ISSN: 0975-766X
CODEN: IJPTFI

Available Online through ***Research Article***
www.ijptonline.com

IN VITRO ANTIDIABETIC, ANTICANCER AND HYPOLIPIDIMIC ACTIVITY OF *COSTUS IGNEUS* N.E.Br

Deva Kalidoss, Sheik Abdulla Shahul Hameed, Sivakumar Ramalingam, Sakthivel Selvaraju
Department of Chemistry and Biosciences, SASTRA University, Srinivasa Ramanujan Centre,
Kumbakonam, Tamil Nadu, India.
Email:sheikhaneef@src.sastra.edu.

Received on: 11-02-2017 *Accepted on: 27-03-2017*

Abstract

Diabetes mellitus is a common and very prevalent disease affecting the citizens of both developed and developing countries. In the present study to investigate the *in vitro* antidiabetic, anticancer and hypolipidimic activity of *Costus igneus. In vitro* antidiabetic assays such as inhibitory activity of α – amylase, glucose adsorbtion, glucose diffusion was studied in the ethanol extract of *Costus igneus*. The results obtained revealed that the plant extracts showed significant inhibitory activity on carbohydrate metabolizing enzymes to reduce the postprandial blood sugar level. *In vitro* anticancer activity on trypan blue dye exclusion and MTT assay and LDH assays were carried out ethanol extract against EAC cell line. The result revealed that the ethanol extract of *Costus igneus* showed pronounced activity against the tested cell line. *In vitro* hypolipidimic activity on pancreatic lipase inhibitory was studied in the ethanol extract. Thus the result revealed that the ethanol extract showed significant inhibitory activity

Keywords: *Costus igneus*, Diabetes mellitus, Anticancer activity, Hypolipidimic activity

Introduction

Diabetes mellitus is a common and very prevalent disease affecting the citizens of both developed and developing countries. It is estimated that 25% of the world population is affected by this disease. Diabetes mellitus is caused by the abnormality of carbohydrate metabolism which is linked to low blood insulin level or insensitivity of target organs to insulin (Maiti R *et al.,* 2004). Despite considerable progress in the treatment of diabetes by oral hypoglycemic agents, search for newer drugs continues because the existing synthetic drugs have several limitations. The herbal drugs with anti diabetic activity are yet to be commercially formulated as modern Medicines, even though they have been acclaimed for their therapeutic properties in the traditional systems of medicine (Wadkar *et al.,* 2008).

Cancer is a group of diseases caused by loss of cell cycle control. Cancer is associated with abnormal uncontrolled cell growth **(**Rishnamurthi, 2007). Cancer is caused by both external factors (tobacco, chemicals, radiation and infectious organisms)and internal factors (inherited mutations, hormones, immune conditions, and mutations that occur from metabolism). Cancer is a significant worldwide health problem generally due to the lack of widespread and comprehensive early detection methods, the associated poor prognosis of patients diagnosed in later stages of the disease and its increasing incidence on a global scale. Indeed, the struggle to combat cancer is one of the greatest challenges of mankind **(**Divisi D, *et al.*, 2006).

The major causes of cancer are smoking, dietary imbalances, hormones and chronic infections leading to chronic inflammation. Age is also a primary risk factor for most cancers, with about 77% of all cancers diagnosed among people aged 55 or older. Breast cancer is the most common form of cancer in women worldwide. According to an estimate, 50% of breast cancer and 37% of prostate cancer patients use herbal products. Several chemo preventive agents are used to treat cancer, but they cause toxicity that prevents their usage. In this context, the natural products derived from medicinal plants have gained significance in the treatment of cancer. (Engel *et al*., 2011).

Diabetes has become one of the devastating disease afflicating health of many people, in recent times, and has accounted for a high proportion of health problems worldwide.(Williams *et al.*, 2000). Hyperlipidemia is considered as a risk factor involved in the development of cardiovascular disease (Frishman, 1998). High lipid le Diabetes mellitus is a chronic metabolic disorder characterized by abnormalities in carbohydrate, lipid, and lipoprotein metabolism, which not only lead to hyperglycemia but also cause many complications, such as hyperlipidemia, hyperinsulinemia, hypertension, and atherosclerosis (Alberti et al.,1997). Control of diabetes mellitus normally involves exercise, diet and chemotherapy. Development and utilization of antidiabetic plants have attracted increasing interest. (Alarcon-Aguilar et al., 1997). The plant kingdom is a wide field to search for natural effective oral hypoglycemic or hypolipidemic agents that have slight or no side effects can harden the arteries or speed up the process of atherosclerosis. Nowadays, there are numerous hypolipidemic drugs for clinical use; however, the pharmacologists and chemists have been perplexing by the characteristic profiles of toxic side effects including numerous harmful syndromes (Kameswara Rao,B *et al*.,2000). The present study is aimed to examine the *in vitro* antidiabetic, anticancer And hypolipidemic effect of the ethanol extract of *Costus igneus* (EECI).

Materials and Method

***In vitro* antidiabetic studies**

In vitro α-amylase inhibition assay was carried out by the method of Apostolidis (2007). The α-glucosidase inhibitory activity was determined according to the method described by Apostolidis et al., (2007). Yeast cells were prepared according to the method of Gupta daksha *et al*., (2013).

