Acta Biomedica Scientia

Journal homepage: www.mcmed.us/journal/abs

ANTI-DIABETIC AND HYPOLIPIDAMIC EFFECTS OF *Costus igneus* LEAVES EXTRACTS AGAINST STREPTOZOTOCIN INDUCED DIABETIC ALBINO RATS

N.Kanivalan[1], R.Rajakumar[1], P.Mani[2*]

[1]Department of Zoology and Biotechnology A.V.V.M. Sri Pushpam College (autonomous), Thanjavur, Tamilnadu, India.
[2]Department of Plant Biotechnology, Sharmila Institute of Medicinal Products Research Academy (SIMPRA), Thanjavur, Tamilnadu, India.

Article Info

Received 29/04/2014
Revised 10/05/2014
Accepted 17/05/2014

Key words: *Costus igneus*, Diabetic, Streptozotocin, Lipid profile, Glucose.

ABSTRACT

The present study investigates the antidiabetic effect of leaf extract of *Costus igneus* on Streptozotocin (STZ) diabetic rats. The rats treated with Streptozotocin showed a significant increased in glucose level and altered level of lipid profile, heamoglobin and insulin were observed. The mechanism underlying STZ hyperglycemia in diabetes mellitus involves over-production (excessive hepatic glycogenolysis and gluconeogenesis) and decreased utilization of glucose by the tissues. Twenty-eight days administration of ethanolic extract of leaf of the *Costus igneus* (200 and 300mg/kg b.wt.) to diabetic ratsresulted in significant reduction in blood glucose level, restored heamoglobin, lipid profile and insulin as compared to diabetic rats. The present study suggests that the *Costus igneus* leaves extracts had synergetic hypoglycemic effect revealed by decreased serum lipid levels, restored insulin, heamoglobin and therefore attribute to therapeutic value of the *Costus igneus* extracts of leaves to combat the diabetic condition in rats. Among the two doses, 300mg/kg of *Costus igneus* leaves extract possess potential anti-diabetic activity.

INTRODUCTION

Diabetes mellitus is a complex and a multifarious group of disorders that disturbs the metabolism of carbohydrates, fat and protein. It results from shortage or lack of insulin secretion or reduced sensitivity of the tissue to insulin [1]. In 2007, the diabetes treatment market worldwide was worth over $25 billion, and had double-digit growth from the year before. According to the World Health Organization (WHO) the total number of people with diabetes was 171 million in 2000, and is projected to rise up to 366 million in 2030 [2].

Several drugs such as, biguanides and sulfonylureas are presently available to reduce side effects and thus searching for a new class of compounds is essential to overcome diabetic problems [3].

Corresponding Author

P.Mani
E-mail:master.maniji@gmail.com

hyperglycemia in diabetes mellitus, but these drugs have In these dangerous condition, alternative strategies to the current modern pharmacotherapy of diabetes mellitus are urgently needed[4]. because of the inability of existing modern therapies to control all the pathological aspects of the disorder, as well as the enormous cost and poor availability of the modern therapies for many rural populations in developing countries. Herbal drugs are mostly out of toxic or side effect than the chemical drug [5]. It is estimated that 70 to 80% of the people worldwide rely chiefly on traditional health care system and largely on herbal medicines [5-7]. In developing countries, the World Health Organization (WHO) estimates that about 80% of the population relies on plant based preparations used in their traditional medicinal system and as the basic needs for human primary health care[8]. In India, traditionally numbers of plants are used to manage the diabetic conditions and their active principles were isolated but few

plants have been scientifically studied. Therefore, the present study was carried out to evaluate the antidiabetic activity of *Costus igneus* leaves in Streptozotocin (STZ) induced diabetes and to probe into the mechanism of its antidiabetic property.

