THE INHIBITORY EFFECT OF *COSTUS IGNEUS* LEAF EXTRACT ON SALIVARY AMYLASE AND PANCREATIC AMYLASE ACTIVITIES

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ABSTRACT

Diabetes mellitus is an endocrinal disorder associated with depleted insulin secretions, damaged pancreatic β-cells with altered carbohydrate, lipid and protein metabolism. It is the most common serious metabolic disorder and is considered to be one of the five leading causes of death in the world. *Costus igneus*, commonly known as insulin plant in India, belongs to the family *Costaceae*. The aim of the study is to compare inhibitory effect of aqueous leaf extract of *Costus igneus* and the anti diabetic drug acarbose on salivary amylase, goat pancreatic amylase and commercial porcine pancreatic amylase activities. The results showed a significant inhibitory effect of *Costus igneus* on amylase activities. Preliminary phytochemical screening of ethanol, aqueous and chloroform extracts revealed the presence of carbohydrates, cardiac glycosides, proteins, flavonoids, saponin and tannins. The results of TLC analysis reported the presence of the flavonoid in *Costus igneus* leaf extract.
Keywords: *Costus igneus*, diabetes mellitus, amylase, acarbose, flavonoid.

INTRODUCTION

Diabetes mellitus (DM) is an endocrinal disorder associated with deficiency of insulin secretion due to damaged pancreatic β-cells with altered carbohydrate, lipid and protein metabolism. It has been estimated that Indian people are more genetically susceptible to diabetes accounting about 30 to 33 million and would go up to 74 million by 2025[1].

Even though the oral hypoglycemic agents and bio-technically engineered insulin preparations are currently available for the treatment of diabetes mellitus, but are not free from undesirable side effects [2]. The management of diabetes mellitus thus is global challenge that demands for alternative therapy. Phytochemicals become more popular due to their innumerable medicinal uses. Phytochemicals play a vital role against number of diseases such as diabetes mellitus, allergy, asthma, arthritis, cancer etc. Since the phytochemicals cure diseases without causing any harm to human beings these can also be considered as “man- friendly medicines”.

Herbal drugs are considered to be less toxic and more free from side effects compared to synthetic drugs. Wide arrays of plant derived active principles representing numerous chemical compounds have demonstrated activity consistent with their possible use in the treatment of non insulin dependent diabetes mellitus (NIDDM). The ethnobotanical information reports that about 800 plants have always been exemplary available drugs and many of the currently available drugs were derived from them.

Costus igneus, commonly known as insulin plant in India, belongs to the family *Costaceae*. This plant is native of Southeast Asia, especially on the Greater Sunda Islands in Indonesia. Commonly known as Step ladder, *Costus igneus* was introduced in India from South and Central America. The plant has large fleshy leaves. The undersides of these large, smooth, dark green leaves have light purple shade. The leaves are spirally arranged around the stem, forming attractive, arching clumps arising from underground rootstocks. The maximum height of these plants is about two to three feets. The flowers are orange in color and are beautiful, 1.5-inch diameter. Flowering occurs during the warm months and they appear to be cone-like heads at the tips of branches [3].

Taxonomy

Kingdom : Plantae

Phylum : Tracheophyta
Class : Liliopsida
Order : Zingiberales
Family : Costaceae
Genus : *Costus*
Species : *igneus*
Binomial name: *Costus igneus*

Figure -1: Insulin Plant

The leaves of *Costus igneus* are used as a dietary supplement in the treatment of diabetes mellitus. In Traditional Medicine it is used to promote longevity, Treats rash, Reduces fever, asthma, bronchitis and to eliminate intestinal worms. The diverse activity of *Costus igneus* inspired us to investigate its antidiabetic activity [4].

The aim of the study was to identify various phytochemicals present in different solvent extracts of *Costus igneus* leaf. Determination of flavonoid in aqueous extract of *Costus igneus* by TLC and to quantitate flavonoids. The amylase inhibitory activity of aqueous extract of *Costus igneus* leaves on salivary amylase, goat pancreatic α-amylase and commercial porcine pancreatic α-amylase was also studied.

MATERIALS AND METHODS

Plant Source

The plant *Costus igneus* was collected from Siddha hospital in Arcot. The leaves were washed thoroughly in tap water and air dried in the shade for six days. The plant sample was grounded into uniform powder using milling machine. The powder was used for extract preparation.