***In-vitro* cytotoxicity assay carried out by Scudiero DA *et al*., 1988**

Trypan Blue (Sheeja KR *et al*., 1997)

Short- term *In-Vitro* cytotoxicity was assessed using Ehrlich Ascites Carcinoma cell lines and Daltons Lymphoma Ascites cells by incubating different concentrations of the ethanolic extract of *Costus igneus*.at 37°C for 3 hours. The tumor cells were aspirated from peritoneal cavity of tumor bearing mice using an insulin syringe and transferred to a test tube containing isotonic saline. The cells were then washed in normal saline and the cell number was determined using a haemocytometer and adjusted to $1x10^6$ cells/ml. For the cytotoxity assay, different concentrations of the extracts (50-1000 µg/ml) were added to each tube and the final volume was adjusted to one ml with normal saline. Control tubes were maintained with the saline and tumor cells but without the plant extract. All the tubes were incubated at 37^0c for 3 hours. After incubation 0.1ml of 0.2% tryphan blue dye in isotonic saline was added to a watch glass along with 0.1ml of test sample and the number of viable (unstained) and non-viable (stained) cells were counted using haemocytometer.

$$\% \text{ Dead cells} = \frac{\text{Total cells counted} - \text{total viable cells}}{\text{Total cells counted}} \times 100$$

MTT ASSAY(Scudiero *et al*., 1988)

Increasing concentrations of WECB were added to the cells and incubated at 37ºC for 24 hrs in CO_2 incubator with 5% CO_2. The media was replaced with a fresh growth medium along with 20 µl of 3(-4, 5-dimethyl thiazol-2-yl) 2, 5 di phenyl tetrazolium bromide (MTT, sigma) MTT reagent was added to it. Again it was incubated for 4 hrs at 37ºc. After incubation purple precipitate was clearly visible under the inverted microscope then the growth medium was removed and 200µl of 0.1% 0.1N acidic isopropyl alcohol was added to the cells to dissolve the Formazan crystals. Then the covered plates were kept in the dark at 18-24º per overnight. The samples were drawn every 2 hrs and observed the

reading at 570nm. If the reading is low returned the plate for incubation. Each experiment was conducted in triplicate form. The average was calculated, and compared with the control test samples. The percentage growth inhibition was calculated using the following formula.

$$\% \text{ Growth Inhibition} = \frac{\text{Control OD} - \text{Treated OD}}{\text{Control OD}} \times 100$$

In Vitro Cytotoxic Assay of EECI Against EAC

Cell Line (LDH ASSAY) (Russo et *al*., 2016)

Lactic dehydrogenase activity is spectrophotometrically measured in the culture medium and in the cellular lysates at 340 nm by analyzing NADH reduction during the pyruvate lactate transformation. Cells are lysed with 50 mM 0.1 ml Tris-HCI buffer, (pH 7.4) + 0.1ml 20mM EDTA + 0.1 of 0.5% Sodium Dodecyl Sulfate (SDS), further isrupted by sonication and centrifuged at 13,000 X g for 15min. The precipitate obtained was added with 33 μl of the plant extract (31.5-250 μg/ml in 4mM PBS), 0.2ml of 1Mm Pyurate and 0.2ml of 0.2mM NADH. After 15 minutes incubation reduction in OD of NADH was observed at 340 nm in UV-Spectrophotometer.

Measurement of *In-vitro* Pancreatic Lipase Activity

A suspension of triolein (80 mg), phosphatidylcholine (10 mg), and taurocholic acid (5 mg) in 9 ml of 0.1 M *N*-tris-(hydroxymethyl)-methyl-2-aminoethanesulfonic acid (TES) buffer (pH 7.0) containing 0.1 M NaCl was sonicated for 5 min. This sonicated substrate suspension (0.1 ml) was incubated with 0.05 ml (10 U) of pancreatic lipase and 0.1 ml of various concentration of sample solutions (50, 100, 200, 300, 400 and 500 µg/ml) for 30 min at 37 ^{o}C in a final volume of 0.25 ml. The amount of release of oleic acid produced was determined based on the method described by **Zapf** with a minor modification. The incubation mixtures were added to 3 ml aliquots of a 1:1 (v/v) mixture of chloroform and *n*-heptane containing 2% (v/v) methanol and extracted by shaking the tubes horizontally for 10 min in a shaker. The mixture was centrifuged at 2,000 g for 10 min, and the upper aqueous phase was removed by suction. Copper reagent (1 ml) was then added to the lower organic phase. The tube was shaken for 10 min, the mixture was centrifuged at 2,000 g for 10 min, and 0.5 ml of the upper organic phase, which contained copper salts of the extracted free fatty acids (FFA),

was treated with 0.5 ml of 0.1% (v/v) bathocuproine in chloroform containing 0.05% (w/v) 3-(2)-tert-butyl-4-hydroxy-anisol. The absorbance was then measured at 480 nm. Pancreatic Lipase inhibition was expressed in percentage (%).

