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Invitro and *insilico* anti hyperglycemic activity of *cadaba fruticosa* leaves - an enteric approach

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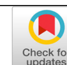
ABSTRACT



In the present study, an effort was made to identify the anti hyperglycemic potential of ethanolic extract of *Cadaba fruticosa* leaves concentrating the enteric system. The glucose-lowering potential was studied by using the *in vitro* methods such as α amylase inhibitory activity, inhibition of glucose diffusion, glucose adsorption and uptake by yeast cells. *In silico* methods comprising of the molecular docking of the selected phyto constituents as antagonistic ligands to the disaccharide digesting enzyme α glucosidase and glucose level maintaining enzyme dipeptidyl peptidase IV. The plant extract inhibited α amylase enzyme and the glucose diffusion considerably, which was found to be concentration-dependent. The uptake of glucose by the yeast cells in the presence of the extract was also found to be increased. In the molecular docking analysis based on the docking score, iso quercetin was found to be a potential antagonistic ligand for both enzymes α glucosidase and dipeptidyl peptidase IV since they exhibited similar amino acid interactions shown by the acarbose and Sitagliptin which are the standard competitive inhibitors of these two enzymes. It can be concluded that the leaves of *Cadaba fruticosa* may serve as a potential anti hyperglycemic drug in future for the management of diabetes mellitus.

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INTRODUCTION

Diabetes mellitus is a metabolic disorder due to the deficiency of the hormone secretion insulin from the pancreas. The symptoms of diabetes mellitus include hyperglycemia dyslipidemia, polyuria, polydypsia , polyphagia and weight loss (Rao and Sreenivasulu, 2012; Plevyak, 2011). Chronic elevated levels of blood glucose leads to complications in vital organs and disturb the carbohydrate, protein and lipid metabolism. Day by day, the diabetic population in India is increasing due to unhealthy food habits and sedentary lifestyle. At present, the disease can be controlled by the allopathic drugs such as biguanides, sulfonylurea, and inhibitors of specific gut enzymes such as α- amylase, α- glucosidase and dipeptidyl peptidase IV. In addition to drug therapy, modification in dietary habit and increased physical activity also helps in the management of hyperglycemia in diabetic patients. Control of diabetes either by drug therapy or by lifestyle modifications is still a difficult task since the existing anti hyperglycemic drugs has several side effects (Anbu

et al., 2012).

Currently, there is a paradigm shift all over the world towards herbal remedies for treating many dreadful diseases due to their low cost as well as less toxicity. From ancient times India is known for its traditional medicine systems and still the tribal and rural population practices these systems for treating their ailments (Mukherjee *et al.*, 2006). Herbal drugs contains several bioactive molecules with synergistic effects and unknown chemical characterization. The systematic scientific and pharmacological evaluation is the need of the hour for the search of lead compounds. Many Indian medicinal herbs have a folklore claim for treating diabetes mellitus. The plant *Cadaba fruticosa* belonging to the family Capparacae is found all over the world mainly concentrated in tropical and sub-tropical regions (Chatterjee, 1993). It is distributed in Gujarat, Karnataka and Tamilnadu in India (Arokiyaraj *et al.*, 2008). The crushed leaf juice has the potential use as anti diarrheal, anti syphilis, anti gonorrheal agent and a rejuvenator (Gayake *et al.*, 2012). According to the existing literature available, leaves possess antidiabetic and antipyretic activity (Arokiyaraj *et al.*, 2008; Mythreyi *et al.*, 2008). With the above scenario, the present investigation is undertaken to evaluate the *in vitro* and *in silico* anti-diabetic activity of the ethanolic extracts of *Cadaba fruticosa* leaves with main focus on enteric system and their enzymes.

MATERIALS AND METHODS

Chemicals

The required routine chemicals such as DMSO, glucose, were purchased from SD Fine Chemicals Ltd. The enzyme α amylase, dialyzing membrane were obtained from Hi-media Ltd. The remaining chemicals and reagent used were of analytical grade.

