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Anti-diabetic potential of *Asparagus racemosus* and docking studies of its active components

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Abstract
The incidence of type 2 diabetes mellitus is tremendously increasing across the globe, especially in India, which is often known as the diabetic capital of the world. As diabetes is a chronic disorder, there is an urgent need to expand the range of effective, safe, and cost-efficient treatments for long-term use. Vascular plants such as *Asparagus racemosus*, commonly known as Shatavari, provide therapeutic opportunities and a rich source of potential anti-diabetic drugs. In the present study, receptor proteins associated with carbohydrate and lipid metabolism (nuclear receptor ROR alpha, aldo-keto reductase 1 family B10, pancreatic alpha-amylase, alpha 2A adrenergic receptor, and cyclic dependent kinases 4 and 6) were selected and interaction studies were performed with *A. racemosus* using Autodock Vina 1.5.6 software. Phytosterols present in Shatavari showed a high affinity for RAR-related orphan receptor (-11.0 kcal/mol). Other active components, namely vitamin P, Isoquercetin and miquelianin showed affinity towards aldo-keto reductase family 1 B10 receptor (-9. -7.3, -7.3 kcal/mol, respectively) and pancreatic alpha amylase (-10, -8.5, -8.8 kcal/mol, respectively). Shatavarin I and shatavarin IV, which are the major steroidal saponins, showed affinity for cyclin dependent kinases. Maximum affinity was shown by shatavarin IV towards cyclin dependent kinases 4 and 6 (-9.1 kcal/mol). These phytoconstituents may be isolated and used for preparing anti-diabetic drugs. A variety of aldose reductase inhibitors are already in use for treatment of diabetic complications. The use of pancreatic alpha amylase inhibitors from natural sources could also be a possible way to block absorption of dietary carbohydrates. An insight into multiple underlying pathophysiologic processes will offer a possibility of controlling diabetes and its complications.

Keywords: Docking, shatavari, *Asparagus racemosus*, diabetes, drug discovery

1. Introduction
The prevalence of diabetes mellitus is increasing at an astronomical rate worldwide. According to International Diabetes Federation (IDF), 463 million people are living with diabetes in the world. and by 2045, these figures are expected to rise to 700 million. India is home to 77 million diabetics, next only to China which leads the list with over 116 million diabetics [2]. Diabetes killed 1.7 million people in 2016, making it the seventh leading cause of global deaths [35]. Diabetes mellitus is a metabolic disease that impairs the body's ability to process blood glucose, resulting in high blood sugar levels. There are three major types – Type 1, Type 2, and gestational diabetes. Type 1 diabetes mellitus (T1DM) or juvenile-onset diabetes is results from the destruction of insulin-making pancreatic cells, called beta cells. Historically, T1DM has been viewed as an autoimmune disease, but new evidence suggests beta-cell heterogeneity may be involved in disease progression [32, 24]. Another condition, similar to T1DM, is caused when the beta cells are destroyed by a disease or an injury to the pancreas, rather than by the immune system. Type 2 diabetes mellitus (T2DM), also known as adult-onset diabetes, occurs either when the body does not produce enough insulin, or it resists insulin. Young-onset T2DM is a more lethal form of diabetes and is associated with more complications and cardiovascular disease risk factors as compared to T1DM [4]. Since T2DM is much more common than T1DM, it is often just called "diabetes".
Changes in lifestyle over the last century, such as reduced physical activity have resulted in a dramatic increase in the incidence of diabetes worldwide. Since it is a life-long disease, the rationale behind its treatment is not just the control of hyperglycemia but also the prevention of long-term complications. In developing countries like India where the cost of treatment is prohibitive, there is a need for anti-diabetic agents of natural origin. Traditional medicines derived from medicinal plants are safer for long-term use and are also cost-efficient.

Asparagus racemosus, commonly known as Shatavari, is one such plant with recorded therapeutic applications in Indian and British Pharmacopoeias and in traditional medicine like Ayurveda, Unani and Siddha. Although the hypoglycemic effect of some herbal extracts has been studied in human and animal models of type 2 diabetes, the mechanism of action for the majority of these remains unexplained as the interaction between the active component of the herb and receptors is yet to be understood clearly. Moreover, medicinal plants have not gained much importance because of a lack of scientific evidence.

