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INVITRO AND INSILICO ACTIVITIES OF COSTUS IGNEUS LEAF EXTRACTS ON ALPHA AMYLASE - AN ANTIDIABETIC STUDY

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ABSTRACT

Diabetes mellitus is a group of metabolic disorders characterized by a chronic hyperglycemic condition due to disturbances in glucose homeostasis. Phytocompounds play a major role in modulating glucose metabolism by inhibition of carbohydrate digesting enzyme like α-amylase. The ethanolic extract of *Costus igneus* had high *invitro* α-Amylase inhibition percentage than all other extracts. Gas Chromatography-Mass Spectrometry analysis was performed for the ethanol extract and the compounds obtained were docked against the target Human Pancreatic Alpha-Amylase. Phytol was found to be an active compound through *insilico* docking with the least E value of –6.51 kcal/mol. The results are expected to be useful in conducting *invivo* screenings on animal model which may lead to the development of more effective and potent new chemical entities with antidiabetic property.

KEYWORDS: α-amylase, *Costus igneus*, Gas Chromatography-Mass Spectrometry, Phytol, antidiabetic property.

INTRODUCTION

In Indian scenario, World Health Organization (WHO) estimates about 70-80% of Indians depend on Indian system of medicine like Unani, Siddha, and Ayurvedha **(Gupta and Shaw, 2009).** Traditional use of herbal medicine is usually an integral part of culture around the world, which has been used in medical practice for thousands of years and has made a great contribution for maintaining human health before spread of modern science **(Verma and Singh, 2008)**.

Although modern medicine may be available in developed and developing countries, herbal medicine has often maintained popularity for historical and cultural reasons. Concurrently many people in developed countries have begun to turn to alternative or complementary therapies including medicinal herbs. Many pharmaceutical companies show interest in plant derived drugs mainly due to the current widespread belief that 'Green Medicine' is safe and more dependable than the costly synthetic drugs, which have adverse side effects **(Tiwari, 2008)**.

Diabetes mellitus, a metabolic disorder characterized by a loss of glucose homeostasis with disturbances of carbohydrate, fat and protein metabolism results from defects in insulin secretion, insulin action, or both. According to WHO, it is estimated that 3% of the World's population have diabetes and the prevalence is expected to double by the year 2025 to 6.3% **(Meenakshi *et al*.,2010)**. The World Health Organization states that ~347 million people worldwide were suffering from diabetes in 2008, which equates to 9.5% of the adult population **(Danaei *et al.*, 2011)**. Diabetes for the whole world is not an epidemic anymore but has turned into pandemic **(Lal *et al.*, 2009)**. About 90% to 95% of diabetic cases are diagnosed with type II diabetes (T2D) **(Shaw *et al*., 2010)**.

Currently available antidiabetic drugs, such as acarbose, in addition to not being effective in maintaining euglycemia, usually present with some side effects. Hence, there is increasing emphasis on the use of plant products rich in phenolic compounds that could be more effective for the management of type II diabetes with few side effects. In addition to their effectiveness and safety, herbal remedies could be a cheaper alternative to the synthetic antidiabetic drugs. Flavonoids and phenolic compounds are part of the secondary metabolites that constitute the active principles in plant products. These active ingredients are responsible for the therapeutic and or pharmacological activities, such as antidiabetic effects of medicinal plants **(Sumbul *et al*., 2011).** Phenolic compounds are known to modulate glucose metabolism by several mechanisms including inhibition of carbohydrate digesting enzyme like α-amylase. The inhibition of carbohydrate metabolizing enzyme such as α-amylase retards the digestion carbohydrates and the subsequent absorption of glucose, leading to a decrease in postprandial blood glucose level **(Hanhineva *et al*., 2010).**

Costus igneus commonly known as Fiery costus or Insulin plant is native to South and Central America. This is a recent introduction to India from America as an herbal cure for

diabetes and hence commonly called as 'insulin plant' **(Jose and Reddy, 2010).** *Costus igneus* leaves have been proven to possess various pharmacological activities.

Thus the current study is focused to evaluate the *invitro* and *insilico* activities of *Costus igneus* leaf extracts on Alpha amylase.

MATERIALS AND METHODS

COLLECTION AND PREPARATION OF PLANT MATERIALS

Healthy fresh leaves of *Costus igneus* were collected from the nearby areas of Coimbatore district. The leaves were rinsed with distilled water and dried at room temperature under well ventilated shade for 10 days. The dried leaves were powdered and stored in air-tight container for further analysis.