Preparation of various solvent extracts

Aqueous extract:
Aqueous extract of the sample was prepared by soaking 2g of dried powder in 100ml of distilled water for 12 hrs. The extract was filtered using the whatmann filter paper no.42.

Ethanol extract:
2g of powdered leaf was used separately for the preparation of extract. Sample was packed between folds of filter paper and placed in Soxhelet apparatus, run between 60-80ºc using ethanol as solvent.

Chloroform extract:
2g of powdered leaf was used separately for the preparation of extract. Sample was packed between folds of filter paper and placed in Soxhelet apparatus, run between 60-80ºc using chloroform as solvent.

Qualitative analysis of phytochemicals

Qualitative analysis of phytochemicals were carried out in the aqueous, ethanol and chloroform extracts of the powdered leaf using standard procedures to identify the constituents as described by Harborne [5].

Determination of flavonoid by TLC method

The aqueous extract of *Costus igneus* were spotted on activated TLC plates of silica gel F 254 of 0.5 mm thickness coating. The plates (10 X 5 cm) were developed with solvent system Toluene: Ethyl acetate: methanol (5:3:2.2) to separate flavonoids. The developed plates were air dried, which was sprayed with chloroform and detected at 254nm. The bands were obtained and retardation factor (Rf) value were calculated.

DETERMINATION OF THE INHIBITORY EFFECT OF AQUEOUS EXTRACT OF *COSTUS IGNEUS* ON SALIVARY, GOAT, PANCREATIC AND PORCINE PANCREATIC AMYLASE ACTIVITIES

To determine the activity of salivary amylase, a calibration curve for maltose is plotted by taking the concentration ranging from 1000µgs to 5000µgs. This is done by taking 0.5ml to 2.5ml of standard maltose solution in the tubes marked as S1 to S5. Then 3ml of phosphate buffer, 0.5ml of 1% Nacl and varying volumes of distilled water are added to equal volume in all the tubes. An appropriate blank is also set by adding 3ml of phosphate buffer. 0.5 ml of 2N NaoH and 2 ml of DNSA are added. All tubes are heated in a boiling water bath for 3 minutes and the absorbance is read at 540nm.

The inhibition assay was performed using the chromogenic DNSA method [6], 1959). To measure the enzyme activity, in a set of tubes marked as B, T and C, buffered starch substrate, phosphate buffer, 1% Nacl and distilled water were added. Then all the tubes were incubated for 10 minutes at 37°C for activation. Then the enzyme source was added only to test tube (T). Then all the tubes were kept for incubation for 10 minutes at 37°C. 2N NaoH was added in the tubes marked as B, T and C, then 0.5 ml of enzyme source was added to the control tube (Tc). Finally 2 ml of DNSA was added to all the tubes and heated in a boiling water bath for 3 minutes, cooled and the absorbance is read at 540 nm.

To determine the inhibitory activity of *Costus igneus* on amylase activity, take set of tubes marked as B, T_C, T, T_{WI} (with inhibitor-Acarbose) and Tp (Aqueous *Costus igneus* plant extract)**.** Buffered starch substrate, phosphate buffer, 1% Nacl and distilled water was added to all the tubes accordingly. Then all the tubes are incubated for 10 minutes at 37°C for activation. Then enzyme source followed by both plant extract and acarbose is added to the tubes T_{WI} and Tp**.** Then all the tubes are kept for incubation for 10 minutes at 37°C. Then 2N NaoH is added in the tubes for B, T, T_{WI} and Tp. Then 0.5 ml of enzyme source and plant extract is added to the control tube (T_C). Finally 2 ml of DNSA is added to all the tubes and heated in a boiling water bath for 3 minutes, cooled and the absorbance is read at 540nm.

The % inhibition of *Costus igneus* on amylase activity was calculated as follows,

$$\% \text{ inhibition} = \frac{[(\text{Control} - \text{Plant Extract})] \times 100}{\text{Control}}$$

RESULTS AND DISCUSSION

Table 1 shows the phytochemical compositions of the aqueous, ethanol and chloroform extracts of *Costus igneus* leaf. The results illustrate the presence of various phytochemicals in *Costus igneus* leaf extracts. The ethanol, aqueous and chloroform extracts show the presence of carbohydrates, cardiac glycosides, proteins, flavonoids, saponins, and tanins.