Result and Discussion

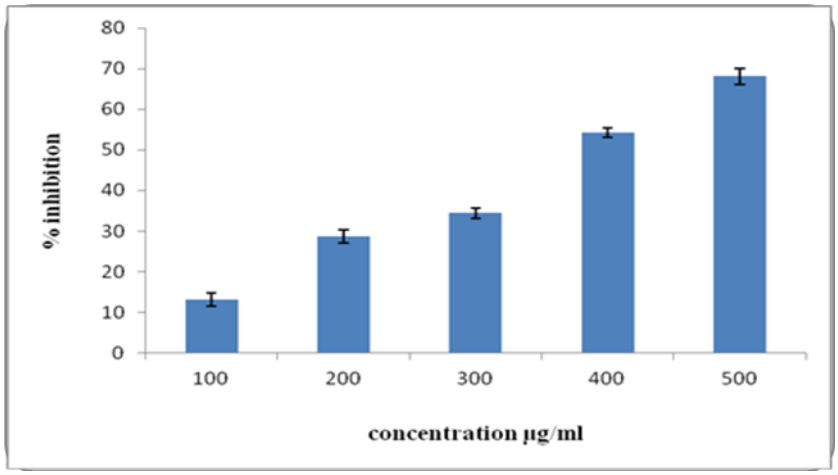
Table-1: Effect of Costus igneus on Alpha Amylase activity.

S.NO	CONCENTRATION(µg/ml)	% INHIBTION
1	100	13.24±1.62
2	200	28.72±1.68
3	300	34.41±1.32
4	400	54.21±1.22
5	500	68.12±1.92

IC_{50}= 368.9µg/m

The inhibitory effect of *Costus igneus* on α – amylase enzyme was given in the **Table 1** and **Figure 1**. From the results it is clear that the ethanol extract of plant were found to exhibit highest inhibition on α – amylase enzyme with increased concentration and maximum activity at 500 µg/ml. The IC_{50} value was found to be **368.9 µg/ml.**

Figure 1- Effect of *Costus igneus*. on alpha amylase inhibitory activity.



α – amylases play an important role in starch breakdown by hydrolysing alpha – bonds of large alpha linked polysaccharide such as glycogen and starch to yield glucose and maltose. The α – amylase inhibitors act as an anti-nutrient that obstructs the digestion and absorption of carbohydrates. The carbohydrate metabolizing inhibitors such as *α*-amylase, *α*-glucosidase and sucrase delay carbohydrate digestion causing a reduction in the rate of glucose absorption and consequently blunting the postprandial plasma glucose rise hence, play an important role in controlling postprandial blood glucose levels.

Table-2: Effect of *Costus igneus* on Glucose Diffusion.

	GLUCOSE CONTENT IN DIALYSATE			
SAMPLES	**60 Minutes**	**120 Minutes**	**180 Minutes**	**240 Minutes**
CONTROLE	0.11	0.81	1.12	1.42
PLANT values	0.04±0.01	0.18±0.04	0.21±0.03	0.28±0.02
GDRI values	33.42%	21.14%	16.31%	9.37%

The effect of the plant extract on retarding glucose diffusion across the dialysis membrane is shown in **Table 2**. The rate of glucose diffusion was found to increase with time from 60 to 240 min. In the present study, the movement of glucose across the dialysis membrane was monitored once in 60 min till 240 min and it was found that, the sample of plant extract demonstrated significant inhibitory effect on movement of glucose into external solution across dialysis membrane compared to control. The retardation of glucose diffusion by *Costus igneus*. These effect was reflected with increasing order of GDRI values.

Table-3: Effect of *Costus igneus* on Glucose Adsorbtion.

	GLUCOSE CONCENTRATION (mMole/L)			
SAMPLE	**5mM**	**10mM**	**50mM**	**100mM**
PLANT EXTRACT ADSORBTION CAPACITY	**8±0.57**	**12.33±1.20**	**33±0.57**	**66.3±1.20**

A series of different concentrations of glucose (5-100mmole/l) were used to study glucose adsorption capacity of plant extract. As seen in **TABLE-3**, adsorption capacities of *Costus igneus* at a different glucose concentration. At high glucose concentration (100mMole/l) adsorption capacity of *Costus igneus* (66.3±1.20). When low glucose concentration used (5mMole/l) the glucose-adsorption capacity extract was found to be (8±0.57). Thus, the results indicated that the fibers could effectively bind to glucose even at low concentrations of glucose thereby reducing the amount of accessible glucose in small intestinal. It is observed that the glucose adsorption capacity of fibers is directly related to available

glucose concentration. Thus the plant extract may be beneficial with respect to reducing amount of accessible glucose in the small intestine.

Figure -2: Effect of *Costus igneus* on Trypan Blue Dye Method.