MATERIAL AND METHODS

Animals

Albino wistar male rats 7 – 8 weeks old, weighing 190-220 g, were used for the present study. Animals were housed under standard conditions of temperature (24±2°C) and relative humidity (30-70%) with a 12:12 light: dark cycle. The animals were fed with standard pellet diet (Chakan Oil Mills, Sangli) and water *ad libitum*. Animal handling was performed according to Good Laboratory Practice (GLP). Ethical clearance was obtained from Institutional Animal Ethics Committee and conducted according to the Indian National Science Academy guidelines for the use and care of experimental animals (CPCSEA/265).

Chemicals

Streptozotocin (STZ), Ethylene Diamine Tetra Acetic Acid (EDTA)), Glibenclamide (Prudence Pharma Chem, India), Chloroform were purchased for Sigma chemical company, Mumbai All other chemicals and reagents used in this study was of analytical grade with high purity and were obtained from Glaxo laboratories and Sisco Research laboratories, Mumbai, India.

Plant materials

Fresh plant leaves of *Costus igneus* was collected from Kottyam District of Kerala and identified to confirm by the Taxonomist Botanical Survey of India, Tamilnadu, India.

Plant sample extraction

The leaves were cut into pieces and shade dried at room temperature. The dried leaves were subjected to size reduction to a coarse powder by using dry grinder and passed through sieve. 100 g of crushed leaves were continuously extracted with 95% ethanol using soxhlet up to 48 h. The extract was filtered and concentrated in rotatory evaporator at 35-40 ºC under reduced pressure to obtain a semisolid material, which was then lyophilized to get a powder (28.5%, w/v).

Induction of Diabetes in Rats

After fasting, diabetes was induced by intraperitoneal (*ip*) injection of Streptozotocin dissolved in 0.1 M cold sodium citrate buffer, pH 4.5, at a dose of 55 mg/kg [9]. The control rats received the vehicle alone. The animals were allowed to drink 5% glucose solution overnight to overcome the drug induced hypoglycemia. After a week time for the development of diabetes, the rats with moderate diabetes having glycosuria and hyperglycemia (blood glucose range of above 250 mg/dl) were considered as diabetic rats and used for the experiment.

Experimental Design

The animals were divided into six groups of six animals each as follows. Each animal was marked for identification and regularly monitoring. The animals of normal control (Group I) were injected with citrate buffer alone. Group II served as diabetogenic rats (Control). Group III and IV rats treated with *Costus igneus* leaves at a dose of 200 and 300mg/kg were orally given once a day for 4 weeks. Group IV rats treated with Glibenclamide at dose of 5mg/kg and served as reference stranded treatment continued for 4 weeks.

Collection of sample

After the termination of the experiment all the animals were anesthetized using ketamine chloride (24mg/kg bw) and sacrificed by cervical dislocation after an overnight fast. Blood was collected with and without EDTA. Plasma and serum were separated after centrifugation and used for various biochemical estimations.

Biochemical estimations

Serum glucose was estimated by the oxidase method [10]. The total cholesterol was estimated by the method of [11]. Triglyceride was estimated by the method of [12]. HDL cholesterol was separated by adding phosphotungsti magnesium chloride to the fresh samples to precipitate other lipoproteins and the HDL cholesterol was estimated. The concentration of LDL cholesterol was calculated by using the Friedwald formula and VLDL cholesterol was calculated by dividing the triglycerides value (in mg/dl) by 5. Heamoglobin estimated by the method. Plasma insulin was assayed by the solid phase system amplified sensitivity immunoassay

Histological Assay

On the 28th day, pancreatic tissues were taken from animals which were fasted overnight under ether anesthesia. The whole pancreas from each animal was removed after killing the animals, was placed in 10% formulation solution and immediately processed by the paraffin technique section of 5 µm thickness were cut and strained by haematoxylin and Eosin (H and E) for histological examination. The photomicrographs of histological studies are taken.

Statistical Analysis

All results are presented as mean ± SEM Data were analyzed by the student's T test. Groups for the pair of observations depended upon each other. Results were

considered statistically at P < 0.001.