Table – 1: Qualitative analysis of the phytochemicals of aqueous, ethanol and chloroform extracts of *Costus igneus* leaf

Solvents / Phytochemicals	Aqueous Extract	Ethanolic Extract	Chloroform Extract
Flavonoids	++	++	++
Saponins	++	++	++
Tannins	++	++	—
Terpenoids	—	+	—
Cardiac Glycosides	++	++	++
Steroids	—	—	—
Carbohydrates	+	+	—
Amino acids	+	—	—

Saponins and flavonoids present in *Costus igneus* are responsible for the therapeutic properties. It was reported that *Costus igneus* possess diuretic, hypotensive, anti-obesity and hypoglycemic activities. The active constituents responsible for anti diabetic properties are found to be flavonoids and Saponins[7]. Flavonoids are found to act on various molecular targets and regulate different signaling pathways in pancreatic β- cells, hepatocytes, adipocytes and skeletal myofibres.

The ability of saponin to reduce elevated plasma blood glucose makes it an excellent candidate in the treatment of diabetes mellitus. The hypoglycemic action of saponin is by the restoration of insulin response, improvement in insulin signaling, Inhibition of α- glucosidase activity [8].

Figure 2 shows the identification of flavonoids by TLC. The plant extract was separated and identified with quercitin as standard. The result indicates the presence of flavonoids. Flavonoids, such as flavanones, flavones, and flavonols, are among the most widespread groups of plant secondary metabolites and show a broad diversity of biological functions [9, 10].
Costus igneus appears to be a very good source of flavonoids. Polyphenolic compounds such as flavonoids (quercetin, kaempferol) are normally found in *Costaceae* family. Flavonoids may exert beneficial effects in diabetes by enhancing insulin secretion and reducing apoptosis, promoting proliferation of pancreatic β- cells and inhibition of amylases activity.

Figure -2: Identification of flavonoids in aqueous extract of *Costus igneus* by TLC method

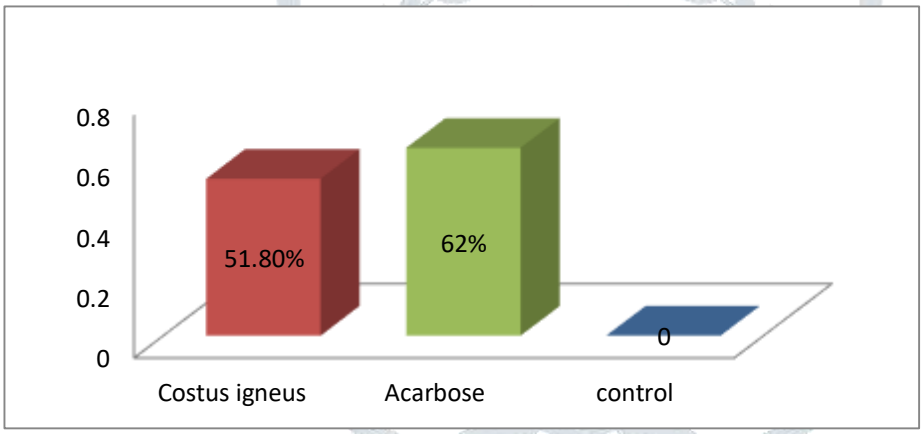
"S" - indicates standard flavonoids (Quercetin)
"U" - indicates unknown (*Costus igneus* plant extract)

The Inhibitory effect of *Costus igneus* and acarbose on salivary amylase activity is shown in Table-2. The present investigation indicates that the crude aqueous extract of *Costus igneus* shows 51.8 % inhibition. The antidiabetic drug acarbose shows 62% inhibition. The control enzyme source shows no inhibition. The % inhibition of *Costus igneus* on salivary amylase enzyme activity is significantly higher when compare to that of enzyme control. The % inhibition of *Costus igneus* on salivary amylase enzyme activity is lower than that of the % inhibition of acarbose. These findings suggest that *Costus igneus* has got the delaying effect on intestinal glucose absorption. Natural remedies used since ancient times became popular as effective, inexpensive and safe mode of treating diabetes. It is recognized that there are more than 1,200 species of plants with hypoglycemic activity. A review on the medicinal plants has reported the use of approximately 126 antidiabetic plants including Costus speciosus leaves [11].

