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IN SILICO DOCKING STUDIES ON SGLT 2, FOXO1, GLYCOGEN SYNTHASE ACTIVITY BY ISOLATED ACTIVE PRINCIPLES OF *STEREOSPERMUM TETRAGONUM* DC

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ABSTRACT

Background: *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 evaluated from *S. tetragonum* with SGLT 2, FOXO1, Glycogen Synthase by insilico docking studies. **Methods:** Compounds isolated from active fraction of water extract of *S. tetragonum* which are 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 is activated with SGLT 2, FOXO1, Glycogen synthase by insilico docking studies. The two bio active 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 *insilico* docking approach with SGLT 2, FOXO1, Glycogen synthase with the receptors. The glide score for SGLT 2 for compound 1 is -8.765 for compound 2 is -7.056 and for metformin -1.711. In FOXO1 the glide score for standard metformin -0.381 and for compound 1 is -4.377 and compound 2 is -2.715. In glycogen synthase the glide score of compound 1 is -7.558, compound 2 is -5.454 and

standard metformin is -3.39 with the receptors. **Conclusion:** The work establishes the isolated compounds from the fraction *of S. tetragonum* as a potential source for diabetes mellitus thus enabling a possibility of this plant as new alternative to existing diabetic approaches.

KEYWORDS: SGLT 2, FOXO1, Glycogen Synthase *Stereospermum tetragonum.*

INTRODUCTION

Diabetes mellitus (DM) is one of the major public health problem which is encountered in the modern society, with increasing incidence and prevalence worldwide.[1,2] Among different types of diabetes mellitus, Type 2 diabetes accounts for 90-95% of adult diabetes.[3] In healthy individuals, the glucose homeostasis is tightly maintained by closely regulating glucose production, reabsorption, and utilization.[4] In people without diabetes, about 180 g of glucose is filtered daily by the renal glomeruli, and is then reabsorbed in the proximal convoluted tubule (PCT). Due to reabsorption, glucose in the urine is either absent or present at very low concentrations (0.03 to 0.30 g/dL).[5] This is achieved by passive transporters, namely, facilitated glucose transporters (GLUTs), and active cotransporters, namely, sodium-glucose cotransporters (SGLTs). There are six identified SGLTs, of which two (SGLT1 and SGLT2) are considered most important.[6] The sodium and glucose linked transporter (SGLT) is a sodium/glucose cotransporter membrane protein. Type 2 (SGLT-2) is present in segment 1 of the proximal convoluted tubule and is the main glucose transporter, whereas type 1 is found in segment 3 of the proximal convoluted tubule and the small intestine.[7]

In liver, insulin resistance increases glucose production because of an impaired ability of insulin to suppress the expression/activity of gluconeogenic enzymes. Fasting-induced gluconeogenesis prevents hypoglycaemia, while the loss of insulin-dependent suppression of glucose production in diabetes causes fasting hyperglycaemia.[8] Forkhead transcription factors of the FoxO subfamily (FoxOs) regulate metabolism, proliferation, and differentiation[9] In liver, FoxO1 acts in concert with PPARγ coactivator 1α (Pgc1α) to stimulate glucose production through glucose-6-phosphatase, catalytic (G6Pc) and phosphoenolpyruvate carboxykinase 1 (Pck1), in cooperation with the cAMP/Creb pathway.[10]

Glycogen synthaseis the rate-limiting enzyme in glycogen biosynthesis, which catalyses the elongation of 1,4-or- linked glucose chains. Glycogenin, a self-glycosylating protein acts as a

primer upon which the glycogen molecule is constructed.[11] Glycogen synthase is considered to exist in two inter- convertible forms, synthase b (or D) and synthase a (or I). The b form of glycogen synthase which is more phosphorylated has little activity in the absence of G-6-P and is less likely to be active in vivo. Synthase a which is less phosphorylated does not depend on G-6-P for its activity. This form of glycogen synthase is considered to be fully active in liver cells. Glycogen synthase activation during in vivo studies is dependent on many factors such as the route of administration of the meal, diurnal variations in glycogen metabolism, composition of the meal and the use of anaesthesia.[12]

MATERIALS AND METHODS

Collection of plant materials

The root parts 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.

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]

Isolation of an 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 were used for elution of two compounds. Based on IR, H^1NMR, ^{13}C NMR and Mass spectrum the compound was 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 (Figure 1) 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 SGLT 2, FOXO1, Glycogen Synthase activity were obtained from Protein Databank (PDB): PDB ID:2WLK, 2B4s, 2Y7J.

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 SGLT 2, FOXO1, Glycogen Synthase activity 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 SGLT 2, FOXO1, Glycogen Synthase 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[18,19,20] of the Schrodinger suite.