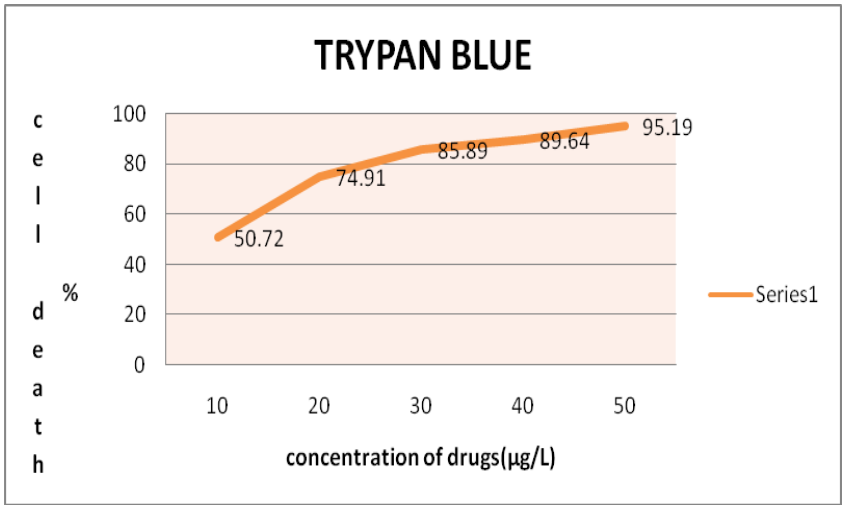


Table-4: Anti canceer effect of ethanol extract on EAC cells (Trypan blue method).

S.No	CONCENTRATION (µg/mL)	VIABLE CELLS(%)	DEAD CELLS(%)
1.	control	96.15	3.80
2.	10	49.28	50.72
3.	20	25.09	74.91
4.	30	14.11	85.89
5.	40	10.36	89.64
6.	50	4.81	95.19

Dead cell = stained with Trypan blue dye

Viable cell = Not stained with trypan blue dye

Figure-2 and Table-4 The ethanolic extract of leaves of Costus igneus was tested against EAC cell lines. Different concentration of plant extract was inoculated with selected cell line and the cytotoxicity was assessed using trypan blue dye exclusive. The test based on the principle that the dead cell accepts dye and stain withblue color. The plant drug may disturb the membrane integrity and caused the cell death, which is one of the hall marks of apoptosis. The ethanol extract showed 95.19% of cytotoxicity(1000µL) against EAC cell line.

Table-5: Anticancer effect of Ethanol extract against EAC cell line (MTT assay)

S.No	CONCENTRATION (µg/mL)	VIABLE CELLS (%)	DEAD CELLS (%)
1.	Control	97.00	3.00
2.	31.25	85.52	14.48
3.	62.5	66.98	33.02
4.	125	42.91	57.09
5.	250	31.97	68.03

IC 50 values of ethanol extract = 95 µg/mL

TABLE-5 and **FIGURE -3** represent the result of anticancer evaluation of ethanol extract of costus igneus was examined by MTT assay using EAC cell line. The extend of cytotoxicity can be measured by MTT dye reduction assay. The percentage viability was analyzed by MTT assay after treatment of ethanol extract of *Costus igneus* at 31.25 µg/mL,62.5µg/mL, 125µg/mL, and 250µg/mL concentration.

Figure-3: Anticancer effect of Ethanol extract against EAC cell line (MTT assay).

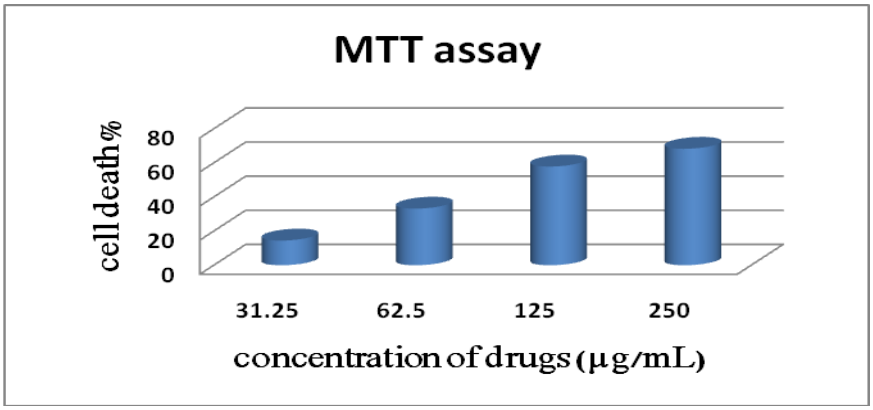


Table- 6: Anticancer effect of Ethanol extract on LDH Assay.

S.No	CONCENTRATION(µg/mL)	VIABLE CELLS	DEAD CELLS
1.	Control	96.00	4.00
2.	31.25	93.86	6.14
3.	62.05	69.16	30.84
4.	125	52.69	47.31
5.	250	33.88	66.12

IC 50 value of ethanol extract =140 µg/ml

LDH Leakage (%) Related To Control Wells Containing Cell Culture Medium Without Extracts Was Calculated By [A] Test / [A] Control X 100. Where [A] Test Is The Absorbance Of The Test Sample And [A] Control Is The Absorbance Of The Control Sample. The percentage viability was analyzed by LDH assay after treatment of ethanol extract of *Costus igneus* at 31.25 µg/mL,62.5µg/mL, 125µg/mL,and 250µg/mL concentration.

Figure-4: Anticancer effect of Ethanol extract on LDH Assay.

