# FABRICATION AND EVALUATION OF ANTI- DIABETIC SUSTAINED RELEASE HERBAL TABLETS

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## ABSTRACT

The present study was to fabricate and evaluate oral anti- diabetic sustained release herbal tablets by direct compression method with dry extracts of Costus Pictus., Trigonella Foenum- Graecum and Abelmoschus Esculentus were used as the polymers and binding agent respectively in the preparation of Costus Pictus sustained release tablets.Costus Pictus were subjected for isolation and identification of active compound. The isolated compound were identified by UV, IR, Mass and NMR spectroscopy and the compound identified as the β-Amyrin of triterpenoids, which may be responsible for the anti diabetic activity. Physico chemical properties of dried powdered blend were studied. Prepared tablets were evaluated for thickness, hardness, friability, uniformity of weight, swelling behavior and release ate characteristic were studied. The extract of Trigonella Foenum- Graecum and Abelmoschus Esculentus showed sustained release of the drug Costus Pictus with the concentration of 15 % of the binder and the polymer. The in-vitro release data was applied to various kinetic models and the optimized formulation (F6) was to follow zero order kinetics. In- vivo studies were done to evaluate anti diabetic activity in rabbits and the formulation F6 was found to be the best among all the formulation. Studies indicated that the extracted drugs is a good pharmaceutical adjuants and possess anti – diabetic activity. Finally it is concluded that the herbal tablets of Costus Pictus has reduced side effects, low cost and improved bio availability of the drug.

**KEYWORDS:** Costus Pictus, Trigonella Foenum- Graecum and Abelmoschus Esculentus, Controlled release., Matrix Tablets.

## INTRODUCTION

Herbal medicines are the oldest remedies known to mankind. herbs has been used by all culture throughout history but India has one of the oldest , richest and most diverse cultural living traditions associated with the use of medicinal plants.[1] .Designing of oral herbal formulations is till date of challenge in modern pharmaceutics. There are number medicinal herbs in traditional system of medicine which are time tested and useful for the number of aliment. Diabetes is a chronic disorder of the metabolism of carbohydrate, proteins and fat are caused due to absolute or relative degree of insulin resistance.[2] It has become an epidermic and there are more than 30 million people with diabetes mellitus in India and the incidence is increasing. Insulin and various types of hypoglycemic agents such as biguanides and sulphonyl ureas, old and new are available for the treatment of diabetes. However none of these medications are ideal due to toxic side effects.[3]

There is an increased demand by patients to use natural product with antidiabetic activity.[4] Herbal medicines are considered to be less toxic and more free from side effect than synthetic ones . In the traditional system of Indian medicinal plant formulation and several cases, combined extract of plant are used as drug of choice rather than individual. Many of these have shown promising effect.[5] Many plants are reported useful for the treatment of diabetes mellitus in ayurveda system of medicine have been tested on experimental animals. [6] So with increasing incidence of diabetes mellitus in rural population, and due to its adverse effects of synthetic medicine,there is a clear need for development of indigenous inexpensive botanical source of antidiabetic cure drug.[7]

Tigonella foenum-graecum L (Fenugreek) belongs to the Leguminosae family. Fenugreek seed have been widely used in food as a flavor component and seasoning in folk medicine as tonic .[8, 9] In clinical pharmacology and biological tests, extracts and fractions of Fenugreek seeds are reported to have glucose and lipid lowering properties of antioxidant and antiphlogistic effects. Since Fenugreek seeds produce high viscosity mucilage at low concentration levels, the binder effects of this mucilage was used in tableting. A binder holds powders together to form granules and also provides the cohesive required for the binding of the granules under compression to form a tablet.[10]

Abelmoschus esculentus (Malvaceae family) is an annual or perennial climber; growing up to a 2m tall. The fruit is a capsule up to 18cm long. Fruits are cooling, stomachic, astringent and aphrodisiac used in chronic dysentery. These fruit mucilage are used as a release retardant for making controlled release matrix tablets.[11]

Costus Pictus D. Don (Fingiberaceae) commonly known as spiral Ginger, grown in gardens as an ornamental plant known to possess anti diabetic property.

Plants have always been great source of drugs and many of the currently available drugs have been derived directly or indirectly from them. The aim of the present work is to fabricate and evaluate oral anti- diabetic sustained release herbal tablets by direct compression method using dry extracts of Costus pictus Trigonella foenum-graecum and Abelmoschus esculentus as polymerand binding agent respectively. The optimized formulation was selected and evaluated for anti- diabetic activity in alloxan- induced diabetic rabbits.There has been increasing demand for the use of plant products with anti-diabetic activity due to low cost, easy availability and lesser side effects.

## MATERIALS AND METHODS

### Ingredients used

Costus Pictus, Trigonella foenum- graecum, Abelmoschus esculentus (Green chem, Bangalore), Magnesium stearate, Aerosil, micro crystalline cellulose (S.D fine chemicals).

### Methods

### Collection and Preparation of plant extract

The plant *Costus pictus* was collected from the Nandha college campus, Erode. The dried leaves of the plant were sent and extracted in the Green chem laboratories, Bangalore.     The powder form of leaves of *Costus pictus* was taken and subjected to successive solvent extraction. The extraction was carried out with the following solvents petroleum ether, and aqueous methanol. Then finally it is dried and made into the powder form. The extract was then distilled, evaporated and vacuum dried. The successive aqueous methanolic extract value of *Costus pictus* was 28.5%w/v. which was done by prescribed monograph specified in I.P 1996.

## Phytochemical Investigation[12]

The phytochemical tests were carried out to find the presence of Phytoconstituents using the standard procedures. Chemical test for carbohydrates, proteins, alkaloids, steroids/ triterpenoids, flavonoids, tannins, glycosides, saponins, fixed oils and fats were performed by using the various tests for each of the above.