Asparagus racemosus (Fig. 1a, 1b) belongs to the family Asparagaceae. Genus Asparagus is of medicinal importance because of the presence of steroidal saponins and sapogenins in various parts of the plant [21]. The major bioactive constituents of Asparagus are a group of steroidal saponins known as shatavarin. Shatavarin I and shatavarin IV are the major saponins present in roots, leaves and fruits of Asparagus species [11]. Roots of *Asparagus racemosus* contain shatavarin I to X, schidigerasaponin [12], shatavaroside A, shatavaroside B, filiasparoside C [28], phytosterols, triterpinoids, alkaloids, flavonoids, tannins, glycosides, and lactones [26]. Many flavonoids are present in flower, fruit, and leaf of *A. racemosus*, some of which are kaemferol, quercetin, rutin, hyperoside, quercetin-3-glucunoride, cyanide-3-galatodise [30]. Quercetin-3-glucunoride is also known as 'miquelianin'. Three steroidal saponins, racemoside A, B and C, are present in the fruits of *A. racemosus* [17]. Various studies conducted on experiment animals show that Shatavari has antioxidant activity [14], antitussive propertie [18], gastroduodenal ulcer protective properties [27], antidiarrheal activity [34], anticandidal property [33], antiurolithic property [13], anticancer activity [19] and antihyperglycemic activity [9].

Asparagus racemosus may be useful as a source of novel antidiabetic compounds or a dietary adjunct for the management of diabetes [9]. The present study is aimed at identifying bioactive constituents of *Asparagus racemosus* that may play a role in controlling hyperglycemia by their affinity towards receptors that are involved in T2DM pathogenesis. Anti-hyperglycemia activity of *A. racemosus* is partly mediated by the inhibition of carbohydrate digestion and absorption, together with the enhancement of insulin secretion and action in the peripheral tissue [10]. In the present study, molecular docking was performed between active components of this plant with receptors that play a role in carbohydrate and lipid metabolism. The *in-silico* approach utilized in this study can further be investigated to generate more effective phytomedicines through a ligand-based designing approach.

2. Materials and Methods

2.1 Preparation of ligand

List of ligands present in *A. racemosus* was obtained from IMPPAT, a database on medicinal Indian plants containing more than 9500 phytochemicals [20]. Compound ID number for phytochemicals were collected from IMPPAT database along with their 3D structures (SDF and PDB) and canonical SMILE. Canonical SMILES obtained from IMPPAT were used for this purpose to evaluate the phytochemical properties of the obtained phytochemicals using the SwissADME database [5]. Phytochemicals given in Table 1 were selected for further evaluation.

2.2 Preparation of Binding Site

Canonical SMILES and 3D SDF files were utilized for the prediction of binding sites using 'Swiss Target Prediction' webserver [7] and 'Binding DB' [8]. Target sites obtained from Swiss Target Prediction and Binding DB were compiled on an Excel worksheet. The target sites were individually studied and only those receptors that played a role in carbohydrate and lipid metabolism were selected. Uniprot DB was used to obtain information about 3D structures of selected binding sites, namely, retinoic acid receptor-related orphan receptor (ROR) alpha, aldo-keto reductase 1 family B10, pancreatic alpha amylase, and cyclic dependent kinases 4 and 6 (Table 2). PDB files for these receptors were downloaded from RCSB protein data bank [25] and prepared for molecular docking by removal of water molecules, heteroatoms, any side chains using Discovery Studio version 4.0.

2.3 Docking Simulation

Auto Dock Vina (Version 1.5.6.) was used for molecular docking. Autodock tool was applied to build a complete pdbqt file name of ligands and receptors. Receptor preparation was carried out by four major steps, *viz.* addition of polar hydrogen, removal of water molecule, addition of Kollman charges and location of grid box. For setting the ligand, the 3D structure in PDB type file was loaded into Autodock tool to detect the root and convert it to pdbqt. Size of grid box was set in 60X60X60 points and number of modes were 10. Affinity scores were generated.