Collection and extraction of *Cadaba fruticosa* leaf extract

The C. *fruticosa* leaves were collected from Thiruvanamalli district of Tamil Nadu, and authentication was done by Dr. Mythreyi, Professor of Pharmacognosy department K.K. College of pharmacy, Chennai, India. The voucher specimen is deposited in abovementioned venue for future reference. The shade dried leaves were crushed into a coarse powder after shade drying was extracted using 90% ethanol at room temperature by cold maceration process for three days. The ethanolic extract was subjected to filtration and evaporated using rota flash evaporator for solvent evaporation. The yield of the sample was calculated, and the extract was preserved in the refrigerator till investigations. The yield was found to be 0.53%w/w.

In vitro anti-diabetic activity

Assay of α-Amylase inhibitory activity

The effect of the plant extracts in inhibiting the α-amylase enzyme is studied by the method of (Jayasri and Radha, 2009). Different concentrations of plant extract (100μg-1000 μg/ml) were dissolved in 500μl of 0.02M sodium phosphate buffer (pH 6.9 with 0.006M sodium chloride) containing α-amylase solution and made up to 500 μl with the same buffer. The above mixture was incubated at 25°C for 10 minutes. Once this pre-incubation is over 500 μl of 1% starch solution in 0.02M sodium phosphate buffer was added to all the test tubes and incubated at 25°C for 10 minutes, followed by the addition of 1ml of DNSA reagent to stop the reaction. Finally, all the test tubes were incubated for 5minutes in a boiling water bath, and the color intensity was measured in colorimeter at 540nm. A control without plant extract and a positive control acarbose were also used in the present investigation. The enzyme inhibitory activity in terms of percentage was calculated using the formula [(A_0–A_1/A_0) x 100], Where A_0 is the absorbance of the control and A_1 is the absorbance of plant extract or the standard sample

Glucose diffusion inhibitory assay

The ability of the extract to inhibit the diffusion of glucose to the external solution at time intervals was calculated based on the method of (A.Gallagher *et al.*, 2003). 1 ml of various concentrations of plant extract (100μg-1000 μg/ml) was placed in the dialyzing membrane along with 15 ml solution containing 0.22mM glucose and 0.15M sodium chloride. This dialyzing tube was kept in a beaker containing 45ml of 0.15mM sodium chloride solution. The beaker were placed in an orbital shaker at 37°C and glucose concentration in the beaker was measured for every half an hour for the period of 3hrs using DNSA method. The positive control acarbose was also treated in the same way.

Measurement Glucose uptake by yeast cells

The uptake of glucose by yeast cells was performed based on the method of (Cirillo, 1963). Briefly, a 10%(v/v) suspension of baker's yeast was prepared in distilled water. Aliquots of plant extract (100μg-1000 μg/ml) were added to 1ml of 5mM glucose solution and incubated at 37°C for 10 minutes followed by the addition of yeast suspension and incubation for 60 min at 37°C. After incubation the tubes were centrifuged, glucose estimation was performed in the supernatant. Metronidazole

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acts as a standard drug in the present investigation. Increase in percentage glucose uptake is calculated as Abs sample−Abs control ÷ Abs sample ×100 Where, Abs sample is the absorbance of the test sample, and Abs control is the absorbance of control reaction.

Measurement Glucose adsorption potential of plant extract

This assay was performed based on the method of (Ou *et al.*, 2001). About 1% of the extract was added to 25ml of a solution containing different concentration (5, 10, 20, 50 and 100 mM) glucose. This mixture is mixed well and incubated at 37°C, in the shaker water bath for a period of 6 hrs. The supernatant is collected after centrifuging for 20 minutes at 4000 rpm, and the glucose content is measured using DNSA reagent. The amount of bound glucose is calculated using a given formula

$$Glu\cos e \quad bound = \frac{G1-G6}{Weight\ of\ the\ sample} \times volume\ of\ the\ solution$$

The G1 is the glucose concentration of the original solution. G6 is the glucose concentration after 6 hours.