## Physico-Chemical Parameters

The organoleptic characters[13] of the samples were evaluated based on the method described by Siddiqui *et al*. Organoleptic evaluation refers to evaluation of the formulation by color, odour, taste and texture etc.1% solution of polyherbal formulation was prepared with distilled water and pH was determined using pH meter. Solubility of isolated mucilage was studied using different types of solvents like water, alcohol, acetone, polyethylene glycols, propylene glycol, glycerin, sorbitol, ethyl alcohol, methanol, benzyl alcohol, isopropyl alcohol, etc.[14] The crystals are powdered finely and charged into a capillary tube seated at one end. The capillary tube is tied to the thermometer and thermometer is now lowered in a beaker containing paraffin oil and the beaker is heated slowly and the temperature of the bath kept uniform by gentle but constant stirring with a ring stirrer. When the substance in the capillary just shows signs of melting, the burner is removed and the stirring continued. The temperature at which the substance just melts and becomes transparent is noted.[15]

Loss on drying is the loss of weight expressed as percentage w/w resulting from water and volatile matter of any kind that can be driven off under specified conditions as specified in the individual monograph.[16] Moisture content was determined by loss on drying (LOD) method.[17] 3 gm of the weighed quantity of the drug was taken and kept in oven at 1050C till a constant weight was obtained. Amount of moisture present in the sample was calculated as reference to the air dried drug.

## Determination of Swelling Index[18]

The natural suspending agent 1g was taken in a china dish and then 10 ml of distilled water was added and the mixture was shaken and allowed to stand for 1 hour. After 1 hour the remaining water in china dish was discarded and the weight increase of the natural suspending agent was rated.

**Swelling Index % (SI) = (W2 – W1/W1) x 100**
W1= Weight of tablet at time '0'

W2= Weight of tablet at time't'.

**Determination of viscosity and density**

Density and viscosity of the 1% aqueous polyherbal formulation was estimated.[19]

**Total Ash[16]**

Heat a platinum or silica crucible to red heat for 30 minutes; allow cooling in a desiccators and weigh it. Weigh accurately about 1 g of the substance under examination and evenly distribute it in the crucible. Dry at 1000C to 1050C for 1 hour and ignite to constant weight in a muffle furnace at 6000C ± 250C. Allow the crucible to cool in desiccators after each ignition. Ignite to constant weight. Calculate the percentage of ash on the dried basis.

**Acid-insoluble ash**

Boil the ash with 25 ml of 2M hydrochloric acid for 5 minutes, collect the insoluble matter in a grouch crucible or on an ash less filter paper, wash it with hot water, ignite, cool in a desiccators and weigh. Calculate the percentage of acid-insoluble ash on the dried drug basis.

**Water-soluble ash**

Boil the ash for 5 minutes with 25 ml of wate*r*, collect the insoluble matter in a gooch crucible or an ash less filter paper, wash with hot water, and ignite for 15 minutes at a temperature not exceeding 4500C. Subtract the weight of the insoluble matter from the weight of the ash; the difference in weight represents the water-soluble ash. Calculate the percentage of water-soluble ash on the dried basis.

**Heavy Metals[16]**

The limit for heavy metals is indicated in the individual monographs in terms of ppm, i.e., the parts of lead, Pb, per million parts (by weight) of the substance under examination. Take 2 cylinders containing the standard solution (1.0 ml of lead standard solution) and the test solution (specified quantity of the substance) add 5 ml of dilute sodium hydroxide solution, 5 drops of sodium sulphide solution and make up to the required volume with water mix, allow to stand for 5 minutes and view downwards over a white surface; the color produced with the test solution is not more intense than that produced with the standard solution.

**Particle size determination by microscope[16]**

The eye piece micrometer has to be standardizing before going to the procedure. A small amount of sample is taken with liquid paraffin. A few drops of this transferred to a glass slide

and focused in a microscope, diameter of 200 particles are determined randomly. The number of particles in each size range is then counted and tabulated.

## TLC of plant extract

The TLC plates are prepared by pouring method and they are activated for 1 hour at $100^0$c. The aqueous methanolic extract is dissolved in few ml of methanol and the sample is spotted using a capillary tube. The solvent system is saturated and the plates are immersed in the chamber. After the solvent front runs 3/4th of the plate, then the plates are removed and dried and then the spots are detected using the detecting agent and the Rf value is calculated.

$$Rf = \frac{\text{distance travelled by solute from origin}}{\text{distance travelled by solvent from origin}}$$

## Isolation and identification of active compound

Silica gel G was used as the Stationary phase and the solvent system used was n-butanol: 2M ammonium hydroxide in the ratio of (1:1) with the plate thickness of 0.1mm and saturated with iodine vapours for a period of 45 mins. The Rf value obtained was 0.48 .Plant active constituents responsible for anti-diabetic properties were isolated by thin layer chromatography (TLC). Acid hydrolysis was carried out on vaccum-dried aqueous methanol extract of *Costus pictus* to liberate aglycones, if any glycosides were present. The concentrates were spotted on activated TLC plates of silica gel GF 254 (60-120 mesh) of 0.5 mm thickness coating. The plates (20cm × 5cm) were developed with solvent system n-butanol-2M ammonium hydroxide (1:1) to elute α and β-Amyrin. [20, 21]The developed plates were air-dried and detected by Carr price reagent, 20% antimony chloride in chloroform was sprayed and dried in a chromatographic oven at 1050C for 30 min. The resolution bands were obtained and retardation factor (Rf) values calculated. The β-Amyrin found in the concentrate was identified by comparing the Rf value with earlier-reported study. [22] The fractions of similar TLC patterns were combined, concentrated and chromatographed repeatedly over silica gel GF 254 (100-200 mesh) columns of 60cm × 3cm to isolate active compound and confirmed by qualitative chemical analysis.[23] The crystals are identified by UV, IR, NMR and MASS.

## Preformulation Studies

Preformulation studies were performed to assess the physicochemical properties and release characteristics of the developed formulations.

## Compatibility studies of drug and polymers[24]

The pure drug, the mixture of polymers and a mixture of drug with polymer were mixed with IR grade KBr in the ratio of 100:1. The base line correction was done using dried KBr. Infrared spectra of the mixture were taken over a wave number range of 4000-400 cm-1 also the infrared spectra of the drug and polymers were run individually. Then it was investigated for any possible interaction between polymer and drug.

## UV- Spectral analysis of drug

*Costus pictus* drug solution in phosphate buffer PH 6.8 was scanned using UV-Spectrophotometer between the range 200-400nm using phosphate buffer PH 6.8 as blank and the range of the drug was found to be 273.44nm.

