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Glycolytic enzyme inhibitory and antiglycation potential of *Gymnema sylvestre* R. Br.: An *in silico* approach

K. Kamalakkannan◆, N. Kiruthiga, V. Balakrishnan*, T. Sivakumar, G. Liji Martina, M. Janani** and S. Ramya*****

Department of Biotechnology, Nandha College of Pharmacy, Erode-638052, Tamilnadu, India

**Department of Botany, Arignar Anna Govt. Arts College, Namakkal-637002, Tamilnadu, India*

***Nandha College of Physiotherapy, Erode-638052, Tamilnadu, India*

****Nandha College of Nursing, Erode-638052, Tamilnadu, India*

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Abstract

Traditional herbal medicines are practiced worldwide for treatment of type II diabetes mellitus since ancient times. *Gymnema sylvestre* R. Br. has been used in ayurvedic medicine for the treatment of diabetes. Our target was to investigate the α-amylase, α-glucosidase and aldose reductase inhibitory along with antiglycation potential of methanolic extract of *G. sylvestre* and molecular docking of dihydroxy gimnemic triacetate. The enzyme inhibitory study revealed that *G. sylvestre* showed prominent α-amylase, α-glucosidase and aldose reductase inhibitory potential (42.49%, 47.21% and 38.93%) at concentration 200 µg/ml, respectively. In glycation inhibitory activity, fructosamine inhibitory potential was found to be 36.22%, protein carbonyls were inhibited up to 15.56% whereas protein thiols were inhibited up to 67.84%. It shows, *G. sylvestre* significantly inhibited (p<0.001) activity of α-amylase, α-glucosidase and aldose reductase. The interaction of dihydroxy G. triacetate with these enzymes were validated by docking studies. The docking study showed the remarkable binding of α-amylase, α-glucosidase and aldose reductase with Dihydroxy G. Triacetate. Molecular docking study, which can be concluded that the phytochemicals have enhanced binding sites, interactions and potent inhibitory effect on enzyme activity. The results indicate that the significant antidiabetic properties of these *G. sylvestre* and its phytochemicals may attribute to its α-amylase, α-glucosidase and aldose reductase.

1. Introduction

Herbal plants have been utilized to cure a variety of diseases around the world before the arrival of contemporary clinical medications, and they are known to contain chemicals that can be employed for therapeutic purposes or as precursors for the production of beneficial drugs (Sofowora, 1982). As a result, natural products account for more than half of all modern medications, and they play an essential role in drug discovery in the pharmaceutical business (Jeyachandran and Mahesh, 2007). Traditional herbal medicines are practiced worldwide for treatment of type II diabetes mellitus since ancient times. The traditional medicines are mainly used which are obtained from plants, it plays important role in the management of diabetes mellitus (Patel and Srinivasan, 1997).

G. sylvestre (Asclepiadaceae) is a large tropical liane native to central and western India and can be also found in tropical Africa and in Australia (Stocklin, 1969). *G. sylvestre* is a popular antidiabetic herb. There is a growing demand for *G. sylvestre* leaves in pharmaceutical trade (Tiwari *et al*., 2013). The blood sugar lowering activity of *Gymnema* leaves was first documented in the late 1920s (Mhasker and Caius, 1930). dihydroxy gimnemic Triacetate, the main active ingredient of this plant, is used widely as an antidiabetic (Shanmugasundaram *et al*., 1983), antisweetner (Kurihara, 1992) and antihypercholesterolemia (Bishayee and Chatterjee, 1994). It also has stomachic, diuretic and cough suppres-sant properties (Kapoor, 1990).

Diabetic mellitus is a disorder of carbohydrate and lipid metabolism in the cell. The condition is linked to a decreased quality of life as well as increased mortality and morbidity risk factors. Long-term hyperglycemia is a significant contributor to the onset and progression of micro and macrovascular problems (Strojek, 2003). Overproduction of ROS in endothelial cells of both big and small arteries has been linked to metabolic problems in diabetic patients. The aetiology of diabetes problems such as neuropathy, nephro-pathy, and retinopathy is hypothesised to be influenced by an imbalance in ROS (Wada and Ou, 2002; Genet *et al*., 2002).