In silico anti-diabetic activity

The in silico antidiabetic activity was performed by molecular docking using patch dock analysis to find a suitable ligand from the plant compound as a competitive inhibitor for the enzyme α glucosidase and dipeptidyl peptidase IV. The corresponding enzymes three-dimensional structures were taken (α glucosidase and dipeptidyl peptidase IV) from protein data bank (http://www.rcsb.org/pdb/). Gas chromatography-mass spectrometry (GC-MS) studies conducted by the researchers (Amudha and Rani, 2014) as well as (Telrandhe and Uplanchiwar, 2013) have revealed the presence of several compounds. From the literature, 13 compounds were taken for the Patch dock analysis.

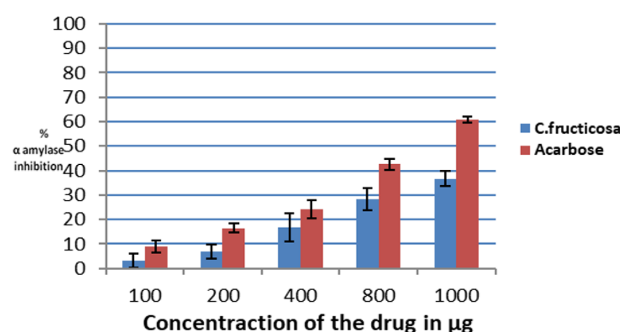

Figure 1: α Amylase inhibitory activity of *C.fruticosa* and Acarbose at different concentration. Each value represents the mean ± SD (n = 3)

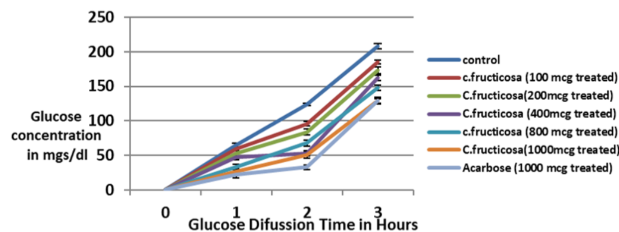

Figure 2: Inhibitory activity of C.fruticosa extract and Acarbose on Glucose diffusion. Each value represents the mean ± SD (n = 3)

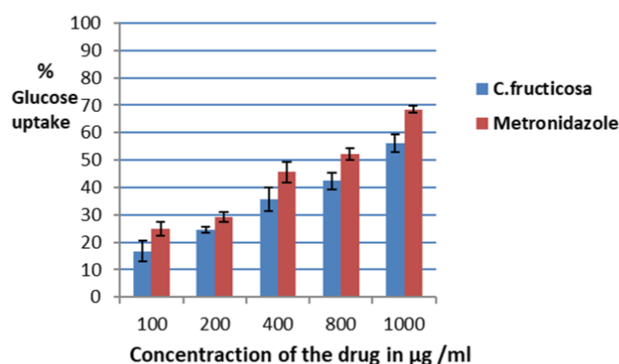

Figure 3: Effect of C.fruticosa extract and Metronidazole on Glucose uptakeby yeast cells. Each value represents the mean ± SD (n = 3)

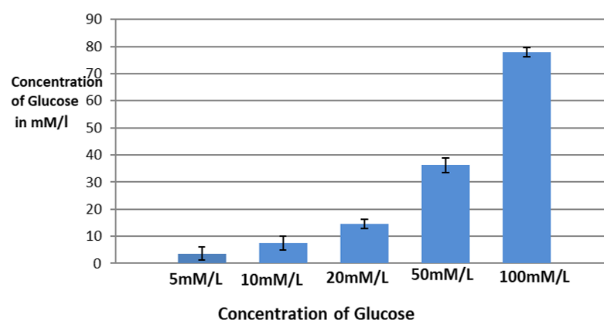

Figure 4: Glucose adsorption capacity of C.fruticosa extract at different concentration of Glucose. Each value represents the mean ± SD (n =3)

The two-dimensional structures of the *Cadaba fruticosa* phyto components were retrieved from PUB chem. site and using the Corina 3D converter, their three-dimensional structures were obtained. The enzyme and ligand interaction were obtained as docking score using the patch dock server. Based on the docking score the best ligand was selected from the phyto constituents of *Cadaba fruticosa* and their inhibitory potential was analyzed in terms of their interactions with the amino acid residues present in the active site were visualized and further confirmed by LIGPLOT. Standard drugs such as acarbose and Sitagliptin were also compared in the present study.