## Preparation of calibration curve of c*ostus pictus*

*Costus pictus* can be estimated spectrometrically at 273.44 nm as it obeys Beer's –Lambert's law limit is the range of 10-50µg/ml.100 mg of *Costus pictus* was dissolved in small quantity of phosphate buffer PH 6.8 and made up to 100 ml using phosphate buffer PH 6.8 which is the stock solution. From this solution, 1ml was taken and dissolved in 10 ml of phosphate buffer PH 6.8 and once again 1 ml was taken and dissolved in 10 ml to obtain a concentration of 10 mcg/ml. Similarly, other concentrations were made.

## Pre - compression parameters[25]

### Angle of repose

Angle of repose was determined by using funnel method. The accurately weighed blend was taken in a funnel. The height of the funnel was adjusted in such a way that the tip of the funnel just touches the apex of the heap or head of blend. The drug excipients blend was allowed to flow through the funnel freely on to the surface. The diameter of the powder cone was measured and angle of repose was calculated using the following equation.

**Tan θ = h/r**

Where h = height of powder cone formed

r = radius of the powder cone formed

### Bulk density

Apparent bulk density was determined by pouring a weighed quantity of blend into graduated cylinder and measuring the volume and weight.

**Bulk density = Mass of the powder (g) / Bulk volume of powder (cc)**

**Tapped density**

It was determined by placing a graduated cylinder, containing a known mass of drug excipient blend. The cylinder was allowed to fall under its own weight on to a hard surface from the height of 10 cm at two second intervals. The tapping was continued until no further change in volume was noted.

**Tapped density = Mass of the powder (g) / Tapped volume of powder (cc)**

**Carr's index**

The Compressibility index of the blends was determined by Carr's compressibility index.

**Compressibility index (%) = (Tapped density - Bulk density) x 100 / Tapped density**

**Hausner ratio**

It is the measurement of frictional resistance of the drug. The ideal range should be 1.2-1.5. It is determined by using the following formula,

$$\text{Hausner ratio (IH)} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

*In-vitro* **absorption study**[26]

**Calibration curve of c*ostus pictus***

Weigh accurately 100mg of *Costus pictus* and transfer into a 100ml volumetric flask. Add small quantity of pH7.4 krebs bicarbonate fluid and shake the solution thoroughly to dissolve salicylic acid .Finally make up the volume with pH 7.4 krebs bicarbonate fluids. Transfer 10ml of the primary stock solution to 100ml volumetric flask and make up the volume with pH7.4 krebs bicarbonate fluid. Prepare dilute solutions with 10, 20, 30, 40 and 50 mcg/ml from the above solution in 10ml standard flask. Measure the absorbance of the solution at 273.44nm using pH 7.4 krebs bicarbonate fluids as a blank.

**Everted gut sac technique**

Isolate a small segment of the intestine of a laboratory animal usually a rat or hamster. Wash the small intestine thoroughly with 0.9%w/v saline at room temperature. Remove the distal to the ligament of traits and evert over a glass rod. Transfer the everted intestine into oxygenated saline (0.9%) at room temperature. Cut 6cm of sac from the jejuna or proximal ilea region of the small intestine and large intestine. Fill small volume (3ml) of drug free

physiologic buffer medias to the everted segment of sac. Tie both ends of the segment and immerse the sac in an Erlenmeyer flask containing a relatively larger volume of buffer solution that contains the drug (1mg/ml).

Inoculate each sac for 120 minutes. The solutions inside the sac are termed as serosal fluid and the drug solution bathing the luminal surface of the intestine is termed as mucosal fluid. Oxygenate the contents and agitated continuously at $37^0$C for a period. Collect the serosal fluid after incubation and the drug content is estimated using uv/visible spectrophotometer.

### Identification of active buffer for *Costus pictus*

Identification of active buffer for c*ostus pictus* were conducted using dissolution testing apparatus II (Paddle method). The dissolution test was carried out using 900 ml of acidic buffer pH1.2 and phosphate buffer pH 6.8, 7.2 and 7.4 at $37 \pm 0.5^0$c and 50 rpm. 10ml of the sample was withdrawn from the dissolution apparatus at 120 minutes and withdrawn sample were replaced with fresh dissolution medium. The samples were filtered through 0.45 μ membrane filter and the absorbance was measured at 273.44nm using UV spectrophotometer.[27]

### Formulation of costus *pictus* herbal tablets

Herbal tablets of costus pictus were prepared separately by direct compression process using different concentrations of polymers 10% and 15% of Abelmoschus esculentus as prolonged release polymer and Trigonella foenum-graceum as a binder. The composition of various formulations is given in Table 1. All the ingredients were passed through mesh no. 100 and mixed with aerosil, microcrystalline cellulose and magnesium stearate. The micromeritic properties were determined for all the mixtures. The powder mixtures possess good flow properties and good packing ability. Thus, the mixtures were directly compressible. Tablets were compressed each of 300 mg on a 16 stage stationary rotary punching machine fitted with 8-mm flat-shaped punches. No manufacturing defects were observed in tablets like capping, lamination and chipping.

**Table1: Composition of herbal tablets of *Costus pictus*.**

| Ingredients (mg/tablet) | Formulation code | | | | | |
|---|---|---|---|---|---|---|
| | F1 | F2 | F3 | F4 | F5 | F6 |
| *Costus pictus* | 170 | 170 | 170 | 170 | 170 | 170 |
| *Trigonella foenum- graecum* | 17 | 25.5 | _ | _ | 17 | 25.5 |
| *Abelmoschus esculentus* | _ | _ | 17 | 25.5 | 17 | 25.5 |
| **Magnesium stearate** | 5 | 5 | 5 | 5 | 5 | 5 |
| **Aerosil** | 5 | 5 | 5 | 5 | 5 | 5 |
| **Micro crystalline cellulose** | 103 | 94.5 | 103 | 94.5 | 86 | 69 |
| **Total weight (mg)** | 300 | 300 | 300 | 300 | 300 | 300 |

**Post Compression Parameters[28, 29]**

**Thickness**

Control of physical dimension of the tablet such as thickness is essential for consumer acceptance and tablet uniformity. The thickness and diameter of the tablet were measured using vernier calipers. It is measured in mm.

