SYNOPSIS

FOR THE THESIS TO BE SUBMITTED TO

THE UNIVERSITY OF MUMBAI

FOR

Ph.D. DEGREE

Title of the Thesis : Micropropagation and Isolation of Bioactive Molecules and Studies in Medicinal properties of Costus pictus D. Don

Subject : Biotechnology

Degree : Ph.D.

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Registration Number and Date : 52 / 07-12-2009

Date of Submission : 12/02/2013

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Title of the Thesis

Micropropagation and Isolation of Bioactive Molecules and Studies in Medicinal properties of *Costus pictus* D. Don

The research work undertaken and the subsequent analysis on the above mentioned topic will be presented in the thesis as follows

CHAPTER I: INTRODUCTION

The World Health Organization (WHO) projections on global health and related risks responsible for deaths globally are; High Blood Pressure (13%), Tobacco use (9%), High Blood Glucose (6%), Physical Inactivity (6%), Overweight and Obesity (5%). These risks manifest themselves into chronic diseases such as Heart Disease, Diabetes and several types of Cancer and affect all countries across all sections of population. In India, 32.7% of mortality in adults in the age group 30 to 70 was by Cardiovascular Diseases and Diabetes in the year 2008 (WHO, 2012). The exponential increase in the population affected by these diseases and the associated disorders has posed the greatest challenge for the world.

There has been a constant quest to make life healthier by discovering new drugs. The traditional systems of medicine are based on ancient wisdom and empirical data collected over the generations and bank upon products derived from natural resources. Traditional Asian, Chinese and homoeopathic medicines involve a very wide range of herbal products (Trease and Evans, 1987). Approach based on herbal medicine takes into consideration, the promotion of health and treating ailments in a holistic manner. WHO is now taking interest in traditional systems of medicine and plant remedies in particular, as a very large population uses herbal medicines (Trease and Evans, 1987).
With the advent of Biotechnology, plant kingdom is being increasingly manipulated to provide products for improving the health of the human beings. Presently the research in drug development is directed at finding new molecules or lead molecules for new drugs from plant kingdom as plants are capable of synthesizing an amazing variety of secondary metabolites. With the opening up of world markets and explosion of information, ancient wisdom is getting tested with advance analytical techniques. The pharmaceutical industry has to adhere to stringent norms before any new drug is launched in the market. There is now a clear focus on evidence based therapeutic evaluation of herbal products using statistical tools and standardizing the herbal products with the help of advancements in Pharmaceutical Biotechnology (Kayser and Muller, 2003).

In traditional systems of medicine, several plants have gained importance in the treatment of Diabetic Mellitus (Erasto P. et al., 2005; Jaradat N. 2005; Mohamed Bnouham. et al., 2006; Malaviya Neelesh. et al., 2010; Kavishankar G.B. et al., 2011; Patel D.K. et al., 2012). There are many effective synthetic and plant derived Oral Hypoglycemic Agents as a substitute for Insulin. (Mankil Jung et al., 2006). But looking at the endemic proportion of the disease Diabetic Mellitus; there is always a scope for study of another plant as a potential source of a new drug. Costus pictus D. Don, besides being an ornamental plant has become a potential source of Anti Diabetic Herbal Medicine having Hepatoprotective action. (Nandkumar Jothivel et al., 2007; Geerish G. et al., 2009). As per reports, active compounds in Costus pictus D. Don also display anti-oxidant /anti-cancer, anti-microbial, anti-inflammatory, hepatoprotective and diuretic effects (Meléndez-Camargo ME. et al., 2006; Nandkumar Jothivel et al., 2007; Nadumane et al., VK, 2011; Majumdar M. et al., 2012).

The plant, Costus pictus D. Don, is commonly known as Spiral Ginger, Stepladder or Insulin Plant. The plant has origin in Mexico. It belongs to
Zingiberaceae family. The plant can be recognized by its yellow flowers with beautiful red striations. The stem is reddish and has spiral leaves.

The present study is focused on comprehensive investigation in several aspects of the plant, *Costus pictus* D. Don.

When any plant is to be exploited as a source of new commercially viable drug, there is a requirement of continuous and sufficient supply of raw material of standard quality. In practice, the plant may be difficult to cultivate, may have slow growth or may vary in chemical constituents as quantity and quality of secondary metabolites vary a great deal with environmental factors. To combat inconsistent and insufficient supply of raw material for commercial viability, Plant tissue culture technique is of greatest help (George and Sherrington, 1984). The present investigation incorporates development of efficient protocol for *in vitro* rapid propagation of *Costus pictus* D. Don (Bhave SP et al., 2010).

