EXPLORING ANTI-DIABETIC COMPOUNDS FROM THE ETHANOLIC EXTRACTION OF STRYCHNOS POTATORUM SEEDS: LIGAND- BASED DESIGN, MOLECULAR DYNAMICS

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ABSTRACT

In this classical lifestyle, people are mostly affected by various diseases. The 2:3 ratios of men and women are affected by diabetics because of food habits. Currently, it is necessary to identify the particular compounds for diabetics. Type 2 diabetes mellitus is a multi factorial, multi systemic chronic disorder characterized by insufficient insulin secretion. S. potatorum seeds have long been utilized in traditional medicine to treat a variety of non-communicable disorders, including diabetes. S. potatorum is a widely utilized plant used in ayurvedic medicine. The seeds of S. potatorum are utilized in the treatment of diabetics and it contains numerous bio-active compounds such as alkaloids, which have valuable outcomes for the treatment of patients with various sicknesses in India. In this present study, an effort will be made to confirm the presence of anti-diabetic compound in the ethanolic extract of S. potatorum by using TLC and GC-MS. Molecular docking will be done with the activated protein kinase (6YYE & 6Y6C) & GC-MS results of the S. potatorum compounds. PyRx software will be used for docking studies. The anti-oxidant and the anti-bacterial activity of the ethanol extract also done for identifying the anti-diabetic compounds.

Keywords: Diabetics, *Strychnos potatorum*, molecular docking.

1. INTRODUCTION

In the next ten years, diabetes will become a major threat. Diabetes mellitus affects at least 30 million people around the world at any given time. Diabetes is one of the most common diseases, afflicting millions of individuals worldwide. The problem is expected to worsen in the coming year as a result of food addiction and the prevalence of sedentary lifestyles in India. While diabetes is not the most lethal disease, it is a main cause of adult blindness, kidney failure, neuropathy, heart attacks, and strokes. Long-term headaches, abnormal insulin and glucagon secretions, massive disruptions in carbohydrate, protein, and lipid metabolism, thickening of capillary basement membrane via the main frame to Microangiopathy and macroangiopathy, and thickening of capillary basement membrane via the main frame to Microangiopathy and macroangiopathy, and thickening of capillary basement membrane via the main frame to Microangiopathy and macroangiopathy, and thickening of capillary The eye, kidney failure, and the circulatory system will all be affected by abnormal secretions and insulin and glucagon actions [1]

There are two forms of diabetes: type 1 and type 2. Diabetes insipidus is a condition in which the urine has a low specific gravity and contains a large quantity of ADH deficiency. A tumour in the posterior pituitary is generally to blame, however it can also be idiopathic. Diabetes mellitus is a metabolic disorder marked by a high blood glucose level. It's not a matter of not having enough insulin or having insulin that isn't working properly. There are two forms of diabetes. One is insulin-dependent diabetes mellitus, whereas the other is insulin-independent diabetes mellitus.

At this time, there is no drug that can completely cure diabetes. Diabetes can be managed by following a healthy diet and exercising on a regular basis. The use of hypoglycemic tablets relieves the headaches associated with diabetes, which is a critical and life-saving situation. These, on the other hand, aren't therapeutic and have a slew of negative side effects. As a result, in recent years, searching for anti-diabetic energy components in plants has become more essential [2].

Strychonos potatorum Linn (Loganiaceae), also known as katakam in Ayurveda and Tettankottai in Tamil. It's a little tree that grows in India's southern and central regions, as well as Sri Lanka and Burma. Emetic, diaphoretic, and alexiteric, with inflammation, anaemia, jaundice, and biliousness as therapeutic options, the ripe fruit is emetic, diaphoretic, and alexiteric [3]. *S. potatorum* seed is used as a stomachic demulcent emetic and for acute diarrhoea, diabetes, gonorrhoea, and eye problems such as conjunctivitis, lachrymation, or copious watery eyes. Since the Rigveda (5600 BC), where over 67 medical works were documented, medicinal plants have played a significant role in Indian culture [4]. Traditional medications are used by 80 percent of the world's 4 billion people due to high costs, a lack of viable therapies, and personal preferences [5]. India is one among the world's 12 biodiversity hotspots, with more than half of advanced shops having medicinal potential. Traditional societies are thought to use over 7500 species, with about factories having good therapeutic potential [6].