Statistical analysis

Table 1: Docking score values of compounds with α Glucosidase and Dipeptidyl peptidase IV

S.no	Compound name	α Glucosidase	Dipeptidyl peptidase IV
1	3,(4 formyl phenoxy) 4 methoxy	-0.08	-0.06
2	Cadabicine	-0.01	-0.02
3	Cadabicine triacetate	-0.04	-0.04
4	Isoquercetin	-0.03	-0.01
5	Thiazolidine	-0.04	-0.07
6	1,2 Benzene dicarboxylic acid mono 2 ethyl ester	-0.02	-0.03
7	9.12 octa decatrienoic acid	-0.08	-0.09
8	Benzene 1 methyltrdecayl	-0.10	-0.04
9	n-Hexadectrinoic acid	-0.05	-0.05
10	Hexadecanoic acid ethyl ester	-0.08	-0.06
11	cadabacilione	-0.05	-0.08
12	Cadabacine methyl ester	-0.13	-0.12
13	Strchidine	-0.12	-0.04

All experimental procedures were performed in triplicates, and the values reported as Mean $\pm$SD. ED50 value for the analysis were done by using a linear regression method.

RESULTS AND DISCUSSION

α-Amylase inhibitory activity

The chronic usage of synthetic drugs will produce side effects, and at one point of time, they become ineffective in treating a particular ailment. There is a change all over globally into plant-based herbal drugs from allopathic medicines due to their easy availability and cost-effectiveness. Among several plants derived anti-diabetic drugs, the phyto drugs which serve as α amylase inhibitors can be developed into a potential drug for the management of diabetes mellitus (Ahmed *et al.*, 2012).

In the present investigation, the ethanolic extract of *C. fructicosa* inhibited α amylase enzyme effectively from lower concentration itself considerably (Figure 1). The IC 50 value was found to be 367μg/ml. According to (Arokiyaraj *et al.*, 2008), the anti-diabetic activity of the ethanolic extract *C. fructicosa* is due to the presence of several bioactive substances such as steroids, alkaloids, gums and saponins. Probably any of these biomolecules or many of them collectively may act as a competitive inhibitor for α amylase enzyme to exert this inhibitory activity.

Glucose diffusion inhibitory assay

The decrease in diffusion of glucose due to the presence of phyto constituents in the form of fiber is considered as one of the *in vitro* technique for the assessment of anti-diabetic effect in terms of glucose absorption in the gastrointestinal tract (López *et al.*, 1996). (Mythreyi, 2008), in their pharmcognostical studies, have reported the presence of fibers in the leaves of *C.fruticosa*. (Maier *et al.*, 2002) In the present study (Figure 2) the plant extract at higher concentrations completely retarded the diffusion of glucose, which can be compared with the positive control acarbose. This may be attributed the fact that either the fiber present in the plant might slow down the diffusion rate or the phyto constituent present in the *C.fruticosa* may inhibit α amylase enzyme thereby slow down the glucose production by delaying carbohydrate digestion.

Measurement Glucose uptake by yeast cells

The measurement of glucose uptake by yeast cells is one of the succesful *invitro* technique for evaluating the hypoglycemic effects of drugs since the cells needs insulin for transport of glucose inside them (Mythreyi, 2008). The transport of glucose across the cell membrae of yeast cells is considerd as a complex process, where facilitated diffusion occurs towards concentration gradient based on the intracellular concentration of the glucose (Ahmed *et al.*, 2009). In the present investigation, there is an effective absorption of glucose by yeast cells, which increases with an increase in the drug concentration (Figure 3). At200μg/ml drug concentration the glucose absorption was almost similar to the positive drug metronidazole used in the present study.

