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IN SILICO DOCKING STUDIES ON PYRUVATE KINASE, GLYCOGEN PHOSPHORYLASE, SULFONYLUREA RECEPTOR ACTIVITY BY ISOLATED ACTIVE PRINCIPLES OF *STEREOSPERMUM TETRAGONUM* DC

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ABSTRACT

Objective: *Stereospermum tetragonum* is a potential source of metabolites such as coumarines, glycosides, tannins, terpenoids and traditionally used in the treatment of Diabetes mellitus. The present work attempts to evaluate the interaction of active principles extracted from *S. tetragonum* with Pyruvate kinase, Glycogen phosphorylase and Sulfonylurea receptor by *in silico* docking studies. **Methods:** Compounds isolated from active fraction of water extract of *S. tetragonum* were analyzed by spectral studies and identified as1,4a,5,7a-tetrahydro-5-hydroxy-7-(hydroxymethyl)-1-(tetrahydro-6-(hydroxymethyl)-3,4,5-trimethoxy-2H-pyran-2-yloxy)cyclopenta[c]pyran-4-carboxylicacid and 5,8-dihydro-7-isopentyl-2,3,5,8-tetramethoxynaphthalene-1,4,6-triol. The two bioactive molecules were active in lowering the blood glucose level. These bioactive molecules were activated with Pyruvate kinase, Glycogen phosphorylase and Sulfonylurea receptor by *in silico* docking studies. The two bioactive molecules were active in lowering the blood glucose level at 2mg/kg dose. **Results:** The isolated compounds showed better interaction than standard Metformin through an extensive *in silico* docking approach with Pyruvate kinase, Glycogen phosphorylase and Sulfonylurea receptors. The glide score for Pyruvate kinase for compound 1 is -6.746 for compound 2 is -5.808 and for Metformin is 1.08. In Glycogen phosphorylase, the glide score for standard Metformin is 4.435 and for compound 1 is -7.558 and compound 2 is -5.454. In Sulfonylurea receptor the glide score of compound 1 is -5.914, compound 2 is -5.951 and standard Metformin is -1.878. **Conclusions:** The work establishes the isolated compounds from the fraction *of S. tetragonum* as a potential source of therapy for diabetes mellitus thus enabling a possibility of this plant as new alternative to existing diabetic approaches.

INTRODUCTION

Key enzymes of carbohydrate metabolism have become potential targets for the development of antihyperglycemic drugs in recent years. Bioactive molecules possessing stimulatory effect on glycolytic Key enzymes and inhibitory effect on glycogenolytic enzyme are effective in maintenance of blood glucose levels in hyperglycemic state. Pyruvate kinase is one of the regulatory enzymes of aerobic

glycolysis which catalyses the irreversible transfer of phosphate from phosphoenolpyruvate to ADP resulting in the production of pyruvate and ATP.[1] Pyruvate kinase is a major regulator for controlling the metabolic flux and ATP production in glycolysis and is considered a potential drug target.[2&3] The enzyme is allosterically regulated by various physiological effectors. [4-7] Glycogen phosphorylase catalyses the rate-limiting step in glycogenolytic pathway and releases glucose-1-phosphate from the terminal alpha-1,4-glycosidic bond in glycogen, thus acts as a key enzyme in the utilization of muscle and liver reserves of glycogen.[8] In muscle, glucose-1-phosphate is further metabolized via glycolysis to provide energy and in liver, phosphorylase helps to maintain constant blood glucose level via the action of glucose -6-phosphatase.[9&10] Glycogen phosphorylase is a dimer consisting of two identical subunits and has an essential cofactor, pyridoxal phosphate. Glycogen phosphorylase can be found in two different states, glycogen phosphorylase a (GP*a*) and glycogen phosphorylase b (GP*b*). The difference in the structures is due to phosphorylation of the Ser-14 residue, which results in the active form (GP*a*). Protein phosphatase dephosphorylates the GP*a* to the inactive form, also known as GP*b*. Both forms of glycogen phosphorylase can also be found in T and R states where T is the inactive state because it appears to have a low affinity for substrate and R is the active state where it appears to have a greater affinity for substrate.[11] Sulfonylurea receptors (SUR) are the molecular targets of sulfonylurea class of antidiabetic drugs, which promote insulin release from the pancreatic Beta cells of Langerhans. The Sulfonylurea receptor is a member of the ATP-binding cassette (ABC) family of membrane proteins. It functions as the regulatory subunit of the ATP-sensitive potassium (K_{ATP}) channel, which comprises SUR and Kir6.x proteins. [12] The present *in silico* docking study is a strategic approach to evaluate the role of Pyruvate kinase, Glycogen phosphorylase and Sulfonylurea receptor in lowering blood glucose, which may contribute a therapeutic effect in Type II Diabetes mellitus.

