PHYTOCHEMICAL ANALYSIS AND ANTIDIABETIC ACTIVITY OF *COSTUS PICTUS* BY ALPHA AMYLASE AND GLUCOSIDASE INHIBITOR ACTIVITIES

Dr. Kadam A. B.
Department of Botany,
Dada Patil
Mahavidyalaya, Karjat,
Ahmednagar, M.S., India
ashakadam16@gmail.com

Bhagat Deepali
Department of Botany,
New Arts Commerce and
Science College,
Ahmednagar, M.S., India

Prof. Gaykar B.M.
Department of Botany,
Ahmednagar College,
Ahmednagar, M.S., India

ABSTRACT

Costuspictus D. Don,commonly known as 'insulin plant ' is a member of Zingiberaceae family and is used as a munching dietary supplement for the treatment of diabetes in Southern India. It is a perennial, upright, spreading plant reaching about two feet tall, with spirally arranged leaves and attractive flowers.Costuspictus are reported, which includes anti-diabetic, anti-microbial, anti-cancer, anti-oxidant, anti-fertility, anti-helminthic, diuretic, anti-inflammatory properties.Various phytochemical investigations reveal the presence of carbohydrates, triterpenoids, proteins, alkaloids, tannins, saponins, flavonoids, steroid, and appreciable amounts of trace elements. The present study was carried out to evaluate the phytochemical Analysis and Antidiabetic Activity of CostusPictus leaves by Alpha Amylase and Glucosidase Inhibitor Activities.

Keywords: *Anti-diabetic activity,*Costuspictus*, Insulin plant, Alpha Amylase, Glucosidase activity.*

INTRODUCTION

*CostusPictus*D.Don is perennial herb a wonderful and well known medicinal plant owing to its anti-diabetic property hence is called insulin Secreting plant .it is native of South and Central America it is a recent Introduction to India during 2002-2003 it is being widely cultivated in South India as an ornamental plant especially Kerala[4]. The popularity of the plant of South India is due to "sugar lowering effect and Antidibetic activity [6].Since it is a newly Introduced plant investigation need to be made to examine the growth survival of this plant different agro climatic condition of India. It is used in India to control diabetes, and it is known that diabetic people eat one leaf daily to keep their blood glucose low.[3] Leaves of *Costuspictus*were one among the plants known to be effectively used for treating diabetes by the tribal people

*Costuspictus*also commonly called as spotted spiral Ginger the large smooth dark green leaves of this tropical evergreen plant have light purple undersides and are spirally arranged around stems forming attractive arching clumps arising from underground root stocks the most pronounced effect the anti-diabetic property of *Costuspictus*has not be provide clinically in experiment nevertheless people have started to consume the leaves as a remedy for diabetics since this is recently introduce plant investigation have to made to confirm the morphological ,molecular , biochemical, phytochemical and pharmacological character of plants exploit it beneficially the present study is biochemical characterization of *Costuspictus.*

Plant are used to treat many aliments and India with approximately 45,000 land species its plant wealth seems to include several thousands .which have been cited to possess medicinal properties of India has 15 agro climatic zone 47,000 different plant species and 15,000 medicinal plant include 7.000 plant used Ayurveda.The drugs derived either from whole plant from difference plants like leaves stems bark root flower etc.

Plants as a potential source Anti-diabetic drugs the most common conventional treatment for diabetic's insulin which have prominent side effects neither insulin nor other pharmaceuticals have been shown to modify the course of diabetic complications and here lies the significance of drugs of herbal medicine have been recommended for the treatment of diabetics

Medicinal plants are the local heritage with global importance word is endowed with a rich wealth of medicinal plants of medicinal value are often a basic requirement of treatment certain disorders and health related condition irrespective of education income levels the use of traditional medicine medicinal plants are play in pivotal role not only as traditional herbal medicine used as health care but also as trade commodities which meet the demand of often distant market

The phytochemical analysis of *Costuspictus*plant screening of plant secondary metabolites test for reagent the anti-diabetic activity of plant the determination of alpha amylase inhibitor activity and alpha glycosidase inhibitory activity.