Flavonoids have the ability to scavenge free radicals and chelate metals. Based on the hypothesized relation between diabetes and inflammation and the potential for flavonoids to protect the body against free radicals and other pro-oxidative compounds, it is biologically plausible that consumption of flavonoids or flavonoid-rich foods may reduce the risk of diabetes [12].

Table - 2: The Inhibitory effect of aqueous extract of *Costus igneus* and Acarbose on salivary amylase activity

S.No	Parameters	% Inhibition
1	*Costus igneus* (250µgs/ml)	51.8%
2	Acarbose (250µgs/ml)	62%
3	control	No Inhibition



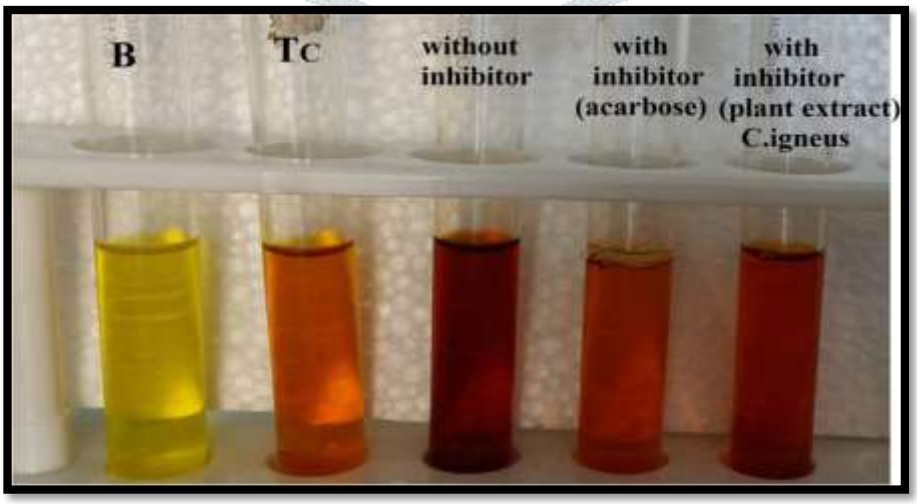


Figure-3: The Inhibitory effect of aqueous extract of *Costus igneus* and Acarbose on salivary amylase activity

The Inhibitory effect of *Costus igneus* and acarbose on goat pancreatic α-amylase activity is shown in Table-3. The results indicate that the crude aqueous extract of *Costus igneus* shows 52 % inhibition. The antidiabetic

drug acarbose shows 56.4 % inhibition. The control enzyme source shows no inhibition. The % inhibition of *Costus igneus* on goat pancreatic α-amylase activity is significantly higher when compare to that of enzyme control.

Costus igneus or insulin plant is being used in the traditional folk medicine of various tribes in south East Asia, especially in southern parts of India for the treatment of symptoms such as unexplained weight loss or gain, excessive thirst, hunger, insomnia, weakness or fatigue together which can be characterized as the indicators of pre-diabetes and diabetes. Studies of various researches have reported the therapeutic efficacy of *Costus igneus* against T2DM [13].

Table - 3: The Inhibitory effect of aqueous extract of *Costus igneus* and Acarbose on goat pancreatic α-amylase activity

S.No	Parameters	% Inhibition
1.	*Costus igneus* (250µgs/ml)	52%
2.	Acarbose (250µgs/ml)	56.4%
3.	control	No Inhibition