RESULTS

Estimation of Body Weight

The body weight changes in control and experimental groups were illustrated in Table1. The body weight of diabetic rats significantly decreased when compared with control group. Supplementation of ethanolic extracts of *Costus igneus* showed a significant improvement in the body weight of diabetic rats. There were no significant changes observed between control treated group animals.

Estimation of Blood glucose, Plasma insulin and Hemoglobulin

Table 2 shows the blood glucose plasma insulin and total hemoglobulin levels of normal and experimental rats. There was a significant increased level of blood glucose and plasma insulin was observed in diabetes animals compared to the corresponding control group. Treatment with *Costus igneus* ethanolic leaves extract restored the levels of blood glucose and plasma insulin of diabetic group of rats and the effect was more pronounced in the group of rats treated with *Costus spicatus.*

Estimation of Serum Lipid Profile

As shown in Table 3 ethanolic extracts of *C. igneus* administration in diabetic rats serum lipids levels. The Triglycerides, Total cholesterol, VLDL, LDL increased and HDL levels were significantly decreased in STZ treated rats. Oral administration of *C. igneus* leaves ethanolic extract in 200 and 300mg/kg of body weight restored the altered parameters, which was compared to that of glibenclamide group. However, no significant changes were observed control treated groups.

Histological Assay

Multiple section of pancreas were taken and studied for histological changes in the plant extract-administered group and control group (Fig.1). Histological findings of pancreas in the extract-administered group and control group were tentatively similar. STZ-induced diabetic rats showed extensive damage on the islets of langerhans cells (Fig.2). The orally administered leaves extracts of *C. igneus* extracts(200 & 300 mg/kg) and commercial drug, Glibenclamide (100 mg/kg) (Fig.3 to 5) were showed restoration of normal cellular population and enlarged size of beta cells with hyperplasia found in islets of langerhans cells in pancreas .The pancreas present in the group of animals treated with the extracts of *C. igneus* extracts(300 mg/kg) clearly showed that the partial restoration of normal cellular population and enlarged size of beta cells with hyperplasia on 30th day. The islets were normal in size, shape and number comparatively similar to that of standard treated drug (Fig.5).

Table 1. Effect of *C. igneus* ethanolic leaves extract on the changes of body weight of control and experimental rats

Groups	Body weight (g)	
	Initial (0 day)	Final (4 weeks)
Control	198.22 ± 19.10	228.21 ± 19.01
Diabetic control	187.13 ± 18.11*	149.11 ± 11.4*
Diabetic + ethanolic leaves extract of *Costus igneus* (200 mg/kg bw)	182.04 ± 12.2	180.11±12.21*
Diabetic + ethanolic leaves extract of *Costus* (300 mg/kg bw)	197.06 ± 19.43*	228.02±11.41**
Glibenclamide (5mg/Kg)	202 ± 11.25 **	225 ±12.35 **

Values are given as mean ± S.D (n=6 rats)
*P<0.01 Vs control; **P<0.001Vs control by students't' test.

Table 2. Effect of *Costus igneus* ethanolic leaves extract on the levels of blood glucose, plasma insulin and Hemoglobin in control and experimental rats

Groups	Blood glucsoe (mg/dL)	Plasma insulin (µg/mL)	Total Hemoglobin (g/dL)
Control	85.45 ± 8.81*	7.24 ± 1.54	13.43 ± 1.35*
Diabetic control	280.11± 19.33	4.24 ±0.13	8.93 ± 0.49
Diabetic + ethanolic leaves extract of *Costus* (200 mg/kg bw)	108.12±4.57*	11.02±1.21*	12.87± 0.45*
Diabetic + ethanolic leaves extract of *Costus igneus* (300mg/kg bw)	90.01±3.12*	17.21±1.05*	13.99±1.58**
Glibenclamide (5mg/kg)	84.21 ±6.23	15.12±2.43**	13.88±3.12**

Values are given as mean ± S.D (n=6 rats)
* P<0.01 Vs control; **P<0.001Vs control by students't' test.

