Powder form or in Methanolic Extract form, possess remarkable anti-diabetic and Hepatoprotective potential against Streptozotocin induced diabetes in Wistar rats. The present investigation is the first such comprehensive and comparative analysis of the Efficacy of *Costus pictus* D. Don.
Summary of the present work:

The present study reported maiden efforts in respect of

- *In vitro* Micropropagation of *Costus pictus* D. Don
- Fingerprinting of Methanolic Extract of leaves of *Costus pictus* with respect to Alkaloids, Flavonoids, Saponins and Triterpenoids
- Isolation of 10 fractions from Triterpenoid group by using Preparative TLC plates
- FTIR data acquisition for 10 fractions
- *In vivo* Acute Toxicity Study, for Powder of the leaves of *Costus pictus*
- *In vivo* Efficacy study for Powder of the leaves of *Costus pictus*
- Comparative evaluation of Efficacy of Powder and Methanolic Extract of the leaves of *Costus pictus*

From the results of *in vivo* Acute Toxicity Studies on animals, the leaves of *Costus pictus* D. Don are found to be Non-Toxic in both the forms, namely Powder and Methanolic Extract. Results of *in vivo* Efficacy Studies prove the Anti-diabetic and Hepatoprotective activity of the leaves of *Costus pictus* D. Don and both Powder and Methanolic Extract show comparable results with each other as well as when compared with Modern Medicine. *Costus pictus* D. Don can be cultivated commercially with the help of PTC technique.

It may hence be emphatically stated that the present study undertaken for *in vitro* Micropropagation of *Costus pictus* D. Don was successful. Isolation of Bioactive Molecules from Methanolic Extract of Leaves resulted into separation of 10 Triterpenoid fractions. The outcome of Study of Medicinal properties of leaves in the forms of Powder and Methanolic Extract as potential sources of drug against Diabetes is very encouraging. The Leaves of *Costus pictus* D. Don are proved to be an important contributor in pursuit of a new oral hypoglycemic agent.