EXTRACTION OF PLANT MATERIAL

The powdered leaves were extracted in various solvents, viz hexane, ethyl acetate and ethanol **(Gayathri and Jeyanthi, 2013).** One part of the powdered leaves were macerated in three parts of hexane, ethyl acetate and ethanol separately and kept for 24 hours at 37°C. Filtered and collected the solvents. The solvents were evaporated to obtain the hexane, ethyl acetate and ethanol extracts.

INVITRO ANTIDIABETIC ACTIVITY

α- AMYLASE INHIBITION ASSAY

Invitro α -amylase inhibition of the extracts were determined using the procedure reported by **Bernfeld *et al.*, 1955** with slight modifications as proposed by **Abirami *et al.*, 2014**. In α -amylase inhibition method, the enzyme solution was prepared by dissolving α -amylase in 20mM phosphate buffer (6.9) at the concentration of 0.5 mg/ml. 1.0 ml of the extract in various concentrations (250, 500, 750, 1000 µg/ml) and 1.0 ml of enzyme solution were mixed together and incubated at 25°C for 10min. After incubation, 1.0 ml of starch (0.5%) solution was added to the mixture and further incubated at 25°C for 10 minutes. The reaction was then stopped by adding 2.0 ml of dinitro salicylic acid (DNS, colour reagent) heating the reaction mixture in a boiling water bath (5min). After cooling, the absorbance was measured colorimetrically at 565 nm. Metformin was used as a standard for the assay. The readings were taken in triplicates. The inhibition percentage was calculated using the given formula, were, Abs control is the absorbance of the control reaction (containing all reagents except the test sample) and Abs sample is the absorbance of the test sample.

% of Inhibition = (Abs of control – Abs of sample)/Abs of control * 100

GAS CHROMATOGRAPHY—MASS SPECTROMETRY

Gas chromatography–mass spectrometry (GC-MS) is an analytical method that combines the features of gas-chromatography and mass spectrometry to identify different substances within a test sample. The ethanol leaf extracts of *Mangifera indica* and *Costus igneus* which had high *in vitro* antioxidant activity were subjected to GC-MS analysis using the software XCALIBUR (ver-2.2). The GC-MS analysis of unknown compounds deals by using a TSQ QUANTUM XLS Gas Chromatography. It ionizes compounds and measures their mass number equipped with the column DB-5MS (30m X 0.25mm X 0.25um) and mass detector which was operated at in EI mode. The experiment was programmed with total run time 34 min, helium was used as the carrier gas at the flow rate of 1 ml / min. The injector was operated at 280˚C and the oven temperature was programmed as follows: 70˚C @ 8˚C /min to 150˚C (1 min) @ 8˚C / min to 280˚C (10 min). Injection volume was 1µl with scan mass range 30m/z – 600m/z having positive polarity (+ve). The identification of components was based on comparison of their mass spectra with NIST-011S library.

INSILICO ANALYSIS

In the first step protein structure file of Human Pancreatic Alpha-Amylase (PDB ID-4X9Y) was retrieved with resolution of about 1.07A°. The target protein was visualized with RasMol. In next step chemical structures were retrieved from PubChem and Lipid maps database. The chemical structures of 4-heptadecanone, 9, 12 Octadecadienoic acid (z,z)-Methyl ester, 3-O-Methyl-d-glucose, Phytol and Palmidrol were used for docking studies by ArgusLab. The docking analysis of ligand with Human Pancreatic Alpha-Amylase was carried by Argus lab docking software using default parameters. The values were obtained in terms of energy (e-value) Kcal/mol. Lesser the E-value greater the acceptability of compound as a drug **(Srivastava *et al.*, 2008).** Docking allows virtually screening of compounds and predicts the strongest binders based on various scoring functions. It explores ways in which two molecules, such as drugs and an enzyme (Human Pancreatic Alpha-Amylase) fit together and dock to each other well. The molecules binding to a receptor, inhibit its function, and thus act as drug.

RESULTS AND DISCUSSIONS

INVITRO ANTIDIABETIC ACTIVITY

α – AMYLASE INHIBITION ASSAY

The intestinal digestive enzymes alpha-amylase plays a vital role in the carbohydrate digestion. One antidiabetic therapeutic approach reduces the post prandial glucose level in blood by the inhibition of alpha-amylase enzyme. These can be an important strategy in management of blood glucose **(Shreedhara *et al.*, 2009).** Alpha amylase is an enzyme that hydrolyses alpha-bonds of alpha linked polysaccharide such as starch to yield high levels of glucose and maltose. Alpha amylase inhibitors bind to alpha- bond of polysaccharide and prevent break down of polysaccharide into mono and disaccharide **(Abirami *et al.*, 2014).** Therefore it is quite important to evaluate the α – Amylase inhibitory activity of the extracts.