2.4 Analysis of docked complexes

The docked complexes were analysed using BIOVIA Discovery Studio version 4.0. The number and length of hydrogen bonds in the complexes and the interacting residues of proteins were analyzed.

3. Result and Discussion

Phytosterols present in Shatavari showed high affinity towards nuclear receptor ROR alpha (-11.0 kcal/mol). Other active components, namely vitamin P, Isoquercetin and miquelianin showed affinity towards aldo-keto reductase family 1 B10 receptor (-9. -7.3, -7.3 kcal/mol respectively) and towards pancreatic alpha amylase (-10, -8.5, -8.8 kcal/mol respectively). Shatavarin I and shatavarin IV, which are the major steroidal saponins, showed affinity for cyclin dependent kinases. Maximum affinity was shown by shatavarin IV towards cyclin dependent kinases 4 and 6 (-9.1 kcal/mol). The affinity of active components towards alpha 2A adrenergic receptor was the lowest.

Anti-hyperglycemic effect of *A. racemosus* could be mediated through pathways involving the above studied receptors. Belonging to the aldo-keto reductase superfamily [1], aldo keto reductase family 1 B1 receptor plays a role in conversion of glucose to sorbitol in polyol pathway. Sorbitol accumulation in lens leads to formation of cataract, which is a major complication caused by diabetes. It has been proven that aldose reductase initiates the cataractous process in diabetic animals [15]. Many aldose reductase inhibitors have been found to control diabetic peripheral neuropathy, retinopathy and cardiomyopathy in various animal models and human clinical trials [36, 23, 22, 29].

Pancreatic alpha amylase is an important digestive enzyme that catalyzes the hydrolysis of starch. If pancreatic alpha amylase is inhibited, then postprandial hyperglycaemia can be controlled through control of starch breakdown [6].

Retinoic acid receptor-related orphan receptor (ROR) alpha, beta and gamma are a subfamily of nuclear receptors that are

involved in lipid and glucose homeostasis. ROR alpha has the typical structure of a nuclear receptor – a ligand-binding domain linked to a DNA-binding domain via a hinge region and an N-terminal region that modulates its transcriptional activity. ROR alpha controls the transcription of the genes encoding glucose 6-phosphatase, which is important in regulation of glucose metabolism. ROR alpha expression modulates insulin gene transcription through action on ROR responsive elements (RORE) site and regulates insulin expression indirectly by stimulating expression of BETA2 [16], which is an active transcription factor of insulin gene. ROR gamma has also been identified as a negative regulator of adipocyte differentiation and a modulator of obesity-associated insulin resistance [31].

Cdk 5-mediated phosphorylation of PPAR gamma (peroxisome proliferator-activated receptor gamma) may be involved in the pathogenesis of insulin resistance and present an opportunity for the development of an improved generation of anti-diabetic drugs through PPAR gamma [3].

The strength of hydrogen bonds can be interpreted according to the Brown and Blessing criteria of relating to the donor-acceptor distance. If donor to acceptor distance is > 2.73Å, the hydrogen bond is considered weak, whereas if the donor-to-acceptor distance is < 2.73Å, the hydrogen bond is considered strong. In the present study, the hydrogen bond lengths ranged from 1.81 to 4.30 (Table 3).

Fig 1(a): Leaves of *Asparagus racemosus*

Fig 1(b): Flowers of *Asparagus racemosus*

Table 1: List of phytochemicals selected for further study from *Asparagus racemosus*

Ligand	Pub Chem Identifier	3-D Structure
Phytosterols	12303662	
Isoquercetin	10813969	
Miquelianin	12004528	
Shatavarin I	101406647	
Shatavarin IV	441896	