2. MATERIALS AND METHODS

2.1 Collection of plant materials: The roots of *S. tetragonum* (family: Bignoniaceae) was collected from Tirunelveli district of Tamil Nadu, India and identified by the taxonomist of TBGRI and a voucher specimen (TBGRI 8282) has been deposited in the institute herbarium.

2.2 Aqueous extraction of dry powder: To prepare water extract, the powder *S. tetragonum* was extracted with distilled water (5 g/100 ml) by stirring for 4 hours and then filtering through filter paper (Whatman No. 1). This process was repeated thrice with the residue. The combined filtrate was freeze-dried in a lyophilizer. [13]

2.3 Isolation of Active Fraction (AF): The water extract of *S. tetragonum* root powder was precipitated with absolute ethanol (1:1 v/v) and separated into precipitate and soluble fractions. The soluble fraction was subjected to the preliminary phytochemical screening and isolation of compounds. In the present study, column chromatography and preparative TLC (Thin Layer Chromatography) were used for elution of two compounds. Based on IR (Infrared), H^1NMR (Nuclear Magnetic Resonance), ^{13}C NMR and Mass spectrum, the compounds were identified as 1,4a,5,7a-tetrahydro-5-hydroxy-7-(hydroxymethyl)-1-(tetrahydro-6-(hydroxymethyl)-3,4,5-trimethoxy-2H-pyran-2-yloxy)cyclopenta [c]pyran-4-carboxylic acid (Figure1) and 5,8-dihydro-7-isopentyl-2,3,5,8-tetramethoxynaphthalene-1,4,6-triol (Figure 2) which was already published from our group.[14] The three dimensional structures of Pyruvate kinase, Glycogen phosphorylase and Sulfonylurea receptor activity were obtained from Protein Data bank (PDB): PDB ID: 4MP2, 3DDS, 4AYT.

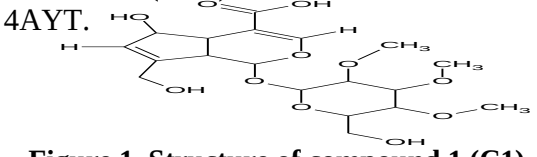


Figure 1. Structure of compound 1 (C1), 1,4a,5,7a-tetrahydro-5-hydroxy-7-(hydroxymethyl)-1-(tetrahydro-6-(hydroxymethyl)-3,4,5-trimethoxy-2H-pyran-2-yloxy)cyclopenta[c]pyran-4-carboxylic acid

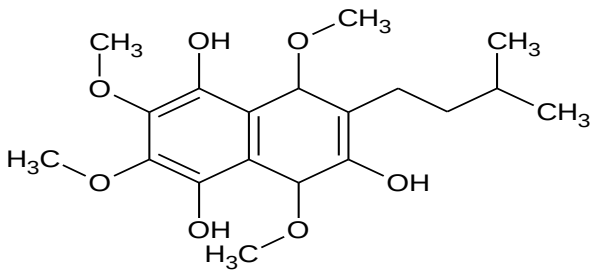


Figure 2. Structure of Compound 2 (C2), 5,8-dihydro-7-isopentyl-2,3,5,8-tetramethoxynaphthalene-1,4,6-triol.