Measurement Glucose adsorption potential of plant extract

In the present investigation, the glucose adsorption potential of the plant extract *C.fruticosa* is depicted in the Figure 4. It is observed that the glucose absorption capacity increases with an increase in

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Table 2: Interaction with the α Glucosidase and Dipeptidyl peptidase enzymes with phytochemicals of C.Fruticosa

S.No	Compound	α -Glucosidase enzyme amino acid binding site		Dipeptidyl peptidase IV amino acid binding site	
		H bonding sites	Hydrophobic contact sites	H bonding sites	Hydrophobic contact sites
1	Isoquercetin	2 Glu 781(A) Asp 812(A)	77 Gln693(A),Arg696(A), Lys697 (A),Glu 689(A), Glu781(A) Asp812(A)	5 Glu 205,206 (A)Tyr631662 (A2)	84 Arg125(A), Glu205,206(A) Tyr(547)(A)Trp629(A), Ser630(A),Tyr631, 632,666(A), Asn710(A), Val711(A) and His740(A)
2	Cadabicine	1 Val 193(A)	33 Asp170(A), Met172(A)Lys184(A) Glu192(A)Val193(A) Pro194(A)	- -	3 Arg696(A) Gln 693(A) lys697(A)
3	1,2 Benzene dicar- boxylic acid mono 2 ethyl ester	1 Arg 463(A)	73 Gly453(A),Arg456(A), Asp459 (A),Glu460(A), Arg463(A),Glu 505(A),	2 Asp243(A) Asn 570 (A)	85 Lys162(A), Asp185(A), Asn188(A), Arg190(A), Tyr191(A), Asp243(A), Gln244(A), Glu537(A), Leu538(A), Tyr569(A), Asn570(A).
4	Acarbose	7 Arg199(A3), Arg203(A), Gln352(A), Asp356(A2)	93 Pro198(A),Arg199(A) Val200(A)Arg203(A), Gln352(A),Asp356(A)	- -	- -
5.	Sitagliptin	-	-	7 Arg125(A2), Glu205(A), Glu206(A), Tyr547(A), Tyr631(A) Tyr662(A),	73 Arg125(A), Glu205(A), Glu206(A), Tyr547(A), Ser630(A), Tyr631(A), Tyr662(A), Asn710(A), His 740