The enzymes involved in the breakdown of polysaccharides which have shown have remarkable results in order to control blood glucose level in type II diabetes mellitus (Kim *et al*., 2005; Mai and Chuyen, 2007; Davis and Granner, 1996). α-amylase is one of the most important enzymes in this process, as it catalyses the breakdown of starch into maltose, which is then transformed to glucose, which is the only sugar that the body can use (Kotowaroo *et al*., 2006; Neerja *et al*., 2017). Another crucial enzyme to be aware of is intestinal α-glucosidase, which is found in the epithelium of the small intestine. The key enzyme aldose reductase, which is the first and rate-limiting enzyme in the polyol pathway, is involved in the conversion of

Corresponding author: Dr. K. Kamalakkannan

Professor, Department of Biotechnology, Nandha College of Pharmacy, Erode-638052, Tamilnadu, India

E-mail: car2203@gmail.com

Tel.: +91-9789502934

glucose to sorbitol. In diabetics, prolonged hyperglycemia causes the development of sorbitol in insulin-insensitive tissues like the lens, retina, nerves, and kidneys *via* the polyol pathway (Ueda *et al*., 2001). These enzymes are now regarded as a therapeutic target for the modulation abnormality that occurs in type II diabetic mellitus (Yao *et al*., 2010; Lalitha and Sivakumar, 2018).

To explore ligand conformations within acromolecular targets' binding sites, molecular docking methods are commonly used in current drug design. This method determines the ligand-receptor binding free energy by analysing key events in the intermolecular recognition process. Given the large range of docking methods available today, knowing the advantages and disadvantages of each method is crucial for developing effective strategies and producing relevant results. When compared to experimental techniques like HTS, molecular docking has a number of advantages, one of which is its ability to screen large compound databases at a low cost (Acharya *et al*., 2011).

Hence, the present study was to investigate the α-amylase, α-glucosidase and aldose reductase inhibitory along with antiglycation potential of methanolic extract of *G. sylvestre* and molecular docking of dihydroxy gimnemic triacetate.

2. Materials and Methods

2.1 Extraction procedure

G. sylvestre leaves were collected in and around Erode, Tamilnadu in June 2019, and the plant was validated by the Botanical Survey of India (BSI) in Coimbatore. The samples were air dried at room temperature. Dried leaves (200 g) were coarsely crushed and subjected to continuous hot extraction followed by sequential solvent extraction (Soxhlet). Methanol was used for the extraction. The marc was air dried each time. The extract was concentrated using a rotary flash evaporator to evaporate the solvent. The extract was maintained in sealed containers at 4°C until it was needed (David and Sudarsanam, 2013).

2.2 Glycolytic enzyme inhibition studies

2.2.1 α-amylase inhibitory assay

The reducing sugar (maltose equivalent) liberated under the assay conditions was used to determine α-amylase inhibition. The inhibitory activity of the enzyme was measured as a reduction in the amount of maltose released. To calculate the maltose equivalent, a modified dinitrosalicylic acid (DNS) technique was used. 1 ml of the methanolic extract of *G. sylvestre* plant was pre-incubated with α-amylase. 1 U/ml for 30 min and thereafter 1 ml (1% w/v) starch solution was added. The mixture was further incubated at 37°C for 10 min. After stopping the reaction, using 1 ml dinitrosalicylic acid reagent, the contents were heated in a boiling water bath for 5 min. At 540 nm, the absorbance was determined. Voglibose was used as positive control (Puratchimani and Jha, 2004). The antidiabetic activity, which was computed using the following equations:

% reaction = (Maltose) test/(Maltose) control × 100 % inhibition
= 100% reaction

2.2.2 α-glucosidase inhibitory activity

The α-glucosidase inhibitory activity was determined using the standard method (Sutedja, 2005: Nupur *et al*., 2019). 0.5 mg glucosidase was dissolved in 10 ml phosphate buffer and 10 mg bovine serum albumin to make the enzyme solution. DMSO was used to make sample solutions with various concentrations (25, 50, 75, 100, 125, 150, 175, 200 g/ml), and 5 µl of each sample solution or DMSO (sample blank) was added to 200 µl of 20 mM p-nitrophenyl d-glucopyranoside and 480 µl of 100 mM phosphate buffer (pH 7.0). It was pre-incubated for 5 min at 37°C before the reaction began with the addition of the enzyme solution, which was then incubated for exactly 15 min at 37°C. The reaction was then halted by adding 1000 µl of 200 mM Na_2CO_3 solution, and the amount of p-nitrophenol released was determined using a UV-visible spectrophotometer by comparing the absorbance of the sample to that of a blank at 400 nm.