2.4 Molecular docking

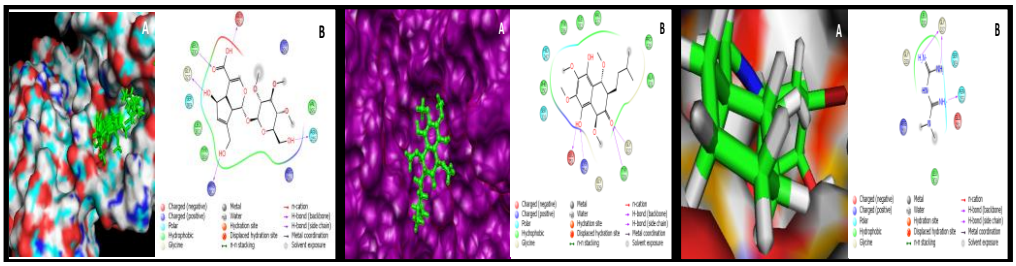
The structures considered for the study were obtained from the isolation of active fraction and from spectral studies. 1,4a,5,7a-tetrahydro-5-hydroxy-7- (hydroxymethyl)-1-(tetrahydro-6- (hydroxymethyl)-3,4,5-trimethoxy-2H-pyran-2-yloxy)cyclopenta [c] pyran-4-carboxylic acid (C1) and 5,8-dihydro-7-isopentyl-2,3,5,8 tetramethoxynaphthalene-1,4,6-triol (C2) were used as ligands and the structures were drawn using CHEMDRAW (Version 11). The structures of Pyruvate kinase, Glycogen phosphorylase and Sulfonylurea receptor were obtained from the protein data bank. Hydrogen atoms were added to the protein consistent with pH 7.0 using the protein preparation wizard in the Schrodinger suite.[15] Further, the protein's hydrogen bond network was also optimized using the wizard. The so-prepared structure was then subjected to energy minimization and the termination condition for minimization was fixed as the step when the root mean square deviation of the heavy atoms in the structure relative to the starting structure exceeded 0.3 A. This process also ensures that the hydrogen atoms are placed in optimized geometries. The protein thus prepared was used for docking of the ligands as described below. Potential binding sites in Pyruvate kinase, Glycogen phosphorylase and Sulfonylurea receptor activity were predicted using the Site Map tool in the Schrodinger suite. [16&17] Lig Prep module (version 2.5) of the Schrodinger suite was used to generate conformers of the ligands. The ligands were then docked using the extra precision mode in the Glide module of the Schrodinger suite. [18-20]

3. RESULTS

Present study provides scientific evidence that bioactive molecules C1 and C2 strongly interact with Pyruvate kinase, Glycogen phosphorylase and Sulfonylurea receptor through different residues. Figure 3 shows the XP Glide score for both the compounds for Pyruvate kinase(-6.746 Kcal/mol for C1 and -5.808 Kcal/mol for C2) which clearly suggests that both the compounds show better interaction than Metformin -1.08. Figure 4 shows the XP Glide score for both the compounds for Glycogen phosphorylase as -7.558 Kcal/mol for C1 and -5.454 Kcal/mol for C2. This clearly suggests that both the compounds show better interaction than Metformin -4.435 (Table.1). Thus, the compounds inhibit glycogen phosphorylase leading to the prevention of glycogen break down which inturn facilitates the lowering of blood glucose level. The XP Glide score for both the compounds for Sulfonylurea receptor is were -5.914 Kcal/mol for C1 and -5.951 Kcal/mol for C2 (Figure 5).