Medicinal herbalists firmly believe that there is synergy between the molecule effective as medicine and other molecules present in the plant. The presence of all constituents together eliminates the side effects. The previous reported efficacy studies on laboratory animals have been on extracts (Nandhakumar Jothivel et al., 2007; Qi Xiang-Yang et al., 2008; Fujii et al., 2009; Jayasri M. A. et al., 2009; Bhanot Abhishek et al., 2010; Paul S. et al., 2011). There is no reported study of using *Costus pictus* leaf powder as an anti diabetic agent. To test the claim of synergy, *in vivo* efficacy study for anti diabetic activity was conducted for *Costus pictus* plant leaf powder and for crude Methanolic Extract of its dried leaves. In order to meet the OECD regulatory guidelines, *in vivo* Toxicity Studies were conducted with *Costus pictus* plant leaf powder as well as Crude Methanolic Extract of the plant leaf powder before the efficacy study.

Plant secondary metabolites act as lead molecules for development of new drugs. Many kinds of natural products belonging to Alkaloids, Flavonoids and
Terpenoids groups have shown Antidiabetic Potential. (Mankil Jung et al., 2006). Secondary metabolites belonging to these groups are currently used as drugs or as dietary supplements. To standardize the plant, Chemical finger printing with respect to Flavonoids, Saponins and Triterpenoids was carried out using HPTLC technique. Isolated fractions were analyzed. Statistical tools were used to critically analyze the observations.

CHAPTER II: In-Vitro PROPAGATION OF Costus Pictus D.Don

INTRODUCTION
Standard protocol for in vitro propagation of the plant Costus pictus D. Don is established using standard plant tissue culture techniques (George and Sherrington, 1984).

MATERIALS AND METHODS
Healthy green plants of Costus pictus D. Don were collected from Aromatic and Medicinal Gardens of S.H.Kelkar and Co. Mulund Mumbai. The dormant buds at the bases of leaves were used as explants. Explants were cleaned and surface sterilized using Mild Detergent, Antifungal and Antimicrobial agents to eliminate dirt and microbial contamination. Explants were then aseptically inoculated on sterile MS medium fortified with growth hormones like NAA, 2,4-D and BAP in various concentrations and combinations. Cultures were maintained under standard tissue culture conditions. Well established plantlets were transferred in pots and shifted to hardening room and later on to Green House and successfully planted in fields.

RESULTS
Costus pictus showed remarkable growth and on an average up to 4 multiple shoots could be established at any one site. Shoots were separated by subculturing and were established into new plantlets. Root initiation was successfully established along with shoot initiation by adding appropriate
combinations of Auxins and Cytokinins. Transfer to the field was achieved. Rate of survival on an average was 90%.

CHAPTER III: ISOLATION AND STUDY OF PHYTOCONSTITUENTS

INTRODUCTION
Dried leaves of Costus pictus D. Don were subjected to Crude Methanolic Extraction of Phytoconstituents using Soxhlet Extractor. Methanolic extract was preferred, as extract in Methanol contains maximum number of groups of Secondary Metabolites (Nandhakumar Jothivel et al., 2007). Methanol was evaporated and residue was analyzed for chemical constituents.

MATERIALS AND METHODS

1. EXTRACTION OF SECONDARY METABOLITES
Healthy and green plant material was collected from the experimental fields of Medicinal and Aromatic Gardens of S.H. Kelkar and Company, Mulund, Mumbai. Leaves free of dust and debris were shed-dried and powdered. The powder was subjected to extraction with Methanol as the solvent. Soxhlet extraction apparatus was used. The extract was subjected to TLC and HPTLC analysis.

2. FINGER PRINTING WITH RESPECT TO SECONDARY METABOLITES
Crude extract was subjected to TLC Analysis. Various solvent systems were used to document finger printing of the plant with respect to major groups of secondary metabolites using HPTLC technique.

3. ISOLATION AND ANALYSIS OF FRACTIONS
The fractions from crude Methanolic Extract were isolated using Preparative TLC technique and were subjected to further analysis (Harborne JB, 1998).
RESULTS
Solvent systems for TLC of broad groups of secondary metabolites were successfully developed and finger printing of the plant with respect to these groups was obtained using HPTLC technique. Collected fractions were successfully analyzed.