**Hardness**

Hardness indicates the ability of a tablet to withstand mechanical shocks while handling. The Monsanto hardness tester was used to determine the tablet hardness. Hardness was expressed in kg/cm2.Three tablets were randomly picked from each formulation and hardness of the tablets was determined.

**Friability**

Tablet strength was tested by using Roche friabilator. 20 tablets were weighed and placed in the friabilator and operated at for 100 revolutions (4min), taken out and were dusted again. The percentage weight loss was calculated by weighing the tablets again. The % friability was then calculated by.

$$F = \frac{(W_{initial}) - (W_{final})}{(W_{intial})} \times 100$$

**Weight variation**

Ten tablets were selected randomly from each batch and weighed individually. The average weight was noted and standard deviation was calculated. The tablet passes the test if not more than two tablets fall outside the percentage limit and none of the tablet differs by more than double the percentage limit.

$$PD = \frac{(W_{avg}) - (W_{initial})}{(W_{avg})} \times 100$$

Where, PD = Percentage deviation,

$W_{avg}$ = Average weight of tablet,

$W_{initial}$ = individual weight of tablet.

**Uniformity of drug content**

The drug content was performed to check the dose uniformity in the formulation. Randomly ten tablets were weighed and powdered. A quantity equivalent to 100mg of *Costus pictus* was taken in a 100ml volumetric flask, dissolved, and made up to the volume with phosphate buffer pH 6.8. After suitable dilutions the drug content were determined by UV spectrophotometer.

**Swelling behavior of herbal tablets[30]**

The extent of swelling was measured in terms of % weight gain by the tablet. The swelling behavior of batches F1, F2, F3, F4, F5 and F6 were studied. One tablet from each batch was kept in a Petri dish containing phosphate buffer of pH 6.8. At the end of 2 h, the tablet was withdrawn, kept on tissue paper and weighed, repeated for every 2 h till the end of 12 h . The % weight gain by the tablet was calculated by following equation.

**S.I = {($M_t$-$M_0$) / $M_0$} X 100**

Where,

S.I = Swelling Index

$M_t$ = Weight of tablet at time 't'

$M_o$ = Weight of tablet at time 0

***In- vitro* dissolution studies[31]**

The release rate of *Costus pictus* herbal tablets were conducted using dissolution testing apparatus II (Paddle method). The dissolution test was carried out using 900 ml of phosphate buffer PH 6.8, at 37 ± 0.50C and 50 rpm. 10ml of the sample was withdrawn from the dissolution apparatus at regular intervals up to 24 hours and withdrawn sample was replaced with fresh dissolution medium. The samples were filtered through 0.45 µ membrane filter and the absorbances were measured at 273.44nm using UV spectrophotometer. The extent of drug

release was estimated by plotting a graph against time versus percentage cumulative drug release.[27]

## Kinetic analysis of *in-vitro* release rates of costus pictus herbal sustained release tablets[32, 33]

To analyse the mechanism of c*ostus* pictus release characteristics from the prepared herbal formulations, the data obtained from *in-vitro* release studies were subjected to zero order, first order, higuchi's and korsmeyer – peppas models.

## Anti-diabetic activity

## Approval of animals for the study

The study was conducted after obtaining the approval from committee for the purpose of control and supervision on animals (CPCSEA) and institutional animal ethics committee (IAEC), proposal number NCP/IAEC/PG/2010-04.

## Selection and maintenance of animals.

Wistar albino rabbits of either sex weighing around 1.5-2.5 kg were selected for the experiment. The animals were checked for the free of any disease, only healthy rodent is accepted for the experiments. The rodents are collected from the animal house of Nandha college of pharmacy and research institute, Erode 52.The selected rodents are brought to the laboratory two days before the commencement of the experiment and provided with standard laboratory rodent chow diet obtained from (Pranav Agro Industries Ltd, Bangalore) and free access of water, 12hrs/ day/ dark cycle and room temperature is maintained $27^0$c.The night before the commencement of the experiment food is withdrawn but free access of water is provided.[34]

## Induction of diabetes

Wistar albino rabbits (1 year old) of either sex, weighing 1.5–2.5 kg, were used as the test animal. The rabbits were fed on a standard pellet diet (Hindustan lever ltd., Bangalore, India) and water *ad libitum* and maintained at $28\text{-}30^0$c. After laboratory acclimation for 7 days, the rabbits were starved for 48 hours and divided into groups. The initial blood glucose levels were checked and injected with alloxan 150mg/kg dose by intra venal route through marginal ear vein in normal saline. The blood glucose levels were checked after 72 hours of alloxan injection. The animals were considered diabetic when the blood glucose level was raised beyond the 200mg/dl; this condition was observed at the end of 72 hrs after alloxan injection.

Alloxan induces DNA fragmentation in pancreatic islets and cell damage has been attributed to the production of toxic free radicals.[35, 36]

**Experimental procedure**
After confirmation of increased hyperglycemia the diabetic rats were divided into different groups as mentioned below.

**Groupings of animals**

| | | |
|---|---|---|
| Group I | = | Control (Normal saline 1ml/kg) |
| Group II | = | Diabetic Control (Alloxan 150mg/kg) |
| Group III | = | Alloxan + *Costus pictus* herbal tablet formulation F1 (170mg) |
| Group IV | = | Alloxan + *Costus pictus* herbal tablet formulation F2 (170mg) |
| Group V | = | Alloxan + *Costus pictus* herbal tablet formulation F3 (170mg) |
| Group VI | = | Alloxan + *Costus pictus* herbal tablet formulation F4 (170mg) |
| Group VII | = | Alloxan + *Costus pictus* herbal tablet formulation F5 (170mg) |
| Group VIII | = | Alloxan + *Costus pictus* herbal tablet formulation F6 (170mg) |
| Group IX | = | Alloxan + Glipizide (5mg/kg) |

The drug was dissolved in normal saline and it was administered orally via a standard orogastric cannula, anti-hyperglycemic activity in diabetic rabbits was assessed by fall in fasting blood glucose level.[37] Blood samples were collected directly from a pinna venule using a syringe carrying a size of 26 needle on day 1, 7, 14 and 21days after the last dose of treatment. Blood sugar was determined using a glucometer (Simple one touch Johnson & Johnson Co., USA).