MATERIAL AND METHOD

Collection of plant material

On the basis of literature and survey of trible people (Gaykar*et al*., 2006) plant was selected for the scientific study of antidiabetic activity. The selected plant was *Costuspictus*.Leaf samples were collected from various areas of Ahmednagar District, India in their natural habitat. They were identified from Botanical Survey of India, Western Circle, Pune. The voucher specimens were deposited in the Herbarium, BSI, Pune as well as in Herbarium of Department of Botany, New Arts, Commerce and Science College, Ahmednagar.

Extraction of the plant materials

The leaf of plants was air dried at room temperature followed by pulverization to powder form using mortar and pestle. The powdered leaves were subjected to aqueous extraction as well as extraction of active components from leaves powder was performed with petroleum ether by using Soxhelet. Polar and non-polar solvent were taken into consideration for extraction. Solvent of each sample was removed by vacuum rotary evaporator at room temperature. The remaining residues were collected and preserved at 4℃ for further experiment. The non-polar Petroleum ether was used which being more effective than mmethanol extracts (Mawahib, 2015), so the extracts were made in this non polar solvent.

Phytochemical screening

Standard procedures were adopted for the phytochemical screening.

Test for tannins: To about two grams of the methanol extract of the sample, a few drops of 5% ferric chloride solution were added. A dark green or bluish-black coloration show the presence of tannins [12].

Test for flavonoids (Shinoda Test): To two grams of the methanol extract, a few fragments of magnesium ribbon were introduced. To this, 6 drops of concentrated hydrochloric acid were added. If a pink or red colour is obtained, the presence of flavonoids is indicated [13].

Test for saponins: To about five grams of methanol extract, 5 ml de-ionized distilled water was added. On vigorous shaking, the formation of a persistent froth that lasted for 15 minutes indicated the presence of saponins [14].

Test for terpenoids (Salkowwski Test): In a test tube 0.5 gm of the extract was taken and about 2 ml of chloroform was added to it. To this, 3 ml of conc. H_2SO_4 was carefully introduced to form a layer. If a reddish brown colour is obtained, presence of terpenoids is indicated [15].

Test for carbohydrates (Molisch's Test): One gram of the methanol extract was dissolved in a few drops of water. Then 1 ml of conc. Sulphuric acid was added along the walls of the test tube. On this addition, if a red or violet zone is visible at the interphase of the oil-water layers, carbohydrates and /or glycosides are indicated in the sample [16].

Test for anthraquinone (Bontrager's Test): To one gram of the methanol extract, 5 ml of benzene was added. Then it was shaken and filtered. Five ml of 10% NH_4OH was added to the filtrate, followed by shaking of the contents. The formation of a red, pink or violet colour in the lower ammoniacal phase confirmed that free anthraquinones are present in the sample [16].

Test for cardiac glycosides (Keller-Kilani Test): To the Methanol extract, 2 ml of glacial acetic acid containing 1-2 drops of 2% solution of $FeCl_3$ was added. This mixture was then introduced into another test tube that contained 2 ml of concentrated H_2SO_4. The appearance of brown ring at the interphase indicated that cardiac glycosides are present in the sample [13].

Test for coumarins: One gram of methanol extract of the sample was taken in a test tube. The test tube was then covered with a filter paper that is moistened with dil. NaOH. The sample was then heated on water bath for a few minutes. The filter paper was then examined under UV (365 nm). If a yellow fluorescence is obtained, the presence of coumarins is indicated [17].

Test for steroids (Liebermann-Burchard Test): One gram of methanol extract was taken. This was followed by the addition of 2 ml of acetic acid. The solution was cooled in an ice bath. After the cooling, conc. Sulphuric acid was added carefully. The development of colour from violet to blue or bluish-green is a positive test for the presence of a steroidal ring [16].