2.2.3 Aldose reductase inhibitory activity

Aldose reductase activity was assayed according to the method described by Haraguchi *et al*. 1997. Using glyceraldehyde as a substrate, the oxidation of NADPH at 340 nm was monitored spectrophotometrically at room temperature to evaluate enzyme activity. Various concentrations (25-200 µg/ml) of alcoholic extract of *G. sylvestre* were added to assay mixtures of aldose reductase and incubated for 5 min before initiating the reaction by NADPH. The percentage inhibition was calculated considering the activity in the absence of methanolic extract of *G. sylvestre* as 100%. Linear regression analysis of the plot of percentage inhibition versus concentration yielded the half-maximal inhibitory concentration values.

2.2.4 Antiglycation potential

Albumin glycation was performed with certain modifications. Glycated BSA samples were made and kept in sealed tubes at 37°C for 4 consecutive days. In the same way, a positive control was maintained. Triplicates of each incubate were created. The solution's unbound form of fructose was kept at 4°C. The resulting was utilised to estimate fructosamine adducts, protein carbonyls, protein thiols, and Congo red absorbance to determine the extract's antiglycation activity (McPherson *et al*., 1988; Kiruthiga *et al*., 2021).

2.2.5 Estimation of fructosamine

Fructosamine was measured using the nitrobluetetrazolium assay (Baker *et al*., 1994). The aliquots of glycated samples and positive control were incubated at 37°C for 30 min with 0.6 ml of nitro blue tetrazolium in sodium carbonate buffer. The absorbance at 530 nm was measured after incubation, and the % inhibition of fructosamines by extract was determined using the formula below.

$$\text{Inhibitory activity (\%)} = [(A_0 - A_1)/A_0] \times 100$$

2.2.6 Carbonyl group estimation

The concentration of protein carbonyls was determined using the molar extinction coefficient and absorbance at 360 nm (Uchida *et al*., 1998). The results were expressed as a percentage inhibition, which was computed using the fructosamine estimation procedure.

2.2.7 Protein thiol estimation

Dithionitrobenzoic acid was used to determine the thiol groups of glycated albumin and a positive control (Ellman, 1959). In this test, 260 µl samples and controls were incubated for 15 min with three volumes of DTNB before the absorbance was measured at 400 nm. The standard curve of varied BSA concentrations was used to calculate

the free thiol concentration in the solution. The formula employed in the estimation of fructosamine was utilized to calculate the % protection.

2.2.8 Binding of congo red

Congo red binding was measured by taking the absorbance at 530 nm (Klunk *et al*., 1999). For this assay, the samples (500 µl) were incubated with 100 µl of congo red (100 µM) in phosphate buffered saline with 10% (v/v) ethanol for 20 min at room temperature.

2.3 Docking studies

Molecular docking was performed on dihydroxy gimnemic triacetate, which is notable active principle present in *G. sylvestre*. A Lamarckian genetic algorithm method implemented in the program AutoDock 4.2 was used to study the molecular interactions between the phytochemical ligands such as dihydroxy gimnemic triacetate against the enzyme α-amylase, α-glucosidase and aldose reductase. Auto dock tools were used to study the docking simulations (trott and olson, 2010; Selvaraj *et al*., 2014). Auto dock relies on pre-calculated grid maps, one for each type of atom in the flexible molecules to be docked. To perform docking calculations, gasteigere-marsili partial charges were applied to the ligands, non-polar hydrogen atoms were merged, and all torsions were allowed to be tested using Bonferroni's test (*p< 0.001). The interactions and binding energy of the docked structure were investigated using the auto dock data.

2.4 Statistical analysis

The results are expressed as mean ± standard error of mean. Experiments were always carried out in three different ways. The analysis of variance was used to compare the two groups, followed by the Bonferroni test (p<0.001).