Table 3. Effect of *Costus igneus* ethanolic leaves extract on lipid profile in control and experimental rats

Treatment	TGL (mg/dl)	HDL-C (mg/dl)	VLDL-C (mg/dl)	LDL-C (mg/dl)	Total Cholesterol (mg/dl)
Control	75.15±5.03	38.6±1.83	15.03±1.06	41.9±4.37	96.16±6.44
Diabetic control	124.32±6.5	42.56±5.52	32.14±1.7	96.47±3.2	215.2±7.4
Diabetic + ethanolic leaves extract of *Costus igneus* (200 mg/kg bw)	97.52±4.67*	38.27±2.56	30.19±2.8*	88.41±2.34	172.3±5.*
Diabetic + ethanolic leaves extract of *Costus igneus* (300mg/kg bw)	91.49±2.45**	33.46±1.36*	25.52±3.4*	81.27±5.28*	152.6±6.9*
Glibenclamide (5mg/kg)	82.04±6.7*	25.38±4.75*	16.92±1.34*	33.9±2.66*	96.2±4.8*

Values are given as mean ± S.D (n=6 rats)

* P<0.01 Vs control; **P<0.001Vs control by student's 't' test.

Fig 1. Histopathology of Islets of Langerhans Normal Rat (Control.)

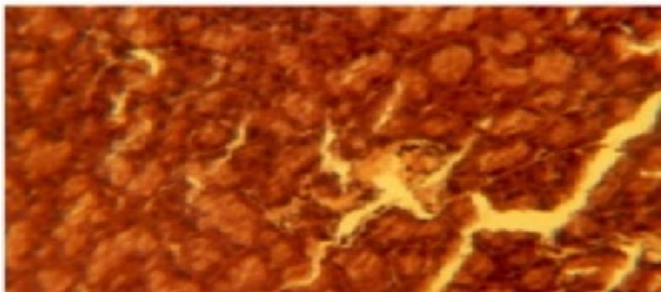


Fig 2. Histopathology of Islets of Langerhans of Diabetic Rat (STZ-Induced)

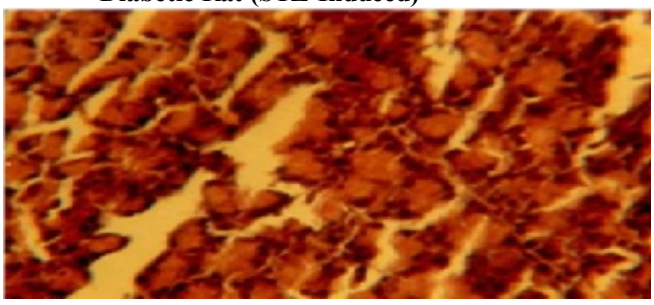


Fig 3. Histopathology of Islets of Langerhans of diabetic Rat Treated With *C.igneus (200* mg/kg)

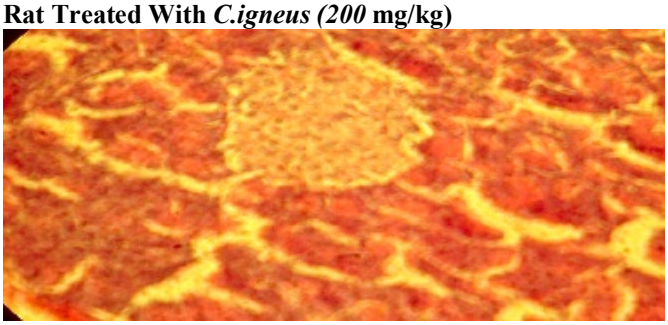


Fig 4. Histopathology of Islets of Langerhans of Diabetic rat Treated with *C.igneus (300* mg/kg)