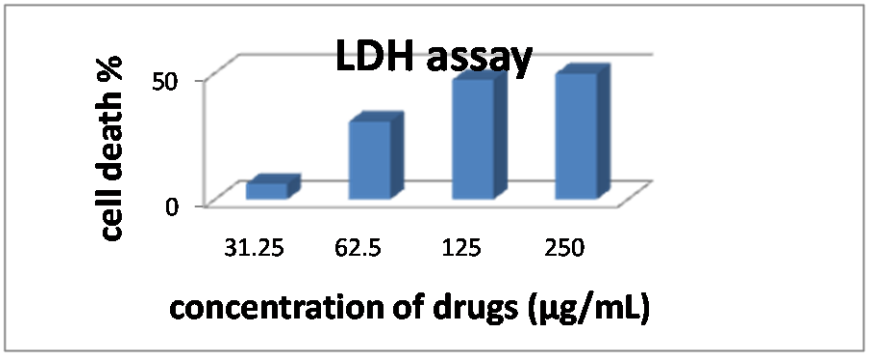


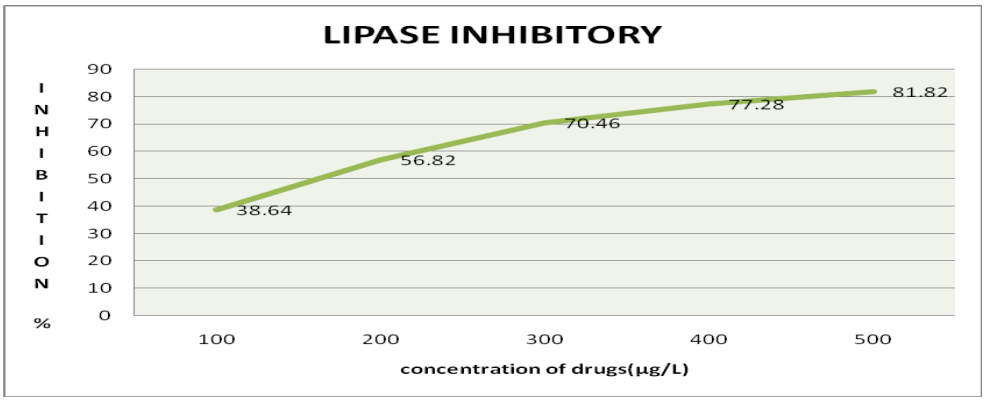
Table-7: Effect of *IN VITRO* pancreatic Lipase Activity.

S.No	CONCENTRATION(µg/mL	INHIBITION%
1.	100	38.64±1.23
2.	200	56.82±1.28
3.	300	70.46±1.37
4.	400	77.28±1.29
5.	500	81.82±1.76

IC 50 VALUES=180.05µg/mL

The inhibitory activity of ethanol extract of *Costus igneus* against pancreatic lipase are shown in TABLE-7. The *Costus igneus* extract inhibited pancreatic lipase activity by 38.64, 56.82,70.46, 77.28, and 81.82% at concentrations of 100,200, 300, 400 and 500 mg/ml *in vitro*, respectively.

Figure-5: Effect of *in vitro* pancreatic lipase activity.



Conclusion

In the present study, research has been carried out to investigate the antidiabetic potential of the ethanol extract of ***Costus igneous.*** *In vitro* antidiabetic assays such as inhibitory activity of α – amylase, glucose adsorbtion, glucose diffusion was studied in the ethanol extract of ***Costus igneus*** . The results obtained revealed that the plant extracts showed significant inhibitory activity on carbohydrate metabolizing enzymes to reduce the postprandial blood sugar level.*In vitro* anticancer activity on trypan blue dye exclusion and MTT assay and LDH assays were carried out ethanol extract against EAC cell line. The result revealed that the ethanol extract of *Costus igneus*. showed pronounced activity against the tested cell line. *In vitro* hypolipidimic activity on pancreatic lipase inhibitory was studied in the ethanol extract. Thus the result revealed that the ethanol extract showed significant inhibitory activity.

References

1. Alarcon-Aguilara, Alejandro Zamilpab,Ma. Dolores Perez-Garciab,Julio C. Almanza-Pereza, Eunice Romero-Nu., Effect of Hibiscus sabdariffa on obesity in MSG mice". Journal of Ethnopharmacology 114 (2007) 66–71.
2. Alberti KGMM, Hockaday TDR. Diabetes Mellitus. In: Weatherall DJ, Ledingham JGG, Warrell DA. "Oxford Textbook of Medicine", Oxford, Oxford University Press, 1987:9.51-9.101
3. Alberti. P. Packer Hypoglycemic and hypolipidemic effects and antioxidant activity of fruit extracts from Lycium barbarum. Life Sciences 76 (1997) 137–149.
4. Ali1 M.A., M. Abul Farah2, F.M. Al-Hemaid1 and F.M. Abou-Tarboush2., In vitro cytotoxicity screening of wild plant extracts from Saudi Arabia on human breast adenocarcinoma cells.,
5. Anand P, Kunnumakara AB, Sundaram C, et al. Cancer is a Preventable Disease that Requires Major Lifestyle Changes. Pharmaceutical Research. 2008;25(9):2097-2116. doi:10.1007/s11095-008-9661-9.res Major Lifestyle Changes"
6. Apostolidis E, Kwon YI, Shetty K, Inhibitory potential of herb, fruit, and fungus enriched cheese against key enzymes linked to type 2 diabetes and hypertension. Inn Food Sci Emerg Technol. 2007; 8: 46-54.
7. Archit Rastogi*, Srihari Shankar, Gayathri Mahalingam., Phytochemical Screening, Anti-Oxidant Activity And In Vitro Anti-Diabetic Activity Of Aqueous, Methanolic, Ethanolic And Chlo Roformic Extracts Of Hygrophilaauriculata., International Journal of Pharmacy and Pharmaceutical Sciences 2014 ISSN- 0975.