The present study aims at identifying the pharmacological compounds of *Strychnos potatorum*, which is effective against diabetics through the phytochemical screening, TLC (thin layer chromatography) & Molecular Docking using the GC-MS result by PyRx software. Antimicrobial activity and antioxidant activity will be checked by using bacterial culture.

2. METHODOLOGY

The seeds of *S.potatorum* seed were collected from local market, Ramanathapuram, Tamilnadu, India and the transported seeds are crushed with mortar and pestle the powder is stored in the laboratory for the further research. Fresh crushed seeds of *S. potatorum*10 g was taken and dissolved in 100 ml of ethanol. The solution was stirred in the magnetic stirrer for 30 minutes. Then the extract was filtered in the Whatman no:1filterpaper and then stored in the room temperature for the further research.

Thin layer chromatography techniques performed to purify substance present in *S. potatorum* seed extracts partially. Antibacterial activity of *S. Potatorum* was tested against pathogenic bacteria such as *Escherichia coli*. The antibacterial activity of ethanol extract utilising *S. Potatorum* seed extract was investigated on nutritional agar using the disc diffusion method. Test pathogens were spread on nutritional agar for bacteria test plates using sterile swabs. Before being placed on the agar plate's surface, the disc was dipped in the sample. To see if there was a zone of inhibition, the test plates were incubated for 24 hours.

Total Antioxidant Capacity assay: According to the procedure, the total antioxidant capacity of the sample extract was determined using the phosphomolybdenum method. A sample of 5 to 10 grammes was obtained, and extraction was carried out with the appropriate solvent.0.3 mL of extract was combined with 3 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). In tubes, the reaction solution was incubated at 95°C for 90 minutes. The absorbance of the solution was measured at 695 nm using a UV-VIS spectrophotometer against a blank after cooling to room temperature. In place of extract, 0.3 mL of solvent was utilised as a control.In gram equivalents of ascorbic acid, the overall antioxidant activity is evaluated. The calibration curve has finally reached its conclusion. Ascorbic acid (1000, 500, 250, 125, 62.5, and 31.25 g/mL) was mixed with solvent to create the calibration curve.

GC-MS analysis: Qualitative and quantitative analysis of phytochemicals can be done using Gas Chromatography Mass Spectroscopy (GCMS). GCMS can be applied to solid, liquid and gaseous samples. First the samples are converted into gaseous state then analysis is carried out on the basis of mass to charge ratio [7].

The ethanolic extract of S. potatorum seeds was GC-MS analysed using an Agilent GC 7890 with a triple axis 5975 MS detector. Agilient HP-5MS (30m x 250m x 0.25m) was the capillary, which was made up of 5% phenyl methyl silox. The initial oven temperature was 55 C for 0 minutes, then 10/C min up to 200 C for 0 minutes, then 5C/min up to 260 C for 5 minutes of hold time. The injector had a 10 litre capacity. The source temperature was 250°C (maximum 300°C), the quad temperature was 150°C (maximum 200°C), and the solvent delay period was 3 minutes. The NIST search library was used to identify compounds based on their RT values and mass spectra.The obtained compounds were searched for detailed pharmacological activities.

Docking studies of *s. Potatorum* compounds with 6yye &6y6c protein: Totally 66 bioactive compound which are belongs to *S.potatorum*, obtained by theliterature study. Those

compounds were revealed by the GC-MS analysis. The crystal of 6YYE &6Y6Cprotein structure was retrieved from Protein Data Bank RCSBPDB (http://www.rcsb.org/pdb/home/home.do)Then the PDB format of protein was subjectedfor docking studies.

Molecular docking: Sixty six ligands were used against the protein 6YYE &6Y6C. These compounds have been studied. Protein and ligands were converted into special file format PDBQT. Ligand preparation included the following steps (i) addition of hydrogen atoms, (ii)neutralization of the charge groups and (iii) removal of any miscellaneous structures from the ligand. Prepared and optimized structures of ligands were used for docking simulation. Grid values for protein the grid point set at 80 x 60 x 95. Before starting the docking study, Vina wizard software was used to perform molecular docking in PyRx virtual screening tool. Discovery studio visualizer I was used to examine the docking poses of the complexes [8].