The inhibitory effect of hexane, ethyl acetate and ethanol extracts of *Costus igneus* on α – Amylase activity are presented in the figure 1.

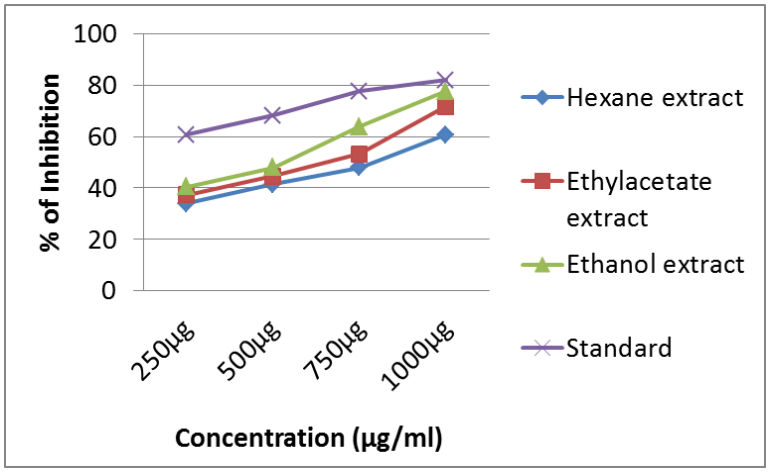

Figure 1: Inhibitory effect of hexane, ethyl acetate and ethanol extracts of *Costus igneus* on α – Amylase activity.

Of all the three extracts, ethanol extracts of *Costus igneus* shows high percentage of inhibition. It is noted that the ethanol extracts of Insulin plant showed maximum alpha amylase inhibitory activity **(Aruna *et al.*, 2014).** The percentage inhibition of ethanol extract of *Costus igneus* is found to be 78% at 1000 µg/ml concentrations of plant extracts. The activity is less than metformin. This showed a dose dependent increase in the percentage inhibition.

GAS CHROMATOGRAPHY—MASS SPECTROMETRY

The study of the organic compounds from plants and their activity has increased by the combination of a best separation technique (GC) with the best identification technique (MS) made GC–MS an ideal technique for qualitative analysis for volatile and semi-volatile bioactive compounds **(Grover *et al.*, 2013).** The ethanol extract of *Costus igneus* which has high *invitro* antioxidant activity were subjected to GC-MS analysis.

In present investigation total fifty eight bioactive chemical constituents are identified in ethanol extract of *Costus igneus* with important therapeutic properties. The GC-MS chromatogram of Ethanol extract of *Costus igneus* is shown in the figure 2.

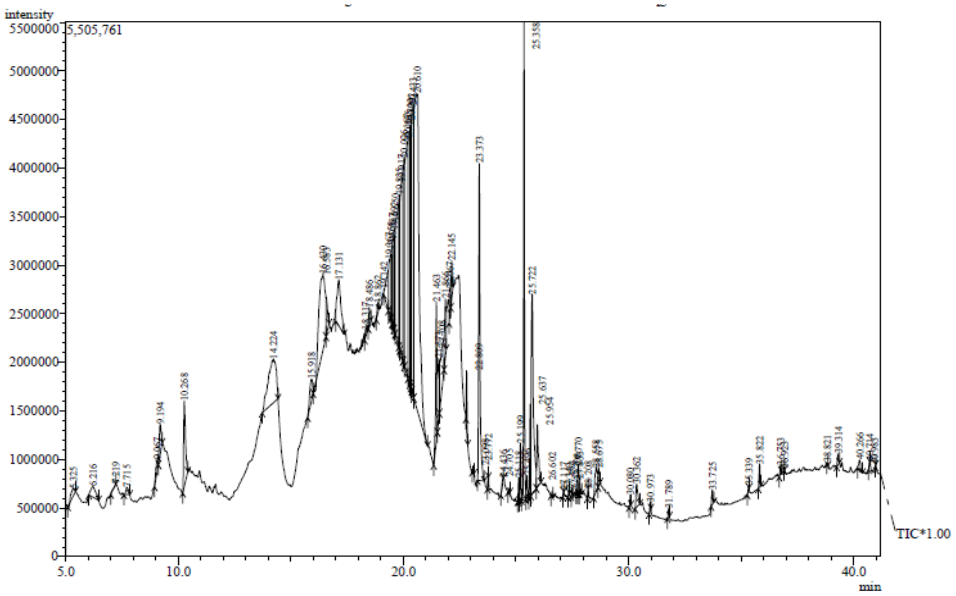

Figure 2: GC-MS chromatogram of Ethanol extract of *Costus igneus.*

The constituents in ethanol extract of *Costus igneus* are presented in the table 1.