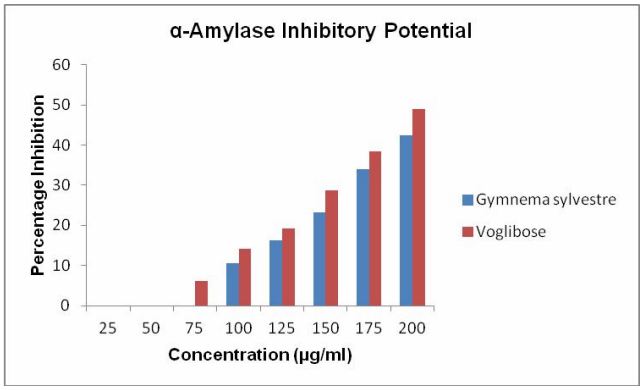

Figure 1: Inhibitory potential of *G. sylvestre* against α-amylase.

3. Results

The present study was to establish the inhibitory activity of *G. sylvestre* against α-amylase, α-glucosidase and aldose reductase. The Figure 1, shown the inhibitory potential activity of methanolic extract of *G. sylvestre*, which justifies that *G. sylvestre* showed prominent α-amylase inhibitory potential (42.49 % at concentration 200 µg/ml). This percentage of inhibition ranged from 10.36 to 42.49 %. The α-glucosidase inhibitory activity of *G. sylvestre* is shown in Figure 2. For all tested concentrations, percent α-glucosidase inhibition increased with increasing concentration of *G. sylvestre*. Inhibition in enzyme activity ranged from 11.05 to 47.21 %. Aldose reductase reveal the range from 8.16 to 38.93 % is shown in Figure 3. In the presence of the extract, the inhibitory activities of α-amylase, α-glucosidase and aldose reductase enzymes significantly rise, according to statistical analysis.

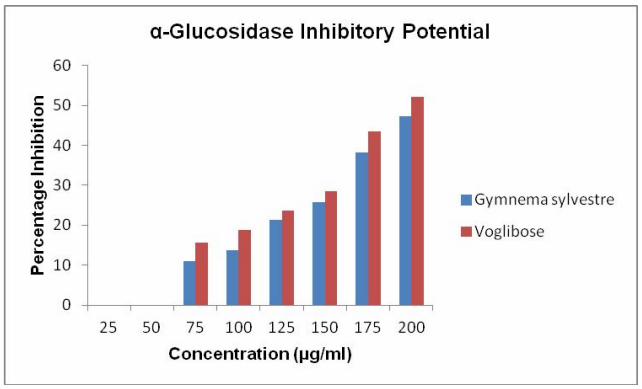

Figure 2: Inhibitory potential of *G. sylvestre* against α-glucosidase.

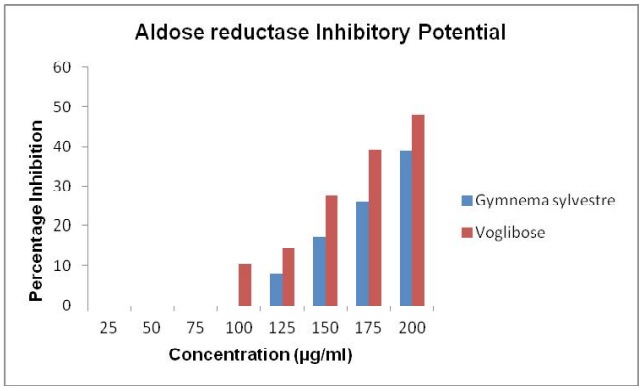

Figure 3: Inhibitory potential of *G. sylvestre* against aldose reductase.

The results on glycation inhibitory activity of *G. sylvestre*, a naturally occurring the therapeutic value of medicinal herbs which was already proven to an antidiabetic, was done using an *in vitro* glucose-bovine serum albumin (BSA) assay. Glucose and BSA were co incubated at 60°C in the presence and absence of *G. sylvestre*. BSA was used to measure the antiglycation potential, and then fructosamine adducts, protein carbonyls, protein thiols, and congo red absorbance were calculated.