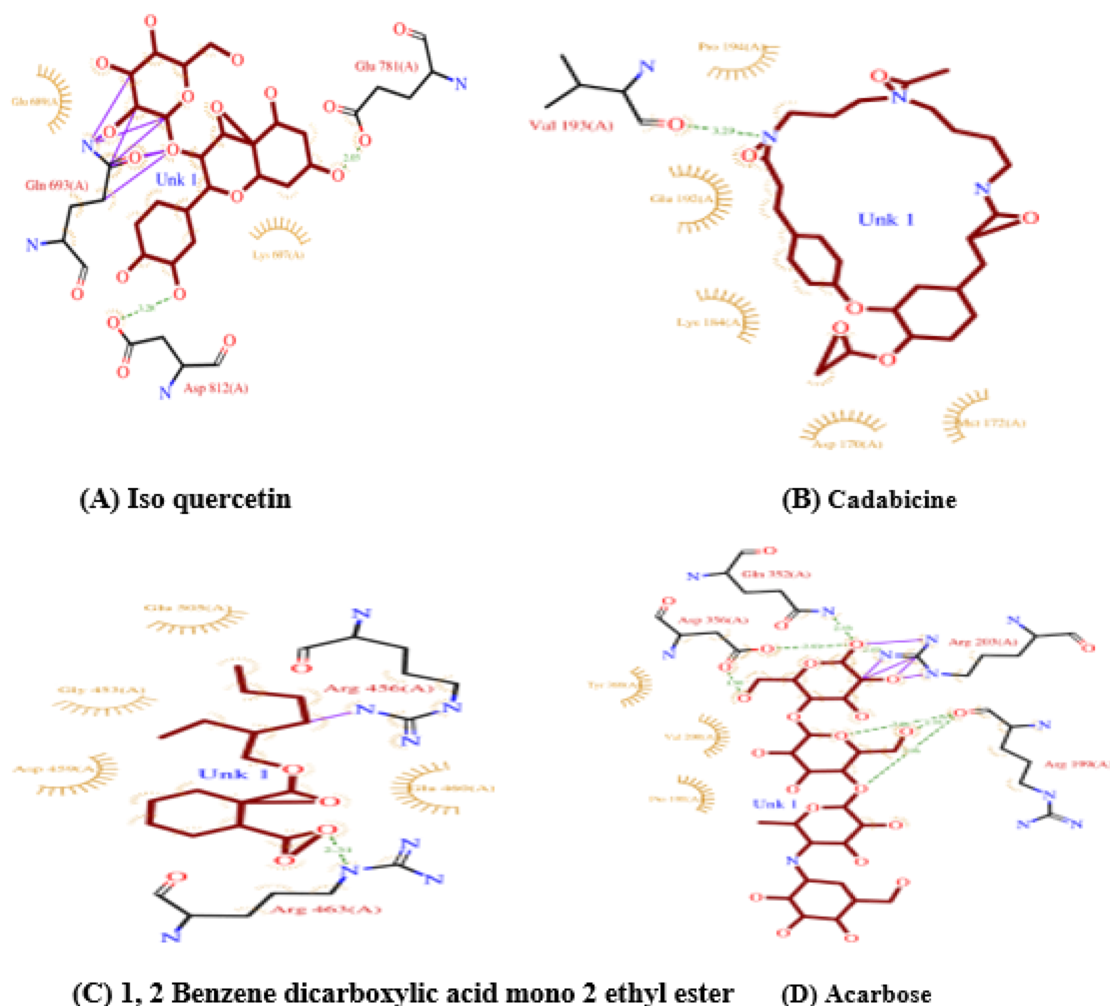

Figure 5: Amino acid Interaction of α glucosidase enzyme with specific ligands

the glucose concentration. Irrespective of the glucose concentration, the drug was able to combine with the glucose and thereby retard the absorption across the intestinal lumen. So it may be of beneficial insoluble fiber-rich fractions in reducing the postprandial blood glucose level (Chau *et al.*, 2004).

In their findings have shown that isolated from *Averrhoa carambola* have the capability of reducing the postprandial hyperglycemia. The active phyto compounds such as Stachydrine and 3-hydroxyl stachydrine, cadabine, terpenoids, flavones may have the capability of adsorption of glucose in the gastrointestinal tract and may account for the hypoglycemic activity (Yousif *et al.*, 1984).

In silico anti-diabetic activity

Molecular docking was performed for the selected 13 compounds with the enzymes α glucosidase and Dipeptidyl peptidase using the patch dock server and the corresponding docking score were obtained (Table 1).

Ligplot analysis was performed to study the enzyme inhibitory activity of the ligands Quercetin, Cadabicine and 1,2 Benzene dicarboxylic acid mono 2 ethyl ester which were selected based on the docking score among the 13 ligand. The types of amino acid interaction in the enzyme and their number of hydrogen and hydrophobic bonding with the selected ligand were explained in the Table 2, Figure 5 and Figure 6. The positive drug Acarbose and Sitagliptin were also subjected to docking analysis for comparison. It is observed that in the case of acarbose there is a hydrophilic interaction exists between the drug and the amino acids arginine and aspartic acid residues of the enzyme which is responsible for the antagonistic action of the acarbose. The same type of amino acid interaction is observed between the two ligands, namely Isoque-