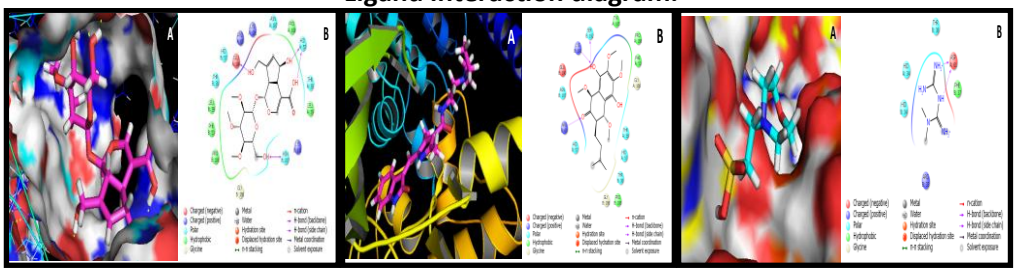
4. DISCUSSION

The results of the above investigation shows the docking of C1 and C2 with Pyruvate kinase increases glucose oxidation leading to the reduction of blood glucose level. This clearly suggests that docking of both the compounds with Sulfonylurea receptor might have inhibited the k+ atp channel causing depolarization of beta cell membrane which in turn might have triggered the opening of ca 2+ channel leading to increased ca 2+ influx resulting in the increased excocytosis of insulin and thus causing reduced blood glucose level (Table 1). In this context, the isolated compounds of *S. tetragonum* is likely to be useful as an alternative medicine or as a combination drug. There is a need to carry on further studies leading to likely development of the isolated compound as a standardized, safe and effective phyto-medicine. These active molecules are very promising for further in-depth studies leading to the development of a novel, safe and valuable anti-hyperglycemic herbal drug medicine for mono-therapy and / or combination therapy for Diabetes mellitus.



C1 **C2** **METFORMIN**

Figure 3. Interaction of compound 1, 2 and Metformin with Pyruvate kinase, A. Surface view, B. Ligand interaction diagram.

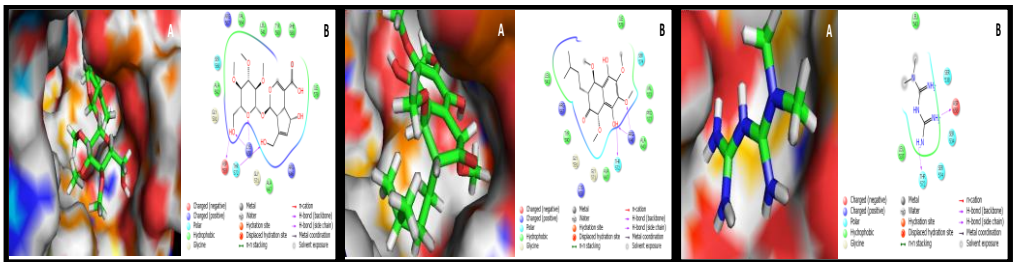


C1 **C2** **METFORMIN**

Figure 4. Interaction of compound 1, 2 and Metformin with Glycogen phosphorylase, A. Surface view, B. Ligand interaction diagram.

Table 1. Glide Score of Compound 1, Compound 2 and Standard.

DOCKING STUDIES							
S. No.	**Name of the protein**	**PDB ID No.**	**Site Volume**		**Glide score**		**Standard**
1	Receptor	Protein data bank	C1	C2	C1	C2	Metformin
2	Pyruvate kinase	4MP2	200.312	200.312	-6.746	-5.808	-1.08
3	Glycogen phosphorylase	3DDS	1097.94	1097.94	-7.558	-5.454	-4.435
4	Sulfonylurea receptor	4AYT	287.777	287.777	-5.914	-5.951	-1.878



C1 **C2** **METFORMIN**

Figure 5. Interaction of compound 1, 2 and Metformin with Sulfonylurea receptor, A. Surface view, B. Ligand interaction diagram.

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Conflicts of interest: All authors have none to declare.

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