**Estimation of biochemical parameters in blood serum[38]**
After the completion of experiment, the blood were collected directly from a pinna venule using a syringe carrying a 26 needle under mild ether anesthesia in Eppendorff's tube (1ml) containing 50μl of anticoagulant (10% trisodium citrate) and the serum was separated by centrifuging at 3000rpm for 15min. The biochemical parameters including liver function test, kidney function test and lipid profile were determined by using the commercial kit. On 21st day the animals were deprived from food overnight and sacrificed by cervical dislocation. The liver, kidney and spleen were dissected out, washed with normal saline and stored in 10% formalin solution for histopathological studies.

## RESULTS AND DISCUSSION

### Phytochemical analysis of aqueous methanolic extract of Costus pictus

Aqueous methanolic extract of Costus pictus extract were subjected to qualitative phytochemical tests for different phytochemical constituents. From the phytochemical analysis, the plant extract shown the presence of carbohydrates, proteins, alkaloids, flavonoids, terpenoids, tannins and saponins and absence of steroids, glycosides, and fixed oils.

### Physico-chemical parameters

**Table2. Physicochemical properties of *Costus pictus, Trigonella foenum- graecum, Abelmoschus esculentus* extracts.**

| S.No | Properties | Observation | | |
|---|---|---|---|---|
| | | *Costus pictus* | *Trigonella foenum- graecum* | *Abelmoschus esculentus* |
| 1 | Organoleptic Evaluation**.**<br>• Colour<br>• Odour<br>• State | Brown<br>Characteristic<br>Amorphous | Cream yellow<br>No characteristics<br>Amorphous | Brown<br>Characteristic<br>Amorphous |
| 2 | pH | 6.2±0.05 | 5.9±0.05 | 6.8±0.05 |
| 3 | Solubility | Slightly soluble in water and greatly soluble in 50% methanol. | Slightly soluble in cold water and insoluble in ether, acetone and chloroform | Slightly soluble in cold water and insoluble in ether, acetone and chloroform |
| 4 | Melting point | 138-140$^0$C | 132-135$^0$C | 125-130$^0$C |
| 5 | Moisture content (%) | 3.2±0.02 | 2.4±0.02 | 6.66±0.03 |
| 6 | Swelling factor (%) | **-** | 150% | 85% |
| 7 | Total ash (%) | 1.37%±0.006 | 0.907±0.015 | 0.2±0.001 |
| 8 | Acid-insoluble ash (%) | 0.92%±0.008 | 0.847±0.015 | 0.15±0.002 |
| 9 | Water-soluble ash (%) | 0.35%±0.004 | 0.01±0.002 | 0.02±0.001 |
| 10 | Heavy metals:<br>Lead<br>Arsenic | *NMT 5 ppm<br>*NMT 1 ppm | *NMT 5 ppm<br>*NMT 1 ppm | *NMT 5 ppm<br>*NMT 1 ppm |
| 11 | Particle size | 45-60 µm | 60-75 µm | 50-60µm |
| 12 | Density | 0.995gm/cc | 0.947 gm/cc | 0.978 gm/cc |
| 13 | Viscosity | 1.81cp±0.005 | 500 cp | 300cp |
| 14 | Yield (%) | 28.5 w/v | 30 w/v | 8 w/v |

*NMT – Not more than

Physicochemical properties of Costus pictus, Trigonella foenum- graecum, Abelmoschus esculentu*s* extracts were studied and all the properties were found to be within the limits

(Table 2). All the extracts showed good flow properties and satisfactory compressibility index.
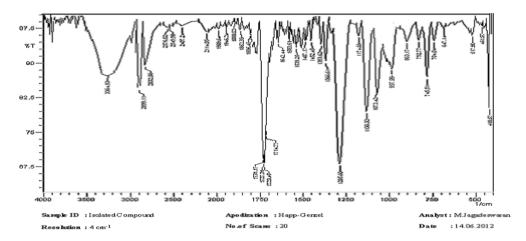
**Identification of the isolated compound**
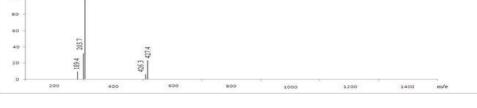


**Fig 1: FTIR Spectrum of isolated compound**
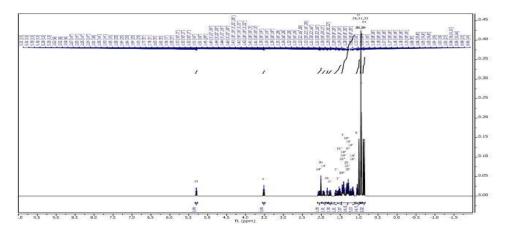


**Fig 2: Mass Spectrum of Isolated compound**



**Fig 3: NMR Spectrum of Isolated compound**

From the IR, Mass and NMR spectrum it was confirmed that the isolated compound present in the *Costus pictus* were found to be β-Amyrin.

**Preformulation Studies.**

**Fourier Transform Infrared Spectroscopy (FT-IR) analysis.**

The FT-IR spectra of *Costus pictus*, *Trigonella*, *Abelmoschus*and there mixtures were recorded. The drug, *Costus pictus*, *Trigonella*, *Abelmoschus*and there mixtures are present in the formulation was confirmed by FT-IR spectra. The characteristics peaks due to pure *Costus pictus* have appeared in mixture without any remarkable change in their position (Table 3) after successful formulation indicating no chemical interaction between drug and polymers (Fig 4, 5, 6& 7). It also confirmed the stability of drug.