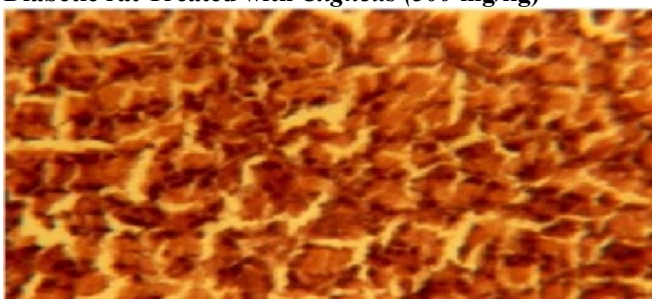
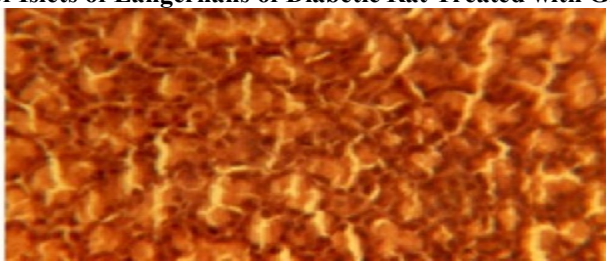


Fig 5. Histopathology of Islets of Langerhans of Diabetic Rat Treated with Glibenclamide (5mg/kg)



DISCUSSION

The present study investigates the antidiabetic effect of leaf extract of *C. igneus* on STZ diabetic rats. The fundamental mechanism underlying hyperglycemia in diabetes mellitus involves over-production (excessive hepatic glycogenolysis and gluconeogenesis) and decreased utilization of glucose by the tissues [13].Twenty-eight days administration of ethanolic extract of leaf of the *Costus igneus* resulted in significant reduction in the fasting blood glucose level compared to diabetic rats. The difference observed between the initial and final fasting levels of different groups revealed a significant elevation in blood glucose in diabetic control group compared to normal. It is evident from these investigations that the leaf

extract is effectively maintaining the blood glucose levels in normal and STZ induced diabetic rats.

Ethanolic extract of *C. igneus* treated groups III, IV and aqueous solution of *C.igneus* treated groups V rats showed a significant ($p < 0.00\ 1$) increase insulin level when compared with group II as well as group V. STZ, a β-cytotoxin, induces chemical diabetes in a wide variety of animal species by damaging the insulin-secreting β - cells of the pancreas. STZ causes time and concentration dependent degenerative lesions of the pancreatic β -cells.

The lipid profiles in control and experimental rats are depicted in STZ-induced diabetic rats, there was a significant ($p < 0.001$) increase of total cholesterol, triglycerides and low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) cholesterol and ($p < 0.00\ 1$) decrease high-density lipoprotein (HDL) cholesterol in serum compared with normal control. The plant extracts used in the study significantly ($p < 0.00\ 1$) decreased the levels of cholesterol, triglycerides, phospholipids, and LDL and VLDL cholesterol and ($p < 0.001$) increase HDL cholesterol.

This indicates that the leaf extract had favorable effects, on lipid metabolism of diabetic rats. Derangement of glucose, fat, and protein metabolism in diabetes results in the development of hyperlipidemia [14]. Significant lowering of total cholesterol is a very desirable biochemical state for the prevention of atherosclerosis and ischemic conditions. The observed hypolipidaemic effect may be because of decreased cholesterogenesis and fatty acid synthesis. Significant lowering of total cholesterol and raise in HDL cholesterol is a very desirable biochemical state for prevention of atherosclerosis and ischaemic condition [15].