Table 1: Percentage inhibition of different glycated proteins by *G. sylvestre*

S.No.	Parameters	Percentage inhibition
1.	Fructosamine	36.22%
2.	Protein carbonyls	15.56%
3.	Protein thiols	67.84%
4.	Protein amyloids	Not found

The values represented are mean ± SE, where n = 3.

In glycation inhibitory activity, methanolic extract of *G. sylvestre* showed remarkable results for antiglycation potential. For, determination of glycation potential, inhibition the various parameters were calculated like fructosamine inhibition was found to be 36.22 %, protein carbonyls were inhibited up to 15.56 %, whereas protein thiols were inhibited up to 67.84 %. The glycation inhibitory potential was shown in Table 1.

Automated docking technique was utilized to determine the 'orientation' of dihydroxy gimnemic triacetate bound with the active pockets of α-amylase, α-glucosidase and aldose reductase enzyme, the interaction of dihydroxy gimnemic triacetate with the active pocket of enzymes were depicted in Figures 4, 5 and 6. Dihydroxy gimnemic triacetate demonstrated minimum binding energy with enzyme *via* non-covalent interaction (Table 2). These *in silico* observation substantiate the *in vitro* α-amylase, α-glucosidase and aldose reductase inhibitory activity of dihydroxy gimnemic triacetate.

The amino acids present in the active site of aldose reductase capable of interaction with dihydroxy gimnemic triacetate are found to be CYS A298, LYS A262, ILE A260, PRO A261, GLN A183, ASN A160, TYR A209, SER A214, HIS A110 and ASP A47. The amino acids responsible for the binding of α-amylase and α-glucosidase with dihydroxy gimnemic triacetate was identified as ARG A337, ALA A324, ASP A297, MET A 328, LEU A329, TYR A321, ILE A391, GLU A385, HIS A386, VAL A325, SER A340, PHE A315 and LYS A523, PHE A543, LYS A524, SER A544, ASP A546, TRP A581, LEU A323, GLU A322, respectively. The data obtained for aldose reductase, α-amylase and α-glucosidase interaction with dihydroxy gimnemic triacetate were illustrate as Figures 4, 5, 6 and Table 2.

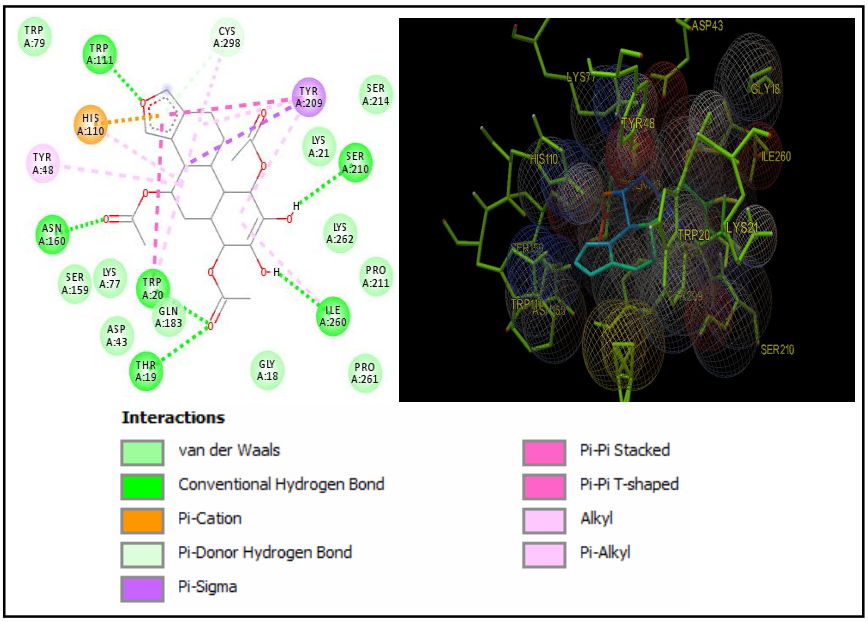

Figure 4: Molecular docking studies of dihydroxy gymnemic triacetate against aldose reductase.