8. Bhalodia Amira A ,Jared L. Crandona,Gregory Williamsb and David P. Nicolaua., In Vivo Efficacy of Humanized Ceftaroline Fosamil-Avibactam Exposures in a Polymicrobial Infection Model., Antimicrob. Agents Chemother. November 2013 vol. 57 no. 11 5674-5678.
9. Birari RB, Bhutani KK. Pancreatic lipase inhibitors from natural sources: unexplored potential. Drug Discov Today. 2007;12:879–889. [PubMed].
10. Cerruti-Sola, S., Sternberg, E.D., Dolphin, D., Levy, J.G. Biodistribution of tritiated benzoporphyrin derivative (3H-BPD-MA), a new potent photosensitizer, in normal and tumor-bearing mice. J. Photochem. Photobiol. B. 1985 ;5:231–244
11. Chandrasekar, Gupta Daksha, Richard Lobo, Yogendra and Gupta Nilesh, "In vitro antidiabetic activity of Stem bark of Bauhinia purpurea Linn", Scholar Research Library Der Pharmacia Letter, 2012, 4 (2):614 – 619.
12. Croce CM1,. 'Oncogenes and cancer'. N Engl J Med. 2008 Jan 31;358(5):502-11. doi: 10.1056/NEJMra072367.
13. Daisy, S Ignacimuthu, V Duraipandiyan., Antidiabetic and antilipidemic effect of eremanthin from Costus speciosus (Koen.) Sm., in STZ-induced diabetic rats., Chemico-biological interactions, 2009., Volume 182 Issue 1Pages 67-72.
14. Devi VD, Urooj A. Evaluation of anti hyperglycemic and anti lipid peroxidative effect of Costus igneus Nak in streptozotocin induced diabetic rats. International Journal of Current Research. 2011;33:4–8.
15. Fifa TD Bothon,Eric DebitonEmail author, Felicien Avlessi, Christiane Forestier,Jean-Claude Teulade and Dominique KC Sohounhloue.,In vitro biological effects of two anti-diabetic medicinal plants used in Benin as folk medicine.,BMC Complementary and Alternative MedicineBMC series ¿ open, inclusive and trusted 2013 13:51.
16. G.saravanan G and Pari L "Hypoglycaemic and Antihyperglycamic Effect of Syzygium cimini Bark in Streptozotocin-induced Diabetic Rats"2008.Acdemicjournals.jpt.3-(1):1-10
17. Ganiyu Oboh, Adelusi Temitope Isaac and Richard Akinlolu Ajani. "Inhibition of Key Enzymes Linked to Type 2 Diabetes and Sodium Nitroprusside Induced Lipid Peroxidation in Rats'' Pancreas by Phenolic Extracts of Avocado Pear Leaves and Fruit,Int J Biomed Sci.2014 Sep;10(3):208-209.
18. Genet. Mol. Res. 13 (2): 3981-3990 (2014) Received June 3, 2013.

19. Gilman EF. Florida: University of Florida, Inc; c2012. Costus igneus. Fact sheet. FPS-151. EDIS-Electronic Data Information Source-UF/IFAS Extension.
20. Gupta daksha, kondongala subraya, chandrashekher, Girish pal. In vitro antidiabetic activity of pentacyclic tritrprnoida and fatty acid ester from Bauhinia purpurea. Int J of Pharmacology and Pharm Technology 2013;2:2277-3436.
21. Gupta daksha, kondongala subraya, chandrashekher, Girish pal. In vitro antidiabetic activity of pentacyclic tritrprnoida and fatty acid ester from Bauhinia purpurea. International Journal of Pharmacology and Pharmaceutical Technology 2013; 2: 2277- 3436.
22. Hong WK1, Sporn MB. "Recent advances in chemoprevention of cancer". Science. 1997 Nov 7;278(5340):1073-7.
23. Hua-qiang D,Fu-jun Wang, MD,Zhen Kang "Effects of endovascular interventions on vWF and Fb levels in type 2 diabetic patients with peripheral artery disease" Asian Journal of Plant Science and Research., 2012: S316 – S335.
24. Jose B, Reddy LJ. Analysis of the essential oils of the stems, leaves and rhizomes of the medicinal plant Costus pictus from southern India. Int J Pharmacy Pharm Sci. 2010;2(Suppl 2):100–1.
25. Jothi, G., and P. Brindha. "Effect of Melia composita willd. on Lipid profile in alloxan induced diabetic rats." Journal of Pharmacy Research 2011; (4.4)
26. Kalimuthu, V. Chinnadurai, R. Prabakaran P. Subramaniam.,Antimicrobial, Antioxidant and Anticancer Activities of Turnera ulmifolia (Yellow Alder) Callus., 10.9734/BJPR/2016/21920 .
27. Kameswara Rao, B., Kesavulu, M.M., Giri, R. and Ap-parao, Ch. (2000) Antidiabetic and hypolipidemic effects of Momordica cymbalaria Hook. Fruit powder in alloxan diabetic rats. Journal of Ethnopharmacology, 67(1), 103-109.[4].
28. Kandaswamy M, Sharma K, Giri S, Rajagopal S., Development and validation of a highly sensitive LC-MS/MS method for simultaneous quantification of acetyl-CoA and malonyl – CoA in animal tissues. Biomed Chromatogr.Volume 25 Issue 12 Pages1352-9.
29. Kiranmayi.Gali, G. Ramakrishnan, R. Kothai, B. Jaykar., In-Vitro Anti-Cancer Activity Of Methanolicextract Of Leaves Of Argemone Mexicana Linn. international journal of pharmtech research coden (usa): ijprif issn : 0974-4304 vol.3, no.3,pp1329-1333, july-sept 2011.