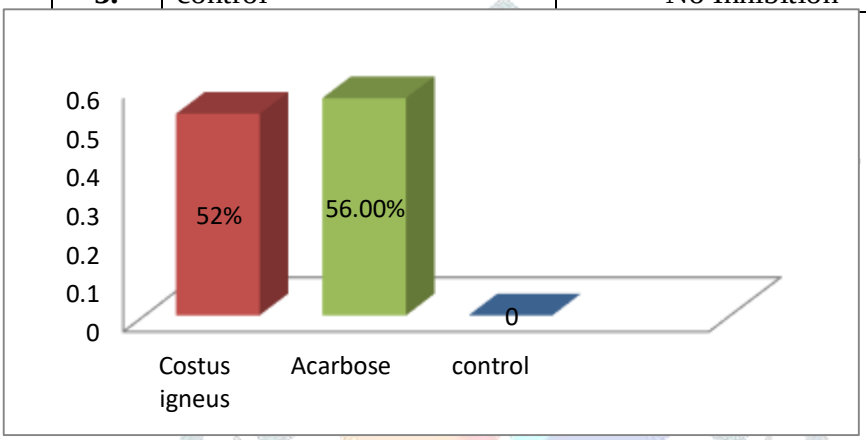
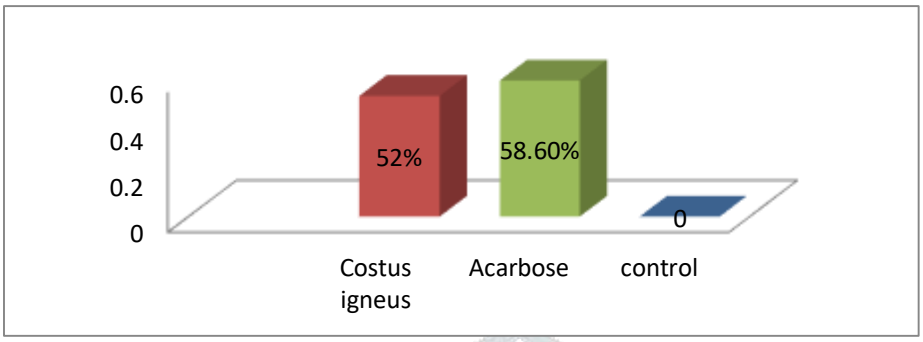


Figure-4: The Inhibitory effect of aqueous extract of *Costus igneus* and Acarbose on goat pancreatic α-amylase activity

The Inhibitory effect of *Costus igneus* and acarbose on commercial porcine pancreatic α-amylase activity is shown in Table-4. The present investigation shows that the crude aqueous extract of *Costus igneus* shows 52 % inhibition. The antidiabetic drug acarbose shows 58.6 % inhibition. The control enzyme source shows no inhibition. The % inhibition of *Costus igneus* on commercial porcine pancreatic α-amylase activity is significantly higher when compare to that of enzyme control. Insulin resistance is the primary cause of development of T2DM, causing significant morbidity and mortality due to various micro and macro vascular changes affecting the quality of life of millions of people all over the world

Table-4: The Inhibitory effect of aqueous extract of *Costus igneus* and Acarbose on commercial porcine pancreatic α-amylase activity.

S.No	Sample	% Inhibition
1.	*Costus igneus* **(**250µgs/ml)	52%
2.	Acarbose (250µgs/ml)	58.6%
3.	control	No Inhibition



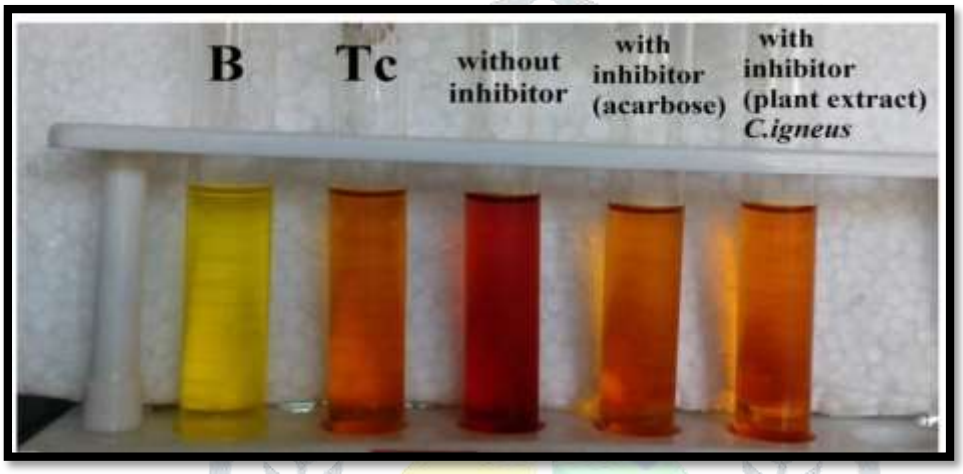


Figure-5: The Inhibitory effect of aqueous extract of *Costus igneus* and Acarbose on commercial porcine pancreatic α-amylase activity

One of most studied natural products has been flavonoids which are found predominantly in several plant sources. Moreover, recent investigations have demonstrated that flavonoids are very promising antidiabetic agents. These polyphenolic compounds had been widely investigated in recent years due to their beneficial properties in cardiovascular disease among their pharmacological effects as anti-inflammatory, antioxidant, antiviral and anticarcinogenic agents [14].