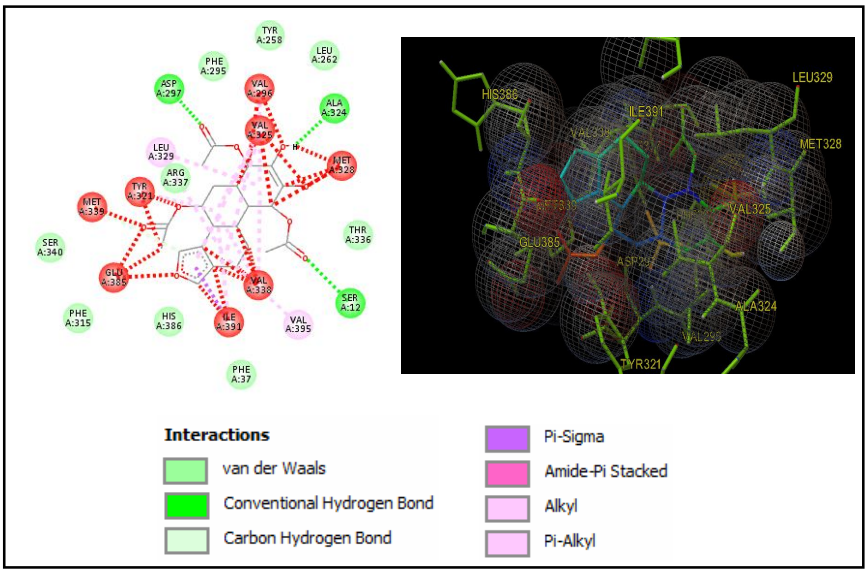

Figure 5: Molecular docking studies of dihydroxy gymnemic triacetate against α-amylase.

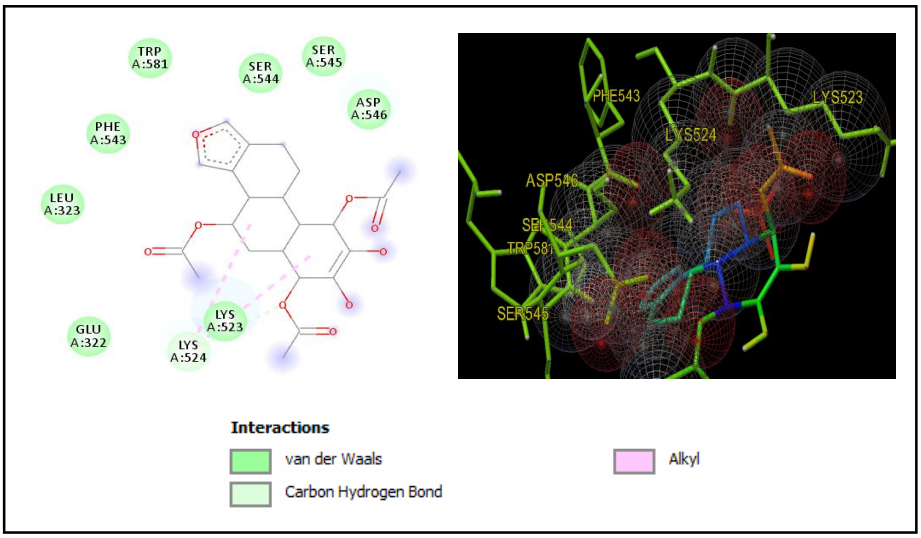

Figure 6: Molecular docking studies of dihydroxy gymnemic triacetate against α-glucosidase.

Table 2: Molecular docking studies of dihydroxy gymnemic triacetate on α-amylase, α-glucosidase, and aldose reductase inhibitors

Parameters	Aldose reductase	α-glucosidase	α-amylase
Binding energy	-3.85	-4.40	-1.24
Inhibition constant (μm)	4.5	75.84	6.4
Intermolecular energy	-6.24	-6.79	-5.48

In the docking studies, the binding energy and intermolecular energy of the dihydroxy gimnemic triacetate was found to be – 3.85, – 4.40, – 1.24 and - 6.24, - 6.79, - 5.48 for aldose reductase, α-glucosidase and α-amylase enzyme, respectively. It is evidenced from the data obtained that the binding energy of dihydroxy gimnemic triacetate antidiabetic ligands possesses significant aldose reductase, α-glucosidase and α-amylase inhibitory activities.