Test for alkaloids: To one gram of methanol extract, 2 ml of 1% HCl was added and the contents were heated gently. This was followed by adding 2-3 drops of Mayer's reagent. The appearance of white or cream precipitate confirm the presence of alkaloids [13,14].

Anti-diabetic Activity of*CostusPictus* Plant

a) Determination of Alpha Amylase Inhibitor Activity

Alpha amylase inhibitor activity was determined by some medication is method described by Narkhede et. [18],the assay mixture Containing 1 ml of 0.02 Sodium phosphate buffer 200ul of enzymes (porcine panacreatic alpha amylase) and the plant extract in conc range 200 to 1000ul mg were incubated for 10 min are room temperature followed by addition of 1ml of starch in all test tubes the reaction was terminated with addition of 1ml DNS Reagent and placed and boiling water bath 5minutes cooled and Diluted with 1 ml of Distilled of water absorbance with measured at 540 nm the control samples with prepared without any plant extract the % inhibition was calculated according to formula .

Percentage of Inhibition =(Control540- Extract540)x100

Control 1540

b) Alpha Glucosidase Inhibitory Activity

The effect of plant on alpha glucosidase activity was determined according to chromogenic methoddescribed by Kadam et .al [19]. Using alpha gliucosidase by using *Saccharomyces cerevisiae* the substrate solution. P-nitrophynlglucopyranosidase as prepare 20 mm phosphate buffer PH 6.9, 200 ul in 5 units of alpha glucosidase were pre incubated with the different concentrations of the plant extract for 10 min. 1ml of 3 mm as A substrate dissolved 20 mm phosphate buffer PH 6.9 added to start the reaction of the reaction at the 37 ^{0}C for 20 min and stopped by adding 1ml of Na_2co_3.The alpha glycosidase activity was determine was measured the yellow coloured p- nitropheoyl released form PNPG AT 405 Nm the results with expressed was as percentage as blank control.

Percentageof Inhibtion=(Control 05-Extract 405) x 100

Control 405

Table 1 shows the presence of phytochemical screening of methanolic extract of leaves of *Costuspictus*.

Sr.No	Chemical Test for Plants	Solvent system Water	Solvent system Methanol
1	Wagner,s Test	+ ve	+ ve
2	Milisch,s Test	+ ve	- ve
3	Kellarkellions Test	+ ve	+ ve
4	Alkaline Reagent Test	+ ve	+ ve
5	Ferric chloride Test	+ ve	+ ve
6	Amino acid and Protein Test	+ ve	+ ve
7	Foam Test	- ve	- ve
8	Bramyer Test	- ve	- ve
9	Salkowskis Test	+ ve	+ ve
10	Quinoes Test	+ ve	+ ve
11	Resins Test	- ve	- ve

B)Alpha amylase

O.D. of Control = 0.8309

Conc.(ug/ml) / Extract	200	400	600	800	1000
Sample O.D.	0.4571	0.4475	0.3388	0.3573	0.3059
% INHIBITION	44.98	46.14	59.22	56.99	63.18

IC50 value for amylase

Sample IC50	426.82 ug/ml

C)Alpha amylase

O.D. of Control = 0.5282

Conc.(ug/ml) / Extract	200	400	600	800	1000
Sample OD	0.266	0.193	0.236	0.195	0.02
Percent inhibition	49.64	63.46	55.31	63.08	92.32

IC50 value for glucosidase

IC50	252.51 ug/ml

RESULT AND DISCUSSION

Costuspictus is reported that synthesis of charactrhizatoin of silver Nanoparticals of insulin plant cost effective and eco-friendly technique for green synthesis of silver nanoparticals from the mmethanolic extract.***Costuspictus***characterhization of synthesis Nanoparticals carried out in this study green synthesis[1].Leaf extract of ***Costuspictus***plant preparing the same a medicinal composition of treating dibeties[2].Phytochemical and hypoglycaemic activity investigation of *Costuspictus*plant from Kerla and Tamilnadu recently introduced potential herbal drug of diabetics[4]