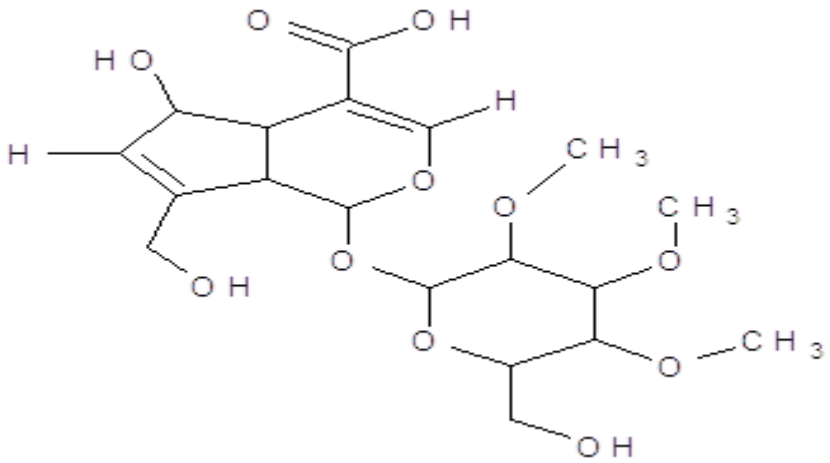
RESULTS AND DISCUSSION

Present studies provide scientific evidence that bioactive molecules C1 and C2 Strongly interact with SGLT 2, FOXO1, Glycogen Synthase through different residues Figure 3 shows the XP Glide score for both the compounds for **SGLT 2** (-8.765 Kcal/mol for C1 and -7.0569.425 Kcal/mol for C2) which clearly suggests that both the compounds show better interaction than metformin -1.711.The docking of C1 and C2 with sglt2 which in turn prevents reabsorption of glucose in renal tubules and thus contributes to lowering of the blood glucose level. Figure 4 shows the XP Glide score for both the compounds for FOXO1**,** and were -4.377 Kcal/mol for C1 and -2.715 Kcal/mol for C2. This clearly suggests that both the compounds show better interaction than metformin (Table.1). thus, the compounds could, reduce the glucagon and glucagen like peptide-1 expression thus leading to the lowering of blood glucose. The XP Glide score for both the compounds for Glycogen Synthase is -7.558 Kcal/mol for C1 and -5.454 Kcal/mol for C2 (Figure 5). This clearly suggests that docking of both the compounds with Glycogen Synthase might have activated the target protein

facilitating the storage of excess glucose in to glycogen and thus contribute in lowering of blood glucose (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.

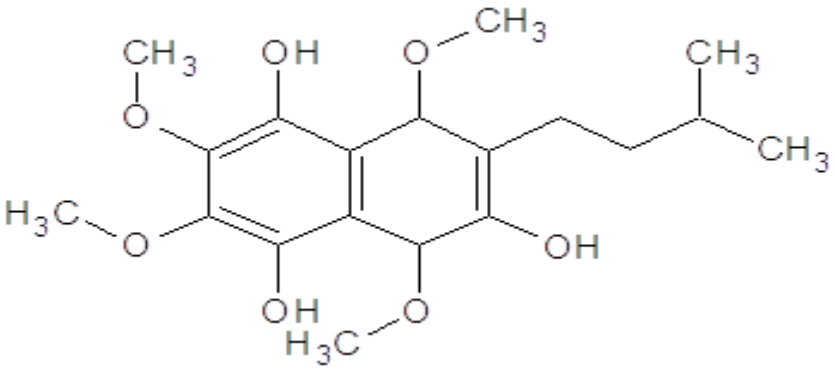
Table 1: Glide Score of compound 1, compound 2 and standard.

DOCKING STUDIES							
Sl.No	Name of the protein	PDB ID No.	Site Volume		Glide score		Standard
			C1	C2	C1	C2	Metformin
1	SGLT 2	2XQ2	763.518	763.518	-8.765	-7.056	-1.711
2	FOXO1	3COX 6	41.503	41.503	-4.377	-2.715	-0.381
3	Glycogen Synthase	3ZRL	332.024	332.024	-7.558	-5.454	-3.39



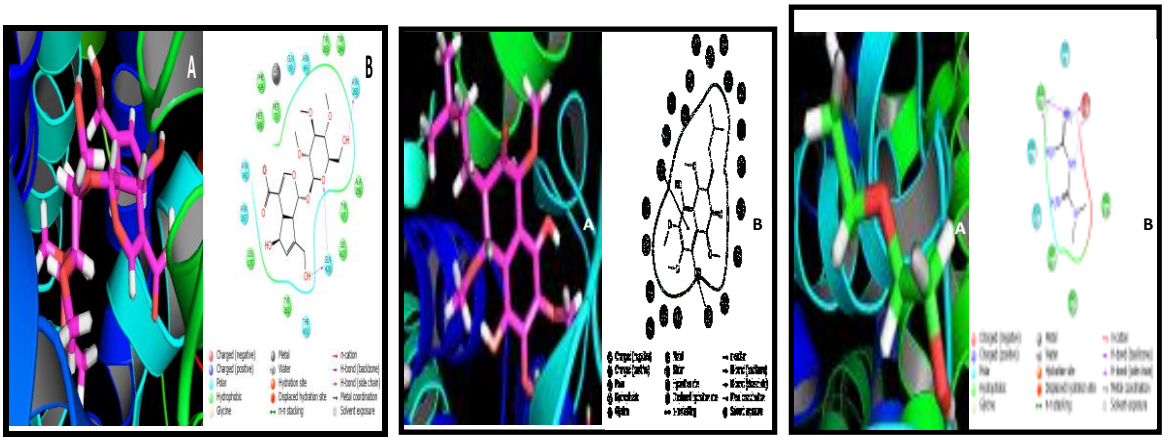
1,4a,5,7a-tetrahydro-5-hydroxy-7-(hydroxymethyl)-1-(tetrahydro-6-(hydroxymethyl)-3,4,5-trimethoxy-2H-pyran-2-yloxy)cyclopenta[c]pyran-4-carboxylic acid

Figure 1: Structure of compound 1 (C1).