Table 1: Constituents in Ethanol extract of *Costus igneus.*

S.No.	Compound name	Molecular formula	Retention time	Area %
1.	2,3-Dihydro-Benzofuran	C_8H_8O	9.067	0.31
2.	2-Methoxy-4-Vinylphenol	$C_9H_{10}O_2$	10.268	1.67
3.	1,2,3,4-Cyclohexanetetrol	$C_6H_{12}O_4$	14.224	3.74
4.	N-Acetyl-L-isoleucine methyl ester	$C_9H_{17}NO_3$	16.430	5.18
5.	1-Octen-3-yl-acetate	$C_{10}H_{18}O_2$	16.583	0.52
6.	1,1-([1,3]Dioxolan)-Octahydro-Azulen-4-One	$C_{12}H_{18}O_3$	18.317	0.14
7.	4,5-Dicarbamoyl-2-methyl-2-oxazoline	$C_6H_9N_3O_3$	18.486	0.25
8.	N-[N-(N-Acetyl-L-Leucyl)-L-	$C_{20}H_{29}N_3O_5$	18.862	0.15

	Alanyl]-3-Phenyl-, L-Alanine			
9.	1-Ethoxy-1-Propene	$C_5H_{10}O$	19.142	0.94
10.	1,1'-Bicyclohexyl-1-Carboxylic Acid, 2-(Diethylamino)Ethyl Ester	$C_{19}H_{35}NO_2$	19.367	1.22
11.	Diisobutylamine	$C_8H_{19}N$	19.458	0.87
12.	4S,6R-Dimethyl-7R-hydroxynonan-3-one	$C_{11}H_{22}O_2$	19.517	1.35
13.	5-Cyclohexene-1,2,3,4-Tetrol	$C_6H_{10}O_4$	19.592	1.23
14.	6,6-Dimethyl-1,4-dioxa-spiro[4.5]dec-7-ene	$C_{10}H_{16}O_2$	19.917	5.00
15.	5-(1,5-Dimethyl-4-Hexenyl)-2-Methylphenol	$C_{15}H_{22}O$	20.026	2.74
16.	Clonitazene	$C_{20}H_{23}ClN_4O_2$	20.167	7.26
17.	5-Cyclohexene-1,2,3,4-Tetrol	$C_6H_{10}O_4$	20.225	3.82
18.	4-Chloro-5-([2-(diethylamino)ethyl]amino)-3(2H)-pyridazinone	$C_{10}H_{17}ClN_4O$	20.300	2.24
19.	4S,6R-Dimethyl-7R-hydroxynonan-3-one	$C_{11}H_{22}O_2$	20.433	5.39
20.	2,6,10-Trimethyl,14-Ethylene-14-Pentadecne	$C_{20}H_{38}$	21.463	1.81
21.	2-Pentadecanone	$C_{18}H_{36}O$	21.573	1.06
22.	3-O-Methyl-d-glucose	$C_7H_{14}O_6$	21.708	1.77
23.	1-Deoxy-Inositol	$C_6H_{12}O_5$	22.067	0.40
24.	Hexadecanoic Acid, Methyl Ester	$C_{17}H_{34}O_2$	22.809	0.41
25.	l-(+)-Ascorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$	23.098	0.06
26.	9,12-Octadecadienoic Acid (Z,Z)-, Methyl Ester	$C_{19}H_{34}O_2$	23.373	4.57
27.	8,11,14-Docosatrienoic Acid, Methyl Ester	$C_{23}H_{40}O_2$	23.772	0.05
28.	Phytol	$C_{20}H_{40}O$	24.436	0.08
29.	Octadecanoic Acid, Methyl Ester	$C_{19}H_{38}O_2$	24.705	0.03
30.	cis-9,cis-12-Octadecadienoic acid	$C_{18}H_{32}O_2$	25.118	0.18
31.	cis-9,12,15-Octadecatrienoic acid	$C_{18}H_{30}O_2$	25.199	0.55
32.	Octadecanoic acid	$C_{18}H_{36}O_2$	25.358	4.46
33.	N-[2-(1-Piperidinyl)Ethyl]-1-[3-(1-Piperidinyl)-1-Propynyl]Cyclohexanamine	$C_{21}H_{37}N_3$	25.496	0.29
34.	Palmidrol	$C_{18}H_{37}NO_2$	25.637	0.95
35.	Heneicosanoic acid, methyl ester	$C_{22}H_{44}O_2$	25.722	4.68
36.	1,1'-(1,2-dimethyl-1,2-ethanediyl)bis-Benzene	$C_{16}H_{18}$	25.954	1.03
37.	Dotriacontane	$C_{32}H_{66}$	26.602	0.03
38.	2-mono- Palmitin	$C_{19}H_{38}O_4$	27.117	0.03
39.	Pentatriacontane	$C_{35}H_{72}$	27.349	0.02
40.	Squalene	$C_{30}H_{50}$	27.494	0.05
41.	5.beta.-Cholestan-3.alpha.-ol, methyl ether	$C_{28}H_{50}O$	27.708	0.15