Many traditional plant treatments for diabetes mellitus are used throughout the world. Management of diabetes without any side effect is still a challenge to the medical system. It has been reported that many herbs and plant products have been shown to have hypoglycemic action. Flavonoids are known to be effective bioactive antidiabetic principles. This has led to an increasing demand for natural products with antidiabetic activity and fever side effects [15].

CONCLUSION

In the present study the aqueous extract of *Costus igneus* have shown significant amount of Inhibitory effect on salivary amylase, Goat pancreatic α-amylase and commercial porcine pancreatic α-amylase activities. The results indicate that the flavonoids present in this plant may responsible for this antidiabetic activity. The possible mechanism for the anti-diabetic activity of this plant is to delay the postprandial rise in blood glucose by inhibiting the amylase activity in the digestive system. This property makes it as a potential candidate in the treatment of diabetes. Further investigations can be carried out to reveal the exact mechanism of the activity of *Costus igneus.*

REFERENCES

1. Porter JR, Barrett TG: Monogenic syndromes of abnormal glucose homeostasis: clinical review and relevance to the understanding of the pathology of insulin resistance and beta cell failure. Journal of Medical Genetics 2005; 42: 893-902.
2. Ibrahim, R.: Diabetes mellitus type II: Review of oral treatment options. International Journal of Pharmacy and Pharmaceutical Science 2010; 2(1): 21-30.
3. Gilman EF. Florida: University of Florida, Inc; c2012. *Costus igneus*. Fact sheet. FPS-1.
4. Brackenridge, Betty, Richard Rubin. Sweet Kids: How to Balance Diabetes Control and Good Nutrition with Family Peace, Alexandria, VA: American Diabetes Association, second edition, 2002.
5. Harborne JB, Baxter H. The handbook of natural flavonoids, Chichester, UK: John Wiley and Sons, Volume 1 and 2, 1999.
6. Miller G. L: Use of Dinitrosalicylic acid reagent for determination of reducing sugar. Journal of Analytical Chemistry 1959; 31(3): 426-428.
7. Mallikharjuna, P.B., L.N. Rajanna, Y.N. Seetharam and G.K. Sharanabasappa: Phytochemical studies of *Strychnos potatorum* L.f.- a medicinal plant. Electronic Journal of Chemistry 2007; 4 : 510-518.
8. Kiessoun Konate, Kassi Yomalan, Okasana Sytar, Patrice Zerbo, Marian Brestic, Van Damme Patrick, Paul Gagniuc: "Free Radicals Scavenging Capacity, Antidiabetic and Antihypertensive Activitiy. Hindawi Publishing coporation 2014; 1-13.
9. Wang, J., Ma, X. M., Kojima, M., Sakakibara, H., and Hou, B. K: N-Glucosyl transferase UGT76C2 is involved in cytokinin homeostasis and cytokinin response in Arabidopsis thaliana. Plant Cell Physiology 2011; 52: 2200–2213.
10. Wang, Y. C., Chuang, Y. C., and Ku, Y. H: Quantitation of bioactive compounds in citrus fruits cultivated in Taiwan. Food Chemistry 2007; 102: 1163–1171.
11. Handunge Kumudu Irani Perera, Walgama Kankanamlage Vindhya Kalpani Premadasa and Jeyakumaran Poongunran: α-glucosidase and glycation inhibitory effects of *Costus speciosus* leaves. BMC Complementary and Alternative Medicine 2016; 16:2.
12. Ramachandran Vinayagam and Baojun Xu. Antidiabetic properties of dietary flavonoids: a cellular mechanism review. Nutrition & Metabolism 2015; 12:60.
13. Murthy EGK Talasila, Hemalatha Bavirisetti, Jithendra Chimakurthy, Mayuren Candasamy: Effect of *Costus igneus*: The insulin plant, on pre-diabetes and diabetes in neonatal streptozotocin rats. Journal of Health Sciences 2014; 4(3):162-168.
14. Ramulu Jadhav, Goverdhan Puchchakayala. Hypoglycemic And Antidiabetic Activity of Flavonoids: Boswellic Acid, Ellagic Acid, Quercetin, Rutin on Streptozotocin-Nicotinamide Induced Type 2 Diabetic Rats. International Journal of Pharmacy and Pharmaceutical Sciences. 2012; 4 (2): 251-256.