Such a phenomenon, its related species *Costus specious* has antidiabetic as well as multipurpose medicinal activity. [16]. investigated the possible protective effects of *Costus speciosus* (Koen.) sm. (*C. speciosus*) rhizome extracts on biochemical parameters in streptozotocin (STZ)-induced male diabetic Wistar rats. STZ treatment (50 mg/kg, i.p.) caused a hyperglycemic state that led to various physiologic and biochemical alterations. Hexane, ethyl acetate, and methanol crude extracts administered at the dose of 250 mg/kg, 300 mg/kg, and 400 mg/kg, respectively, for 60 days to STZ-induced hypoglycemic and normal glycemic rats. The plasma glucose concentration was significantly ($p < 0.05$) decreased by all three extracts compared with controls. In addition, oral administration of hexane extract significantly decreased glycosylated hemoglobin (HbA1), serum total cholesterol, and triglyceride levels, urea, uric acid, and creatinine and at the same time markedly increased plasma insulin, tissue glycogen, serum protein, and high- density lipoprotein (HDL) cholesterol levels. The hexane crude extract also restored the altered plasma enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and acid phosphatase (ACP) levels to near normal. Glibenclamide used as a reference drug (5mg/kg body weight) also produced a significant reduction in the blood glucose concentration in STZ-induced diabetic rats. In summary, the hexane crude extract was found to be more active in comparison with ethyl acetate and methanol extracts. Thus study shows that the *C. igneus* hexane extract has anti-hyperglycemic and hypolipidemic activity, is able to ameliorate the diabetic state, and is probably a source of hypoglycemic compounds.

The hepatoprotective activity of the ethanolic extract of the rhizomes of *Costus speciosus* (Koenig) Sm. is studied on carbon tetrachloride treated rats. The extract registered a significant fall in the levels of serum glutamyloxalacetic acid transaminase (SGOT), serum glutamyl pyruvate transaminase (SGPT), alkaline phosphatase (ALXP), serum bilirubin (SBLN) and liver inflammation supported by histopathological studies on liver, thus exhibited a significant hepatoprotective activity.

In phytochemical analysis of its related species *Costus specious*, Carbohydrates were identified by Molisch's test, proteins were identified by ninhydrin test, triterpenoids and steroids were identified by Libermann-Burchard test, alkaloids were identified by Draggendorf's test, tannins were identified by Braermer's test, glycosides were identified by Legal's test, saponins were identified by haemolysis test, flavonoids were identified by lead acetate test, and fixed oils were identified by the presence of oil stains on the filter paper. Plant active constituents responsible for antidiabetic properties were isolated by thin layer chromatography (TLC). Acid hydrolysis was carried out on vacuum-dried concentrated methanol extract of *C. spiralis* 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 x 5cm) were developed with solvent system n-butanol-2 M ammonium hydroxide (1:1) to elute α- and β-amyrin [17-18].

The developed plates were air-dried and detected by Carr-Price reagent, i.e., 20% antimony chloride in chloroform was sprayed and dried in a chromatographic oven at 105°C for 30mm. The resolution bands were obtained and Retardation factor (R_f) values calculated The β-amyrin found in the concentrate was identified by comparing the R_f value with earlier-reported study [19].The fractions of similar TLC patterns were combined, concentrated and rechromatographed repeatedly over silica gel GF 254 (100-200 mesh) columns of 60 cm x 3cm to isolate active compound and confirmed by qualitative chemical analysis [20]. Hence, the sequential extracts of *Costus igneus* were subjected to qualitative phytochemical screening and GC-MS for the presence of different chemical groups of extract tested, methanol extract was found to contain the highest number of phytochemicals

(unpublished data) such as carbohydrates, triterpenoids, proteins, alkaloids, tannins, saponins, and flavonoids for anti diabetic activity.

During diabetes the excess glucose present in blood reacts with haemoglobin to form glycosylated haemoglobin.. Therefore, the total haemoglobin level is decreased in STZ diabetic rats. So the total haemoglobin level is lowered in alloxan diabetic rats . Administration of *C. igneuss* reversed the total haemoglobin levels in STZ diabetic rats. The present study suggests that the *Costus igneus* leaves extracts had synergetic hypoglycemic effect revealed by decreased serum lipid levels, restored heamoglobin and therefore attribute to therapeutic value of the *C. igneus* extracts of leaves to combat the diabetic condition in rats. Among the two doses, 300mg/kg of *C. igneus* leaves extract possess potential antidiabetic activity. The potential antidiabetic activity of *C. igneus* leaves may be due to the phytochemicals flavonoids, terpenoids etc. present in *C. igneus* leaves. Hence, it might help in preventing diabetic complications and serves as a good adjuvant in the present armamentarium of anti-diabetic drugs.