4. Discussion

Diabetes, a chronic metabolic condition associated with hyperglycemia, caused by insufficient or inefficient insulin production, is currently a global problem. With over 68 million diabetes patients identified in India, the disease is quickly becoming a pandemic. According to ICMR report, diabetes is on the rise in India, particularly among persons of lower socio-economic status in metropolitan areas of economically developed states. According to published figures, India's diabetic population is anticipated to grow from 31.7 million in 2000 to 79.4 million in 2030 (Whiting *et al*., 2011). Diabetes can be readily prevented and controlled by increasing physical activity, reducing calorie intake, and avoiding sedentary behavior. However, altering one's lifestyle is difficult for many people, and they seek a less burdensome alternative.

Plants with sugar lowering effects have been used in traditional medicine for thousands of years without side effects. India is blessed with a large number of plants, which are used to treat diabetes. For ages, *G. sylvestre* has been utilised in Ayurvedic medicine in India to treat diabetes. Diabetes mellitus affects hundreds of million people worldwide (Shabana *et al*., 2015). Over five million deaths occurred in India due to complications associated with diabetes (Goh and Cooper, 2018). This creates an environment highly favorable to increasing protein damage caused by glycation. It has been seen that the inhibition of various glycolytic enzyme can turn as a remedy (Perera, 2016).

Oboh *et al*. (2015) found that inhibiting α-amylase delays the digestion of starch while inhibiting α-glucosidase hinders the digestion of disaccharides, making it an excellent tool for diabetes management by regulating and reducing the rise in postprandial glucose levels. Balaji *et al*. (2015) highlight the usefulness of natural sources of α-amylase, α-glucosidase and aldose reductase inhibitors in folk medicine for the treatment and control of diabetes.

In this present study, we investigated the α-amylase, α-glucosidase and aldose reductase inhibitory activity and glycation potential of *G. sylvestre* . It shows, *G. sylvestre* significantly inhibited ($p< 0.001$) activity of α-amylase, α-glucosidase and aldose reductase. Advanced glycation end products (AGEs) are thought to occur as a result of non-enzymatic glycation between decreasing sugar and protein, and they play a role in the pathophysiology of diabetes and ageing problems (Babu *et al*., 2004).

Docking studies confirmed the interaction of dihydroxy gimnemic triacetate with these enzymes. The docking analysis revealed that α-amylase, α-glucosidase, and aldose reductase have strong affinity to dihydroxy gimnemic triacetate. The phytochemicals exhibit improved binding sites, interactions, and a significant inhibitory effect on enzyme activity, according to a molecular docking research. According to Abdin *et al*. (2003), docking is frequently used to predict the affinity and activity of small molecule drug candidates by predicting their binding orientation to their protein targets. As a result, docking is quite important in the logic.

Madeswaran *et al*. (2011): Lengauer and Rarey (1996) highlighted binding energy, inhibition constant, intermolecular energy, and phytochemical orientation with receptors in their investigation. These molecular docking indices are considered significant. Molecular docking, according to Bissantz *et al*. (2000) is a complicated process that comprises of two parts: ligand prediction and orientation, and affinity for the target.

5. Conclusion

In this study, the inhibitory action and antiglycation potential of critical enzymes such as α-amylase, α-glucosidase, and aldose reductase were investigated using a methanolic extract of *G. sylvestre*. It depicts the enzyme inhibitory and antiglycation potentials that are most prevalent. Based on the report, we may conclude that *G. sylvestre* extract contains herbal bioactive components with enzyme inhibitory ability. Docking studies verified the active components dihydroxy gimnemic triacetate found in *G. sylvestre* with this enzyme. This interaction demonstrates how well enzymes bind to dihydroxy gimnemic triacetate.

The findings suggest that the considerable antidiabetic characteristics of *G. sylvestre* and its phytochemicals are due to their inhibitory effects on α-amylase, α-glucosidase, and aldose reductase, as well as their antiglycation potential. The phytochemicals exhibit improved binding sites, interactions, and a significant inhibitory effect on enzyme activity, according to a molecular docking research.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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