CHAPTER IV: In-Vivo ACUTE TOXICITY STUDY

INTRODUCTION
In vivo Toxicity Study for very fine powder of dry leaves as well as for crude leaf extract of Costus pictus D. Don was carried out. Protocol based on OECD guidelines for Toxicity Study was approved by Animal Ethics Committee and was strictly followed. The animals were housed and tested in an authorized animal house. All norms set by the Governing Body for Animal Experimentation in India, (CPSCEA) were strictly adhered to during the study period.

MATERIALS AND METHODS
In vivo Acute Toxicity Analysis of Crude Methanolic Extract as well as of very fine powder of dried leaves was carried out on female Swiss Albino Mice. Healthy young animals were procured from a laboratory permitted to breed laboratory animals. After acclimatization, animals were randomly selected and caged into seven distinct groups with five animals per group and were marked distinctly. Predetermined Dosages were orally administered to the animals only once on the first day of the study.

Group I was kept as Normal Control and was fed only the vehicle that is water.
Groups II, III and IV were fed Low, Medium and High Doses of powder of dried
leaves, 50 mg/Kg of body Wt, 300 mg/Kg of body Wt and 2000 mg/Kg of body Wt respectively.

Groups V, VI and VII were fed Low, Medium and High Doses of Crude Methanolic Extract of dried leaves, 50 mg/Kg of body Wt, 300 mg/Kg of body Wt and 2000 mg/Kg of body Wt respectively.

The animals were monitored daily during the study period and cage side observations for symptoms of Toxicity were noted.

RESULTS

During the study period, no apparent signs of Toxicity were observed in any animal across the test groups. All the physical parameters were found to be normal. There was no mortality reported.

CHAPTER V: In-Vivo EFFICACY STUDY FOR ANTI-DIABETIC ACTIVITY

INTRODUCTION

As the toxicity study showed no apparent signs of toxicity, in vivo efficacy study to establish Anti-Diabetic and Hepatoprotective Activity was carried out using very fine powder of dried leaves as well as crude extract of dried leaves of Costus pictus D. Don. Protocol for efficacy study was approved by Animal Ethics Committee. All norms for animal experimentation prescribed by CPSCEA were strictly followed and study was conducted in an authorized animal house.

MATERIALS AND METHODS

Efficacy study was carried out on female Albino Wistar Rats procured from a laboratory permitted to breed laboratory animals. After acclimatization, healthy animals were randomly selected and were separated into suitable Control and Test Dosage Groups with six animals per group. Diabetes was induced in the Test Animals. Daily, predeterminded dosages (as mentioned
below) were administered orally. Volume of every dose was 2 ml and the vehicle was water.

Group I Normal Control Rats were fed 2ml Water.
Group II Diabetic Control Rats were fed 2ml Water.
Groups III, IV and V Diabetic Rats received Low, Medium and High Doses of powder of dried leaves Costus pictus, 100 mg/Kg of body Wt, 300 mg/Kg of body Wt and 900 mg/Kg of body Wt respectively.
Groups VI, VII and VIII Diabetic Rats received Low, Medium and High Doses of crude Methanolic Extract of dried leaves, 50 mg/Kg of body Wt, 150 mg/Kg of body Wt and 450 mg/Kg of body Wt respectively.
Group IX Diabetic Rats received doses of modern medicine, Glibenclamide 10 mg/Kg of body Wt.

During the study period, blood was collected on 1st, 7th and 14th days from every animal. Ten Blood Biochemical Parameters like Blood Glucose, AST, ALT, Blood Urea, Cholesterol and Protein were evaluated using standard protocols. Blood Glucose levels were checked on 10th day as well. At the end of the study period, animals were euthanized.

RESULTS

On daily oral administration of the plant leaf powder as well as methanolic crude extract of dried leaves to diabetic animals, statistical analysis of blood sugar and other blood serum parameter values showed tendency towards normalization.

CHAPTER VI: RESULTS AND DISCUSSIONS

The chapter presents comprehensive analysis of the results obtained during the course of present investigation. It tries to define the role; the plant under
investigation, *Costus pictus* D. Don can play in the quest for a remedy for many a disease in the world.

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