It can be concluded that ***Costuspictus***is widely used in some part of community of Kerla to normalize sugar level pharmacoepidmiologicalstudy[3].Anti-diabetic activity of leaf extract ***CostuspictusD.Don***was induced in albino rats by adminstraction of single dosses of alloxan monohydrate of mmethanol extract of ***Costuspictus.***120mg/kg was adminstereted as a single dose per day to diabetic induced rats for a period 21 days high density of lipoprotein serumenzymes alkaline phosphate, total protein in liver glycogen measured diabetic rats in plants [7].Antidiabetic Activity of *CostusPictus* leaves evaluated as 426.82 ug/ml by Alpha Amylase and 252.51 ug/ml by Glucosidase Inhibitor Activities.

The results suggest that methanol extract of *Costuspictus* of leaf efficiently inhibits αglucosidase enzymes in vitro. The antidiabetic action of *Costuspictus* can also be attributed to the intestinal α-glucosidases inhibitory activity.

In the present study ***Costuspictus***revolved the presence of Alkoloides, Carbohydrate , Glycosides,Flavonoides , Phenols ,Amino acid and Proteins and Saponins Terpenoides,Quinones. Resin and Tannin were absent in leaves of *Costuspictus*.It is evident of phytochemical substrate like terpenoides ,alkoloides , phenols, glycosides and flavonoid were shown. Higher concentration of presence Compounds in Steroides , phenol, Quinone has shown positive response phytochemicals presented in elevated amount of secondary metabolites. Secondary metabollities in plant of ***CostusPictus***which showing antidiabetic activity.For this there was carried out determination of alpha amylase inhibitory activity and alpha glucocidase inhibitory activity in ***CostusPictus***plant.

CONCLUSION

In the present study, the qualitative phytochemical analysis and antidiabetic activity of *Costuspictus* were done. *Costuspictus*is shown to exhibit antidiabetic activities by AlphaAmylase and Glucosidase Inhibitor Activities.This study showed that *Costuspictus* leaf extracts have good antidiabetic activity. Phytochemical analysis recorded no. of chemical constituent which may be responsible for antidiabetic activities. Based on the above results it is evident that the leaves of *Costuspictus* have antidiabetic effect and must be considered as a potential candidate for future studies on diabetes mellitus.Further study has to be carried out to determine this bioactive components in the leaves of *CostusPictus*plant and take animal trial.

Our study suggests that methanolic and aqueous extracts of*CostusPictus leaves* may have beneficial effects in treatment of diabetes.The extracts might be useful as a medicinal food or as a source of natural alpha glucosidase inhibitors for suppressing postprandial hyperglycemia in the management of diabetes. Furthermore, the extracts may have other mechanism of actions as well. However, complete phytochemical and pharmacological research is obligatory to uncover the precise mechanism of these extracts for their antidiabetic effect.

ACKNOWLEDGEMENTS

Authors are thankful to Principal and Head, Department of Botany Dada Patil Mahavidyalaya, Karjat for seed money support during the study. Also the authors are thankful to Principal, Ahmednagar College,Ahmednagar and to Botanical Survey of India (BSI), Pune.

REFERENCES

[1]AjithadasArjuna ,Ramraj Nandhini ,vanketachalamkarthikeyanpandibose., Synthesis the characterization of silver nanoparticals of Insulin plants CostusPictusleaves,Asian Journal of biochemical pharmaceutical Sciences.2013, Vol.3.

[2]Shiny, Anuj Saxena and Sharad Prakash Gupta ,Phytochemical and hypoglycaemic activity investigation of CostusPictus plant from Kerala and Tamilnadu. International Journal of Pharmaceutical Science, 2014, Vol.2.