5,8-dihydro-7-isopentyl-2,3,5,8-tetramethoxynaphthalene-1,4,6-triol

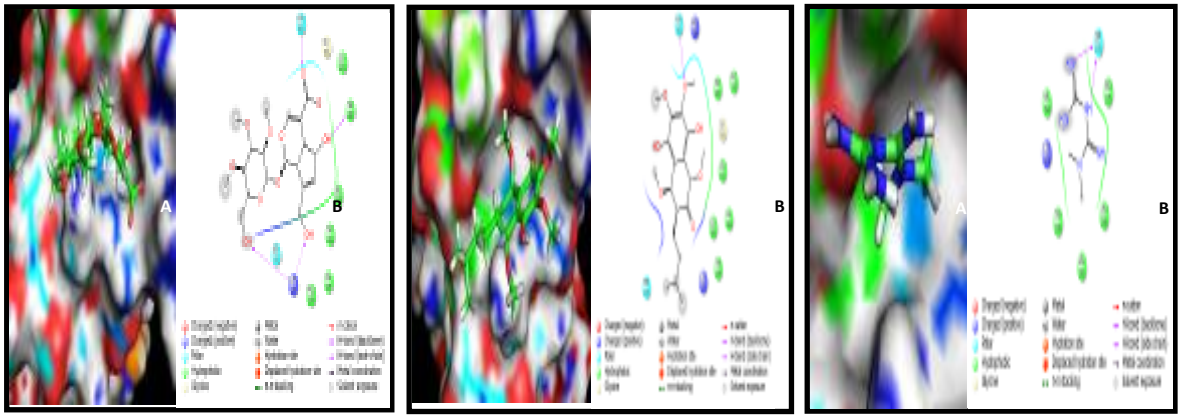
Figure 2: Structure of Compound 2 (C2).



C1 C2 METFORMIN

A. Surface view, B. Ligand interaction diagram

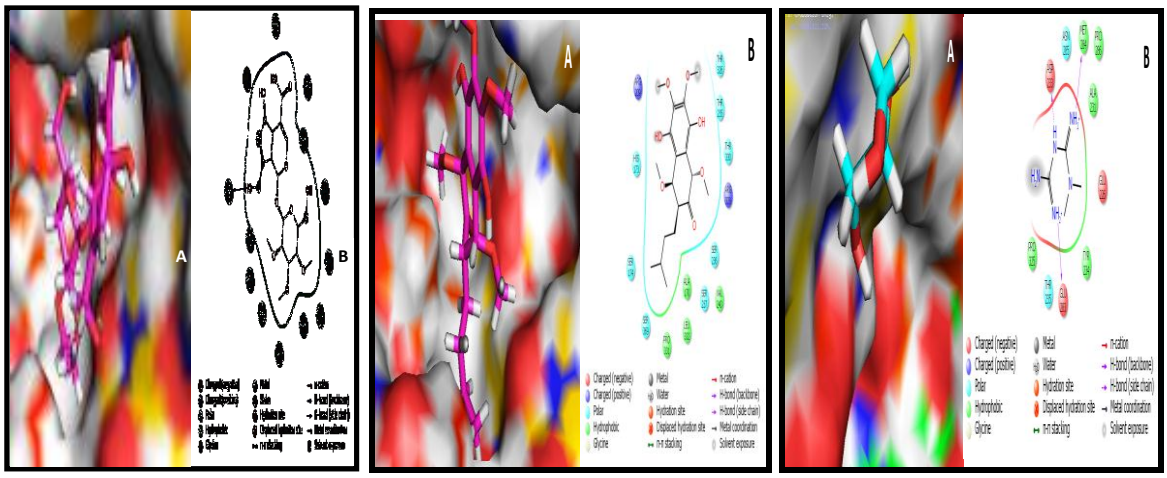
Figure 3: Interaction of compound 1, 2 and metformin with SGLT2.



C1 C2 METFORMIN

A. Surface view, B. Ligand interaction diagram

Figure 4. Interaction of compound 1, 2 and metformin with FOXO-1.



C1 C2 METFORMIN

A. Surface view, B. Ligand interaction diagram

Figure 5. Interaction of compound 1, 2 and metformin with Glycogen synthase.

CONCLUSION

The results of the above investigation shows that 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.

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CONFLICTS OF INTEREST

All authors have none to declare.

ABBREVIATION USED

TLC: Thin layer Chromatography IR: Infrared; NMR: Nuclear magnetic resonance.

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