30. Narkhede M. B., P. V. Ajimire, A. E. Wagh, Manoj Mohan and AT Shivashanmugam., In vitro antidiabetic activity of Caesalpina digyna (R.) methanol root extract., Asian Journal of Plant Science and Research, 2011,1 (2): 101-106.
31. Maiti R1, Jana D, Das UK, Ghosh D. "Antidiabetic effect of aqueous extract of seed of Tamarindus indica in streptozotocin-induced diabetic rats", J Ethnopharmacol. 2004 May;92(1):85-91.
32. Malpani N, Shrikant and KP. Manjunath, "In vitro α-amylase Inhibition and Chromatographic isolation of Cassia Fistula linn. Bark", International Journal of Advances in Pharmacy, Biology and Chemistry 2(1), 2013; 13 – 18.
33. Manikandan R, A. Vijaya Anand and G. Durai Muthumani, "Phytochemical and In vitro antidiabetic activity of methanolic extract of *Psidium gujava* leaves", *International Journal of Current Microbiology and Applied Sciences* 2013; 2 **(2)**: 15- 19.
34. Manikandan R,A.Vijaya Anandand G. Durai Muthumani., Phytochemical and in vitro anti-diabetic activity of methanolic extract of Psidium guajava leaves., Int.J.Curr.Microbiol.App.Sci (2013)2(2):15-19.
35. Manjula K, Pazhanichamy K, Kmaran S, Eevera T, Dale Keefe C, Rajendran K. Growth characterization of calcium oxalate monohydrate crystals influenced by Costus igneus aqueous stem extract. Int J Pharm Pharm Sci. 2012;4(Suppl 1):261–70.
36. Narkhede, M. B., et al. "In vitro antidiabetic activity of *Caesalpina digyna* (R.) methanol root extract." *Asian Journal of Plant Science and Research* 2011; **(1.2)**: 101-106.
37. Nisarg Bhatt1*, Mitali Shrimanker1., Comparative Review of two Anti-Diabetic Herbal Drugs - GymnemaSylvestreand CostusIgneus., Am. J. PharmTech Res. 2014; 4(4) ISSN: 2249-3387.
38. Okolo,PeterAchunike Akah,, SamuelUchnna Uzodinma., Antidiabetic activity of the root extract of Detarium microcarpum (Fabacaee) Guill and Perr., Phytopharmacology,2012, 3(1) 12-18.
39. Patil, K. Vadera 1, D. Patil 1, A. Phatak 1, A. Juvekar 2 and N. Chandra., In Vitro Anticancer Activity And Phytochemical Analysis Of Bacopa Monnieri (L.) Wettst.. IJPSR, 2014; Vol. 5(10): 4432-4438.
40. Pereira SP, Roughton M, Bridgewater J , Cunningham D, Anthoney A, Maraveyas A, Madhusudan S, Iveson T, Hughes S, "Cisplatin plus gemcitabine versus gemcitabine for biliary tract cancer"; N Engl J Med. 2010 Apr 8;362(14):1273-81. doi: 10.1056/NEJMoa0908721 ABC-02