**Table3: Interpretation of FT-IR spectrum**

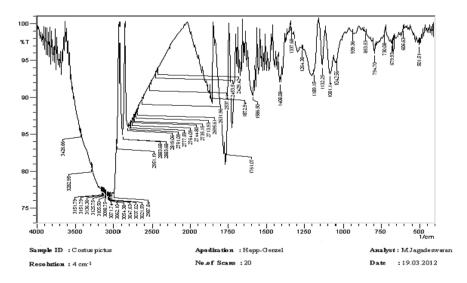| Types of vibrations | IR absorption Ranges (cm-1) | Drug (cm-1) | | | Physical mixture + drug |
|---|---|---|---|---|---|
| | | *Costus pictus* | *Trigonella foenum-graecum* | *Abelmoschus esculentus* | |
| -OH Stretching | 3500-3000 | 3526.66-3021.59 | 3568.43-3000 | 3534.57-3001.30 | 3510.36-3000 |
| SP3 C-H Stretching | 3000-2801 | 2951.19 | 2885.57 | 2949.26 | 2961.80 |
| C = O | 1810-1690 | 1761.07 | 1765.89 | 1766.85 | 1738.29 |
| C = C | 1650 | 1651.19 | 1641.48 | 1659.80 | 1655.94 |
| C - C | 1200 | 1189.15 | 1209.41 | 1203.56 | 1205.12 |
| = C – H Out of plane bending | 1000-650 | 794.70 | 968.55 | 1001.14 | 991.44 |



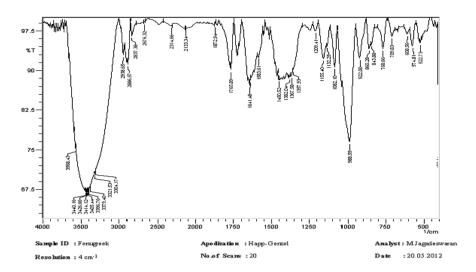**Fig 4: FTIR Spectrum of *Costus pictus***

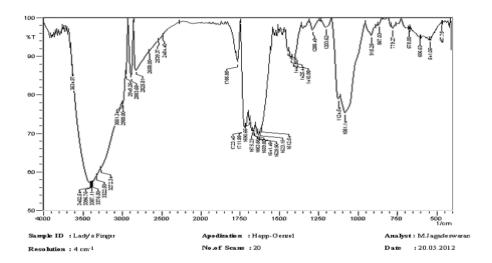**Fig 5: FTIR Spectrum of *Trigonella foenum- graecum***



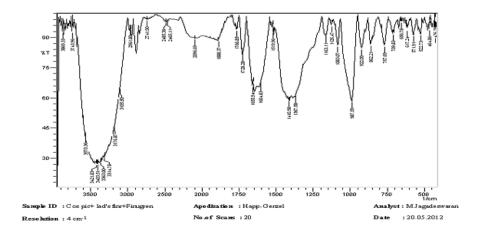**Fig 6: FTIR Spectrum of *Abelmoschus esculentus***



**Fig 7: FTIR Spectrum of mixture of *Costus pictus, Abelmoschus esculentus , Trigonella foenum- graecum***

**Pre-compression study of powder blend**

Herbal tablets of *Costus pictus* were developed to increase the release time of the drug, so that they can help in sustained the release of drug up to 24 hours. Different concentrations of *Trigonella foenum- graecum*, *Abelmoschus esculentus* polymers is known to be beneficial in improving sustain release characteristics. The pre- compression parameters obtained for all formulations are tabled in the Table- 4. The value of angle of repose was found to be in the range of $29^0 32'$ to $32^0 81'$. This indicates good flow property of powder blend. The bulk density was found in the range from **0.36 to 0.42 gm/cc**. The tapped density was found in the range from **0.41 to 0.48 gm/cc**. Hausner ratio value ranges between **1.13 to 1.17** as the results are in the range of < 1.18 indicates good flow. Carr's index value ranges between **12.19 to 14.52%** indicates that the powder blend have the required flow property and good packing ability for direct compression, since the flow properties of the powder mixture is to be analyzed before compression to tablets and all these trials were conducted in triplicates (n= 3).

**Table 4: Precompression evaluation parameters of powder blend**

| Formulation code | Angle of repose | Bulk density (gm/cc) | Tapped density (gm/cc) | Compressibility index (%) | Hausner ratio |
|---|---|---|---|---|---|
| F1 | $29^078'\pm0.5$ | 0.36±0.004 | 0.41±0.003 | 12.19±0.08 | 1.13±0.04 |
| F2 | $32^081'\pm0.1$ | 0.37±0.003 | 0.42±0.009 | 12.38±0.06 | 1.14±0.02 |
| F3 | $31^079'\pm0.2$ | 0.38±0.008 | 0.44±0.011 | 13.63±0.03 | 1.15±0.03 |
| F4 | $29^032'\pm0.4$ | 0.36±0.012 | 0.42±0.007 | 14.28±0.05 | 1.16±0.01 |
| F5 | $29^041'\pm0.3$ | 0.42±0.006 | 0.48±0.008 | 12.51±0.02 | 1.14±0.05 |
| F6 | $32^012'\pm0.4$ | 0.41±0.009 | 0.48±0.006 | 14.52±0.01 | 1.17±0.07 |

**No. of experiments n=3**

*In-vitro* **absorption study**

**Table 5:** *In-vitro* **absorption study of** *Costus pictus*

| S.No | Parts used | Absorbance | Amount absorbed at 120 minutes (mg) |
|---|---|---|---|
| 1 | Stomach | 0.512 | 0.358 |
| 2 | Colon | 0.956 | 0.668 |
| 3 | Jejunum | 0.784 | 0.548 |
| 4 | Ileum | 0.724 | 0.506 |

From the *in-vitro* absorption study it was found that drug *Costus pictus* shows maximum absorption in the colon region (Table 5).

**Table 6: Identification of active buffer for** *Costus pictus*

| S.No | Various buffers (pH) | Absorbance | Percentage release at 120 minutes |
|------|---------------------|------------|-----------------------------------|
| 1 | 1.2 | 0.114 | 5.74 |
| 2 | 6.8 | 0.427 | 21.52 |
| 3 | 7.2 | 0.294 | 14.82 |
| 4 | 7.4 | 0.261 | 13.15 |

The active buffer for Costus pictus was identified and it was found active at phosphate buffer pH 6.8 (Table 6).