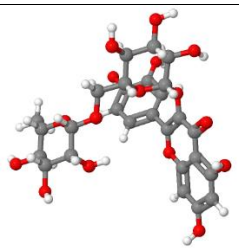
Vitamin P	24832108	

Table 2: Binding sites of phytochemicals of *Asparagus racemosus*

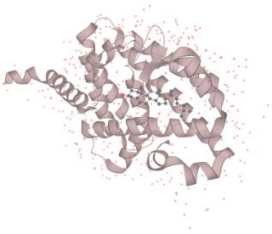
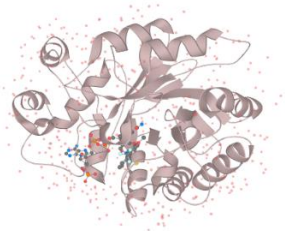
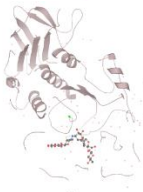
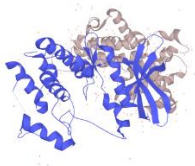
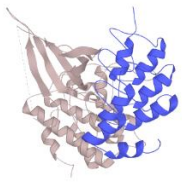
Protein Name	Synonyms	Uni Prot KB ID	3D Structure
Nuclear Receptor ROR alpha	RAR related orphan receptor A, Retinoid-related orphan receptor- alpha	P35398	
Aldo-keto reductase family 1 member B10	Small intestine reductase, Aldose reductase related protein	060218	
Pancreatic alpha amylase	1,4-alpha-D-glucan glucanohydrolase	P04746	
Alpha 2A adrenergic receptor	Alpha 2 adrenergic receptor subtype C10	P08913	
Cyclin dependent kinase 4	Cell division protein kinase 4	P11802	
Cyclin dependent kinase 6	Cell division protein kinase 6	Q00534	

Table 3: Description of hydrogen bonds and their interaction between ligand and receptor

Ligand and receptor	Number of H-bonds	Interacting residues and H-bond length
Vitamin P and pancreatic alpha amylase	6	LYS:200 (3.05), TRP:59 (2.63), GLN:63 (2.18), ASP:300 (1.81 and 2.85), ARG:195 (2.82), GLU:233 (2.30)
Vitamin P and alpha 2A adrenergic receptor	6	LYS:27 (2.76), THR:29 (2.23), ARG:31 (2.18, 2.58, 2.97)
Vitamin P and aldo keto reductase family 1 member B10	11	ARG:269 (2.85), LYS:263 (2.30, 2.31, 2.50, 2.57, 2.38), SER:23 (2.47, 3.35), ASP:217 (2.91) and LYS:22 (2.32, 2.44)
Isoquercetin and pancreatic alpha amylase	2	ASP:197 (2.59), HIS: 305 (2.69)
Isoquercetin and alpha 2A adrenergic receptor	4	GLU:23 (2.32, 2.67), ARG (2.96, 1.84)
Isoquercetin and aldo keto reductase	2	TYR:310 (2.24, 2.28)
Miquelianin and pancreatic alpha amylase	6	GKY:306 (2.12), GLU:233 (2.56), ASP:300 (2.09), HIS:101 (3.04), TYR:62 (2.81) and GLN:63 (2.48)
Miquelianin and alpha 2A adrenergic receptor	4	LYS:27 (2.72), GLU:23 (2.29), ARG:31 (2.89), ARG:28 (4.30)
Miquelianin and aldo keto reductase family 1 member B10	8	ARG:297 (2.56), ASN:300 (2.49, 2.56), GLN:303 (2.78, 2.87), ASP:225 (2.01), TRP:296 (2.66), ASN: 295 (2.01)
Phytosterol and RAR related orphan receptor	1	GLN:286 (2.25)
Shatavarin IV and cyclin dependent kinase 6	2	ASP:163 (2.03), ALA:23 (2.41)
Shatavarin IV and cyclin dependent kinase 4	3	ASN:352 (2.13), ASN:351 (2.31), ARG:378 (1.85)

4. Conclusion

Antidiabetic potential of *A. racemosus* is mediated through interaction of its phytochemicals with receptors that mediate lipid and fat metabolism. Phytochemicals derived from this plant may be used to formulate anti-diabetic medication. Further studies need to be conducted to understand the mechanism of action behind anti-hyperglycemic effect of *A. racemosus.* An insight into multiple underlying pathophysiologic processes will offer a possibility of controlling diabetes and its complications.

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