41. Pradeep Kumar and A. Rama Narsimha Reddy, 2013. Antidiabetic Activity of Methanolic Extract of Hiptage bengalensis Leaves in Alloxan Induced Diabetic Models. Pakistan Journal of Biological Sciences, 16: 844-851.
42. Praylin Singh, Prasanna Kumar Sugirtha., In vitro anti-diabetic activity of compounds from pithecellobium dulce fruit peel., International Journal of Pharmaceutical chemistry., 5.09 (2011), 5.38 (2012), 77.99 (2014).
43. Raju Asirvatham1*, Arockiasamy Josphin Maria Christina2, Anita Murali3., In Vitro Antioxidant and Anticancer Activity Studies on Drosera Indica L. (Droseraceae)., Advanced Pharmaceutical Bulletin, 2013, 3(1), 115-120.
44. Ramachandran, Aiyalu Rajasekaran, and Natarajan Adhirajan., In Vivo and In Vitro Antidiabetic Activity of Terminalia paniculata Bark: An Evaluation of Possible Phytoconstituents and Mechanisms for Blood Glucose Control in Diabetes., Volume 2013 (2013), Article ID 484675, 10 pages.
45. Raman A.Naga Vamsi Krishna., Plants With Antidiabetic Activities And Their Medicinal Values., International Research Journal Of Pharmacy., IRJP 2012, 3 (3).
46. Rinal .Srivastava, D. Saluja, and M. Chopra, "Isolation and screening of anticancer metabolites from Boerhavia diffusa," Indian Journal of Medical Research, vol. 151, supplement 1, p. S19, 2005.
47. Sabajan Khaleel Basha, Vinoji Sugantha kumara, "In vitro antidiabetic activity of *Psidium gujava* leaves extracts" *Asian pacific Journal of Topical Disease* 2012: S98 – S100
48. Saudat Adamson; Fadeyi., In vitro Anticancer Screening of 24 Locally Used Nigerian Medicinal Plants., • FAS Scholarly Articles [9958].
49. Sharma,Christopher R. Ireson, Effects of Dietary Curcumin on Glutathione S-Transferase and Malondialdehyde-DNA Adducts in Rat Liver and Colon Mucosa: Relationship with Drug Levels1., 10.1158/1078-0432.CCR-15-1489 Clin Cancer Res April 15, 2016 22; 1922.
50. Sindhu.S.Nair, Vaibhavi Kavrekar,Anshu Mishra., Evaluation of In Vitro Antidiabetic Activity of Selected Plant Extracts'., International Journal of Pharmaceutical Science Invention ISSN (Online): 2319 –6718.
51. Singh, R. H. Singh, R. G. Singh et al., "Effects of Boerhaavia diffusa Linn. (Punarnava) in experimental acute pyelonephritis in albino rats," Indian Drugs, vol. 26, pp. 10–13, 1988.
52. Strader CD, Hwa JJ, Van Heek M, Parker EM. Novel molecular targets for the treatment of obesity. Drug Discov Today 1998;3:250–256.

53. Sumitra Chanda* and Krunal Nagani., In vitro and in vivo Methods for Anticancer Activity Evaluation and Some Indian Medicinal Plants Possessing Anticancer Properties: An Overview., IC Journal No: 8192, ISSN 2278- 4136.
54. Suzuki Y ,Arase Y, Suzuki F, Akuta N, Kobayashi M, Kawamura Y, et al. Sustained virological response reduces incidence of onset of type 2 diabetesin chronic hepatitis C. H EPATOLOGY 2009;49:739-744.
55. T Eevera ,S Pavithra, S Rubini, B Lavanya, I Ramya, The Efficacy of Costus igneus Rhizome on Carbohydrate Metabolic, Hepatoproductive and Antioxidative Enzymes in Streptozotocin-induced Diabetic rat., Journal of Pharmacy Research Vol 3 (4), 747-752.
56. Thomson M, Al-Qattan KK, Peltonen-Shalaby R, Ali M.:Anti-diabetic and hypolipidaemic properties of ginger (Zingiber officinale) in streptozotocin-induced diabetic rats. British Journal of Nutrition., 2007;96(4):660-666.
57. Tiwari. A K and J. Madhusudana Rao., Diabetes mellitus and multiple therapeutic approaches of phytochemicals: Present status and future prospects.Current Science, Vol. 83, No. 1, 10 July 2002
58. Ukwenya, V. O., "Antihyperglycemic activities of methanolic leaf extract of Anacardium occidentale (Linn.) on the pancreas of streptozotocin-induced diabetic rats", Journal of Cell and Animal Biology 2012; (6.11): 169-174.
59. Varun Kumar Prabhakar, Aakanksha jaidka, Ravinder Singh. In ViThe aqueous extract of Withania somnifera leaf (92.7%) and thetro Study on α -Amylase Inhibitory Activity and Phytochemical Screening of Few Indian Medicinal Plant Having Anti-Diabetic Properties,. IJSRP, Volume 3, Issue 8, August 2013 Edition [ISSN 2250-3153].
60. Velmurugan, C., T. Sundaram, R. K. Sampath, B. Vivek, S. Sheshadri, and A. Kumar. "Anti diabetic and hypolipidemic activity of bark of ethanolic extract of Ougeinia oojeinensis (ROXB.)." ,*Med J Malaysi* 2011; **(66)**: 23.
61. Verma, Prashant R., Prakash R. Itankar, and Sumit K. Arora. "Evaluation of antidiabetic antihyperlipidemic and pancreatic regeneration, potential of aerial parts of Clitoria ternatea." ,*Revista Brasileira de Farmacognosia* 2013; **(23.5)**: 819-829.
62. Victor O Ukwenya et al. (2008), "Profertility Effects of Alcoholic Extract of Sesame in in Male Albino Rats", StudentplusOnline Academic Student Journal, Vol. 3,No. 9, p. 1.117.