[3]C.T Shiny ,Anuj Saxena ,Sharad Prakash Gupta,Phytochemical Investigation of the insulin plant CostusPictus, International Pharmaceutical And Biomedical Research, 2015,Vol.2.

[4]AjithadasAruna, RamrajNadhini,Venkatachalam Karthikeyan,Pandibose, Shanmaganathan ,Insulin Plant Leaves Pharmacognostical Standardization And Phytochemical Evolution., American Journal of Pharmacy and health Research.,2013, Vol. 2.

[5]Mala Majumadar ,Prachi. S. Prihar.,Anti-bacterial Antioxidant and Antiglycation potential of CostusPictus from Southern region.India Asian Journal of plant Science and Research,2012,Vol.4.

[6] Justin. R. Nayagam,Eragastic crystals in identification of CostusPictusa Medicinal spiral Ginger in herbal medicine. Journal of Experimental Biology and Agricultural Sciences., 2012,Vol,4.

[8] Bakrudeen Ali Ahmed , Arun Kumar., In Vitro Propagation Of Monocot An Anti-Diabetic Medicinal Plant.,Journal of Agriculture Technology.,2009,Vol.5.

[9] Altaf. Romaiyan , Stephanie Amienl , Peter.M, Jons and Shanta J. Persad .,CostusPictusStimulates Insulin Secretion From Mouse And Human Is Lets Of Langerhans In Vitro.,JournalCells ,Physiology and Biochemestry.,2009.,Vol.3.

[10]Biji Joseph Sherina ,Jacob Nishant Kumar,Benny Atony.,PharmacoEpidermacologicalSurvey Of The Use Of CostusPictusIn Central Kerla.,International Journal of Pharmacy and Pharmaecutical Sciences., 2011,Vol.2.

[11] Suzan Khayyat, Manal Othman AL-Kattan., Phytochemical screening and antimicrobial activities of Costusspeciosus and Sea Qust., Biomedical Research .,2017,Vol. 28.

[12]Cuilel ,Methodology for the analysis of vegetables and drugs. Chemical Industry Division, NNIDO Romania.,1994,Vol.4.

[13] Yadav RNS, Agarwala M. Phytochemical analysis of some medicinal plants, J Phytol 2011,Vol.3.

[14]Ranjit P, Santhipriya T, Nagasri S, Chowdary Y, Pasumarthy N. Prelininary phytochemical screening and antibacterial activities of methanolic extract of calotropisprocera flowers against human pathogenic strains. Asian J Pharm Clinic Res 2012; 5: 127-131.

[15]Khan F, Iqbal H, Shahid F, Majed A, Muhammad A, Inayat U. Phytochemical Screening of Some Pakistanian Medicinal Plants, Middle-East Journal of Scientific Research.,2011,Vol. 8.

[16] Usman H, Abdulrahman F, Usman A. Qualitative Phytochemical Screening and In Vitro Antimicrobial Effects of Mmethanol Stem Bark Extract of FicusThonningii (Moraceae). Afr J Tradit Complement Altern Med., 2009.Vol.6.

[17]Pochapski M, Fosquiera E, LuísAntônio E, Elizabete S, Paulo V, Fábio A, Francisco C. Phytochemical screening, antioxidant, and antimicrobial activities of the crude leaves' extract from Ipomoea batatas (L.) Lam, Pharmacognosy Magazine., 2011,Vol.7.

[18] M. B. Narkhede, P. V. Ajimire , In vitro antidiabetic activity of Caesalpinadigyna (R.) methanol root extract., Asian Journal of Plant Science and Research, 2011,Vol.1(2).

[19] P. V. Kadam, M. J. Patil., Antidiabetic And Antihyperlipidemic Activity Of Luffa Acutangula Fruit Extracts In Streptozotocin Induced Niddm Rats., Asian Journal of Pharmaceutical and Clinical Research.,Vol.4.