42.	Acetic Acid, Phenyl Ester	$C_8H_8O_2$	27.770	0.33
43.	2-Ethyl-Hexan-1-Ol	$C_8H_{18}O$	27.833	0.14
44.	1-Phenyl-2-Propanone	$C_9H_{10}O$	28.208	0.00
45.	2,3-Dihydro-3,5-Dihydroxy-6-Methyl-4h-Pyran-4-One	$C_6H_8O_4$	28.558	0.68
46	1,2,3,4-Cyclohexanetetrol	$C_6H_{12}O_4$	28.675	0.18
47.	Tetraacetyl Derivative Of 3-Methoxyhexose	$C_{15}H_{24}O_9$	30.080	0.09
48.	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	30.362	0.48
49.	n-hydroxy-3 4-methylenedioxyamphetamine	$C_{10}H_{13}NO_3$	30.973	0.08
50.	Dotriacontane	$C_{32}H_{66}$	31.783	0.09
51.	Pentatriacontane	$C_{35}H_{72}$	33.725	0.18
52.	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)	$C_{30}H_{50}O$	35.822	0.23
53.	Dotriacontane	$C_{32}H_{66}$	36.753	0.07
54.	Cholest-5-En-3-Yl Acetate	$C_{29}H_{48}O_2$	36.923	0.01
55.	Stigmasta-4,7,22-trien-3.alpha.-ol	$C_{29}H_{46}O$	38.821	0.01
56.	5.beta.-Cholestan-3.alpha.-ol, methyl ether	$C_{28}H_{50}O$	39.314	0.14
57.	Stigmast-5-En-3-Ol	$C_{47}H_{82}O_2$	40.266	0.18
58.	Cholesterol	$C_{27}H_{46}O$	40.714	0.04

The bioactive constituents identified possess various therapeutic activities such as antidiabetic, anti-inflammatory, antibacterial, anticancer, antifungal, anti aging activities.

MOLECULAR DOCKING

Docking allows virtually screening of compounds and predicts the strongest binders based on various scoring functions. It explores ways in which two molecules, such as drugs and an enzyme Human Pancreatic Alpha-Amylase fit together and dock to each other well. Human pancreatic α-amylase (HPA) inhibitors offer an effective strategy to lower postprandial hyperglycemia via control of starch breakdown. The compounds binding to a receptor, inhibit its function, and thus act as drug **(Srivastava *et al.*, 2008).**

The compounds were docked against the target Human Pancreatic Alpha-Amylase (PDB ID-4X9Y) using Argus Lab with default parameters. From the table 2, it is clear that phytol, a compound present in the ethanol extract of *Costus igneus* has good docking activity with the least E value of – 6.51 kcal/mol. Lesser the E-value greater the acceptability of compound as a drug and so it is considered as an effective ligand in inhibiting Human Pancreatic Alpha-Amylase.

The docking results are presented in the table 2.

Table 2: Docking Results.

S.No	Compound Name	E value(kcal/mol)
1.	**Phytol**	**-6.51**
2.	9,12 Octadecadienoic acid(z,z)-Methyl ester	-6.09
3.	Palmidrol	-5.93
4.	3-O-Methyl-d-glucose	-4.66
5.	4-heptadecanone	-4.44

CONCLUSION

From the current study it can be concluded that the activitiy against Alpha amylase enzyme may be due to the phytochemicals in the leaf extracts of *Costus igneus*. *Invitro* antidiabetic activity by inhibition of α-amylase assay is higher in ethanol extract than ethyl acetate and hexane extracts. *Insilico* analysis also shows that a compound 'Phytol' present in ethanol extract of *Costus igneus* has a good docking activity with the least E value (– 6.51 kcal/mol) when molecularly docked with Human Pancreatic Alpha-Amylase using ArgusLab. The results are expected to be useful in conducting *invivo* screenings on animal model which may lead to the development of more effective and potent new chemical entities with antidiabetic property.

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