REFERENCES

1. Prasad S K, Kulshreshtha A and Taj N. (2009). Qureshi. Antidiabetic Activity of Some Herbal Plants in Streptozotocin Induced Diabetic Albino Rats. *Pakistan Journal of Nutrition*. 8(5), 551-557.
2. Setacci C, Donato G, Setacci F and Chisci E. (2009). Diabetic patients, epidemiology and global impact. *J CardiovascSurg* (Torino).50(3), 263-73.
3. Noor A, Gunasekaran S, Manickam AS, and Vijayalakshmi MA. (2008). Antidiabetic activity of *Aloe vera* and histology of organs in streptozotocin induced diabetic rats. *Curr Sci*. 94, 1070-1076.
4. Rao BK, Sudarshan PR, Rajsekher MD, Nagarju N and Rao CA. (2003). Antidiabetic activity of *Terminalia pallida* fruit in alloxan induced diabetic rats. *J. Ethnopharmacol*. 85, 169-172.
5. Farnsworth NR and Soejarto DD. (1991). Global importance of medicinal plants.Conservation of Medicinal Plants. Cambridge (United Kingdom), Cambridge University Press. 25 – 51.
6. Farnsworth NR, Akerele O and Bingel AS. (1985). Medicinal plants in therapy. *Bulletin of the World Health Organisation*. 63, 965-981.
7. Shanley P and Luz L. (2003). The impacts of forest degradation on medicinal plant use and implication for health care in Eastern Amazonia. *Bio Science*. 53(6), 573 -584.
8. Gurib-Fakim A. (2006). Review-Medicinal plants, Traditions of Yesterday and drugs of tomorrow. *Mol. Aspects Med*. 27, 1-93.
9. Kakkar P, Das B, Visvanathan PN. (1984). A modified spectrophotometric assay of superoxide dismutase. *Indian J Biophys*. 21, 130-132.
10. Sasaki T, Matsy S, Sorae A. (1972). Effect of acetic acid concentration on the colour reaction in the O-toluidine boric acid method for blood glucose estimation. *Rinsho Kagarku*, 1, 346-353.
11. Allian CC, Poon LS, Chan CSG, Richmand W and Fu P. (1974). Determination of total cholesterol by enzymatic method. *Clinical Chemistry*. 20, 470-475
12. Fossat Lorenzo P. (1982). Serum triglycerides determined calorimetrically with an enzyme that produce hydrogen peroxide. *Clin. Chem*. 28, 2077-2080.
13. Latner A, Majeed M, Badmeev V and Rajendran R. (1998). Piperine as bioavailability enhancer.*US Patent*. 5, 744.
14. Freidmann N. (1984). Hormonal regulation of hepatic gluconeogenesis. *Physiol. Rev*. 64, 170- 176.
15. Luc G and Fruchart JC. (1991). Oxidation of lipoproteins and atherosclerosis. *Am. J. Clin. Nutr*.53, 206-209.
16. Daisy P, Eliza J, and Ignacimuthu S. (2008). Influence of *Costus speciosus* (Koen.) Sm. Rhizome Extracts on Biochemical Parameters in Streptozotocin Induced Diabetic Rats. *Journal of Health Science*. 54, 675-681
17. Harborne JB. (1998). Triterpenoids and steroids.InPhytochemical methods (Chapman and Hall, 3rd Eds.). Rajkamal Electric Press, Delhi, India, 129-137.
18. Karmarkar SH, Keshavachandran R and Augustin A. (2001). Biochemical evaluation of root tubers and in-vitro induced callus of adapathiyan (Holostemmaada-Kodien K. Schum.). *J Trop. Agric*. 39, 108-110.
19. Knapp F, Aexel R and saponifiable constituents of Lettuce. *J Food Sci*. 33, 159-162Nicholas HJ. (1968).The non.
20. Chatwal G. (2004). Terpenoids. In Organic chemistry of natural products (Himalaya Publishing House, 1996 Eds.). Mahalakshmi printer and processors, India, 162-166.