**Post Compression Parameters of the sustained release herbal tablets**

**Table 7: Evaluation Parameters of Formulations.**

| Formulation | Thickness (mm) | Weight variation (%) | Hardness (kg/cm2) | Friability (%) | Drug content (%) | Disintegration time (min) |
|-------------|----------------|---------------------|-------------------|----------------|------------------|---------------------------|
| F1 | 4.33±0.02 | 1.29±0.04 | 5.31±0.03 | 0.80±0.05 | 97.14±0.3 | 22.3±0.2 |
| F2 | 4.56±0.05 | 1.51±0.02 | 5.42±0.06 | 0.96±0.03 | 98.09±0.2 | 23.2±0.3 |
| F3 | 4.26±0.03 | 1.06±0.06 | 5.29±0.02 | 0.71±0.02 | 97.61±0.5 | 21.4±0.1 |
| F4 | 4.53±0.02 | 0.87±0.03 | 5.41±0.04 | 0.83±0.06 | 99.04±0.4 | 22.5±0.02 |
| F5 | 4.43±0.07 | 1.84±0.01 | 5.91±0.05 | 0.93±0.04 | 98.41±0.2 | 27.1±0.3 |
| F6 | 4.36±0.04 | 1.39±0.05 | 5.98±0.03 | 0.87±0.07 | 98.33±0.7 | 28.2±0.2 |

**No. of experiments n=3**

The herbal tablets were prepared by direct compression method using the polymers Trigonella foenum- graecum and Abelmoschus esculentus to provide sufficient drug release retardation to the tablets .The results have shown in the Table 7.The prepared herbal tablets were evaluated for thickness, hardness, friability, average weight variation, drug content and disintegration time all the studies were performed in triplicates and the results were expressed in low standard deviation values indicating efficient mixing of drug and other excipients. The measured hardness for the tablets for each batch arranged between **5.31 to 5.98 kg/cm$^2$**, this ensures the good handling characteristics of all the batches. The % friability was less than **1%** in all the formulations ensuring that the tablets were mechanically stable. The weight variation for different formulations was found to be **0.87 to 1.84%**, indicates consistency in each batch. The drug content was found to be **97.14 to 99.04%**, and with low standard deviation indicates batch to batch consistency. The disintegration time was found in the range **21.4 to 28.2 min** for all batches.

**Swelling Index**

**Table 8: Swelling index (%) of formulations.**

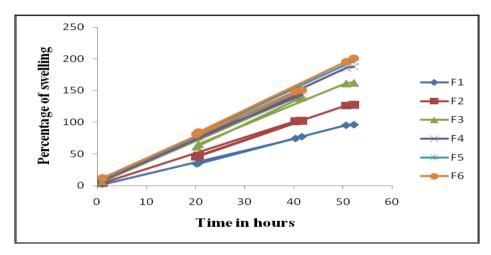| Time (Hrs) | Formulation | | | | | |
|---|---|---|---|---|---|---|
| | **F1** | **F2** | **F3** | **F4** | **F5** | **F6** |
| 1 | 40.3 | 41.6 | 20.2 | 20.6 | 50.6 | 52.2 |
| 2 | 74.4 | 77.3 | 34.1 | 35.3 | 95.3 | 96.3 |
| 4 | 101.6 | 102.3 | 45.3 | 47.4 | 126.2 | 127.6 |
| 6 | 138.2 | 139.4 | 61.4 | 65.2 | 161.3 | 162.4 |
| 8 | 141.6 | 143.5 | 76.2 | 77.5 | 185.6 | 187.5 |
| 10 | 147.3 | 149.3 | 79.3 | 82.6 | 191.6 | 196.2 |
| 12 | 148.6 | 150.6 | 81.2 | 84.4 | 195.5 | 201.1 |



**Fig 8: Showing the variations of swelling index of formulations**

Swelling index for all the formulations was carried out in the phosphate buffer pH6.8. The formulations showed different indices in the swelling media and it is shown in the Table 8 and Fig 8. Tablets containing both Trigonella foenum- graecum and Abelmoschus esculentus showed maximum swelling in 12 hr with sharp increase up to 8 hr .This may due to increased concentration of Trigonella foenum- graecum and Abelmoschus esculentus which retain water and form thick swollen mass.

***In-vitro* drug release studies**

Release rate of Costus pictus from the herbal tablets were evaluated in hydrochloric acid of buffer pH 1.2 for 2 hours and the rest in the phosphate buffer of pH 6.8. Release data has been shown in Table 9. Release study has been performed for six formulations. Cumulative % release has been shown for average of three preparations.

**Table 9:   In-vitro dissolution profiles of Costus pictus from the different formulations.**

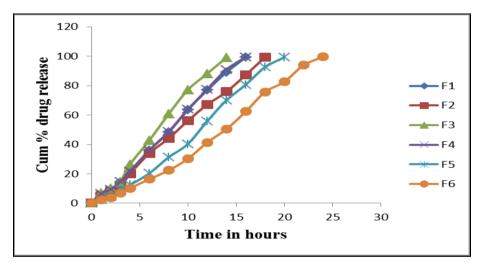| Time (Hours) | Various buffers | Cumulative % drug release | | | | | |
|---|---|---|---|---|---|---|---|
| | | F1 | F2 | F3 | F4 | F5 | F6 |
| 1 | Acidic buffer pH 1.2 | 6.30 | 5.13 | 6.95 | 6.40 | 2.25 | 2.32 |
| 2 | | 9.53 | 8.42 | 9.99 | 9.43 | 4.39 | 3.57 |
| 3 | Phosphate buffer pH 6.8 | 14.38 | 11.35 | 14.48 | 14.68 | 9.38 | 6.87 |
| 4 | | 21.41 | 19.99 | 26.44 | 21.76 | 12.45 | 10.24 |
| 6 | | 35.95 | 33.97 | 42.75 | 36.05 | 20.35 | 16.34 |
| 8 | | 48.29 | 44.05 | 60.59 | 48.55 | 31.22 | 22.37 |
| 10 | | 63.52 | 56.14 | 77.13 | 63.78 | 40.33 | 30.23 |
| 12 | | 76.90 | 67.05 | 88.15 | 77.46 | 55.75 | 41.28 |
| 14 | | 89.04 | 75.80 | 99.13 | 90.95 | 70.03 | 50.45 |
| 16 | | 99.22 | 87.32 | | 99.37 | 80.64 | 62.34 |
| 18 | | | 99.37 | | | 92.53 | 75.68 |
| 20 | | | | | | 99.24 | 82.57 |
| 22 | | | | | | | 93.93 |
| 24 | | | | | | | 99.53 |



**Fig 9: Graph showing the in-vitro dissolution profiles of Costus pictus from the different formulations**

In-vitro dissolution studies were performed for all the formulations using USP dissolution apparatus II at 50 rpm using 900 ml of phosphate buffer pH 6.8 as dissolution medium. The samples withdrawn and were analyzed by using UV spectrophotometer. The drug release from the formulations F1 and F2 prepared with Trigonella foenum- graecum (10% and 15%) was found to be 99.22 and 99.37% at the end of 16th and 18th hour, formulations F3 and F4 prepared with Abelmoschus esculentus (10% and 15%) was found to be 99.13 and 99.37% at the end of 14th and 16th , whereas formulation F5 and F6 prepared with both Trigonella foenum- graecum and Abelmoschus esculentus (10% and 15%) was found to be 99.24 and

99.53% at the end of 20th and 24th hour. As per the results of dissolution study the formulation F6 shows the sustained drug release (Fig 9).