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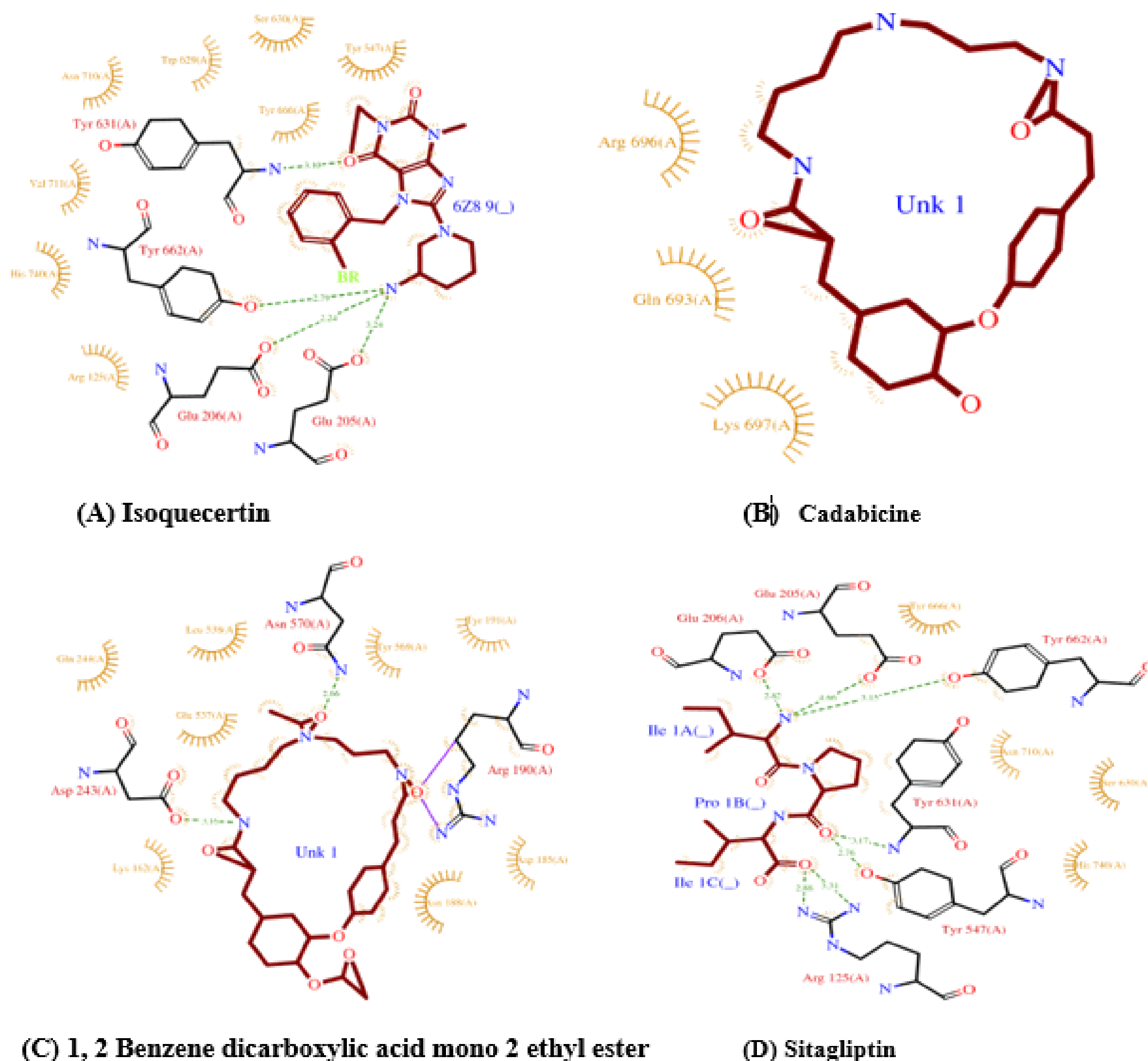

Figure 6: Amino acid Interaction of Dipeptidyl peptidase enzyme with specific ligands

certin and 1,2 Benzene dicarboxylic acid mono 2 ethyl ester to the enzyme α glucosidase. Similarly, in the case of dipeptidyl peptidase enzyme, there is hydrophilic interaction exists between the amino acid residues tyrosine and glutamic acid and the iso quercetin ligand as well as the positive drug Sitagliptin. So isoquecertin act as an antagonistic ligand for both the enzymes thereby exert the hypoglycemic activity as a competitive inhibitor.

CONCLUSION

There is a direct correlation exists between the absorption of glucose from the gut and the concentration of blood glucose level. In the present study, we made an attempt to slow down the absorption process of glucose by the ethanolic leaves extract of *C.fruticosa* so that the postprandial hyperglycemia can be controlled. Additionally, carbohydrate digesting enzymes inhibitors present in the leaves of *C.fruticosa* were identified by *in silico* methods which can be further evaluated in *in-vivo* methods for their potential anti hyperglycemic activity.

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