All the formulations were designed as sustained dosage form.  In order to check the 100% dissolution release profile, formulations were subjected to dissolution studies for 24 hours. Among the six formulations F6 was best and shows 99.53% drug release in the end of 24 hours.

It is evident from the *in-vitro* dissolution data that increase in concentration of polymers Trigonella foenum- graecum and Abelmoschus esculentus decreases the release rate,  this might be due to increase in diffusional path length, which the drug molecule may have to travel. So, formulation F6 was selected as the optimized formulation.

**Kinetic Release Studies**

The in-vitro release data was applied to various kinetic models to predict the drug release kinetic mechanism. The release constant was calculated from the regression coefficient. It was found that the in vitro drug release of herbal tablet was best explained by zero order kinetics as the plots shown highest linearity. The regression coefficients (r) were in the range of 0.9927-0.9952 for various formulations . For formulation F6 (r=0.9927), indicating that the drug release was nearly independent and follows zero order kinetics (r=0.9927).

In the current study, the values of release rate exponent (n), calculated as per the equation proposed by Peppa's and all the slope values ranges from 1.017 to 1.284 revealed the fact that the drug release follows the class II transport system.

**Anti-Diabetic Activity**

The blood glucose levels in rabbits of different groups are shown in Table 11 and in     Fig 10. The glucose level was significantly high in alloxan treated group when compared to that of control and drug treated group. On repeated administration of the formulation and standard drug for 21 days, a significant decrease in blood glucose level was observed in diabetic rabbits.

**Table 10: Anti-diabetic activity of *Costus pictus* herbal tabletin experimental rabbits**

| GROUPS | 0th DAY | 7th DAY | 14th DAY | 21st DAY |
|---|---|---|---|---|
| Normal (Normal saline 1ml/kg) | 112.5 ±3.22** | 112.16±3.48** | 112.33±2.40** | 114±1.57** |
| Control (Alloxan 150mg/kg) | 297.16±3.45 | 295.66±2.56 | 294.5±2.32 | 288.83±1.27 |
| Alloxan+ Glipizide (5mg/kg) | 297.83±2.45 | 282.16±1.40** | 224.83±3.53** | 116.16±1.51** |
| Alloxan + F1 (170mg) | 297±3.24 | 288±3.25 | 281.33±3.27* | 260.5±2.79* |
| Alloxan + F2 (170mg) | 296.83±2.93 | 287.67±2.86 | 282.16±2.62* | 259±2.67* |
| Alloxan + F3 (170mg) | 300.33±2.81 | 291.83±1.88 | 282.33±2.01* | 259.16±2.22* |
| Alloxan + F4 (170mg) | 298.16±2.58 | 288.83±2.48 | 282±2.68* | 259.33±3.60* |
| Alloxan + F5 (170mg) | 296.66±2.69 | 284.5±2.11* | 256±3.41** | 201.33±3.45** |
| Alloxan + F6 (170mg) | 301.5±2.90 | 278.83±1.42** | 224.83±2.89** | 122±2.36** |

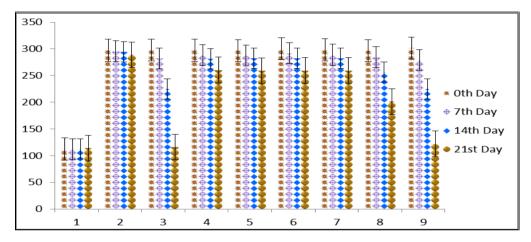Data represents mean ± SEM. (n=6); $*p<0.05$; $**p<0.01$



**Fig 10: Showing Anti-diabetic activity of *Costus pictus* herbal tablet in experimental rabbits**

**Table11. Biochemical parameters of *Costus pictus* herbal tablet (F6)in blood serum of experimental rabbits.**

| Biochemical parameter's | Normal range | Observed value |
|---|---|---|
| **LIVER FUNCTION TEST** | | |
| Bilirubin total | Upto 1mg/dl | 0.91 mg/dl |
| Protein total | 6.4-8.3 g/dl | 7.4 g/dl |
| Albumin | 3.4-5.2 g/dl | 4.2 g/dl |
| Globulin serum | 1.5-3.5 g/dl | 3.3 g/dl |
| SGOT | Upto 37 U/L | 37 U/L |
| SGPT | Upto 40 U/L | 36 U/L |
| Alkaline phosphatase total | Upto 270 U/L | 113 U/L |
| **LIPID PROFILE** | | |
| Cholesterol total | Upto 200 mg/dl | 137 mg/dl |
| Triglycerides | 40-200 mg/dl | 200 mg/dl |
| HDL-Cholesterol | 45-65 mg/dl | 63 mg/dl |
| LDL-Cholesterol | 45-100 mg/dl | 46 mg/dl |
| VLDL-Cholesterol | 8-33 mg/dl | 25 mg/dl |

| KIDNEY FUNCTION TEST | | |
|---|---|---|
| Blood urea | 10-50 mg/dl | 45 mg/dl |
| creatinine | 0.5-0.9 mg/dl | 0.9 mg/dl |
| eGFR | Actual eGFR is not given as per NKDEP recommendation (National Kidney Disease Education Program) | More than 60 ml/minute/1.73 m2 |

The biochemical parameters of the blood serum in formulation F6 is shown in the Table 12. The formulation F6 shows significant balance in the biochemical parameters.

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