Lipinski's rule: Lipinski's rule of 5 was developed by Christopher A. Lipinski in 1997, this rule wasalso called as Pfizer's rule of five or simply the rule of five (R05). This rule was developed toset "draggability" guidelines. In the drug discovery setting, the rule of 5 predicts that poorphysiochemical and structural properties within certain ranges [9].

3. RESULTS AND DISCUSSION

Phytochemicals in medicinal plants have been reported to be the active principles responsible for the pharmacological potentials of plants [10]. Preliminary phytochemical analysis of ethanol extracts of *S. Potatorum*indicated the presence of certain metabolites in a different manner (Table 1). Reports were revealed the identification of major chemical constituents such as alkaloids, protein, coumarin, carbocyclic acid, fixed oils, fats, carbohydrate, gums and mucilages and rest of the metabolites were absent such as Phenolics, flavonoids, quinones, anthraquinone, phlobatannin, glycosides, amino acids, and cardiac glycosides.

Table 1: Phytochemical analysis of *S.potatorum*

S.No	Phytochemical test	Results
1	Alkaloids	+
2	Phenols	-
3	Coumarins	+
4	Terpenoids	-
5	Quinones	-
6	Anthraquinones	-
7	Tannins	-
8	Phlobatannins	-
9	Carbohydrates	+
10	Glycosides	-
11	Cardiac glycosides	-
12	Proteins	+
13	Aminoacids	-
14	Steroids	-

15	Phytosteroids	-
16	Saponins	-
17	Acids	-
18	Carbocyclic acids	+
19	Fixed oils	+
20	Fats	-

The presence of alkaloids, protein, coumarin, carbocyclic acid, fixed oils, fats,carbohydrate,gums and mucilages. Compare to my results, Flavonoids, carbohydrates, and titerpenes were discovered in chloroform extract, carbohydrate in ethyl acetate extract, glycosides, flavonoids, saponin, carbohydrate, proteins, amino acids, and diterpenes in ethanol extract [11].

The ethanolic extract of the *S.Potatorum* seeds was used to done the TLC. The result of the TLC place is given table 2.

Table 2: TLC analysis of *S.potatorum*

Sample	**RF VALUE**
S.Potatorum seed ethanol extract	0.1538
	0.0473
	0.3846
	0.4615
	0.5384

Tannin and flavonoids are present. compare to my results, The approach was used to separate the lipids on silica gel plates. The lipid-containing portions of the gel were agitated with 1 to 9 ml dichromate reagent, 2.5 g K2Cr2O7 in 1 litre 36 N H2SO4, and the solutions were boiled in boiling water for 45 minutes. The absorbance was measured at 350 m after chilling and centrifuging 0.5 ml supernatant solution with 20 ml water. It is possible to estimate as little as 15 g of lipid. The presence of phenolic components was detected in the test sample based on the Rf value [12].

The ethanol extracts of *Strychnos potatorum* showed antibacterial activity against pathogenic gram positive, gram negative, and acid-fast bacteria. *S. potatorum* seed ethanol extract showed maximum antibacterial activity against *Escherichia Coli.* Compare with current results [13] examined the antimicrobial activity in the alkaloid fractions of seeds of *Strychnos potatorum* which exhibited considerable activity against the tested bacteria and fungi. Inhibition of gram-positive bacterium *Staphylococcus aureus* and gram-negative bacteria *Proteus*.

By using a UV-VIS Spectrophotometer, the total anti-oxidant activity of *S. potatorum* seed ethanol extract was found to be 275 g/TAE mL-1 extract. In comparison to my findings, total antioxidant activity of SPP and SPE was determined using FTC and TBA procedures and compared to vitamins E and C.The drugs and standards in the FTC approach showed low absorption values as compared to the control. SPP exhibited the least increase in absorbance,

followed by vitamin E, vitamin C, and SPE. The TBA technique was used to determine the total peroxide levels produced by the oxidation of linoleic acid. The higher the absorption levels, the poorer the antioxidant activity. The absorbance values of SPP, vitamin E, vitamin C, and SPE were all greater than the control. The results were the same as those obtained using the FTC method [14].

GC-MS analysis was performed on an ethanolic extract of S. Potatorum seeds. The GC-MS analysis of an ethanol extract of S. potatorum seed extract revealed the following peaks (graph 1), indicating the presence of different phytoconstituents. The mass spectrum GC-MS interpretation was done with the help of a database (NIST) (Sathyaprabha,G et al.2010). Sixty six bio active compounds in the GC-MS results.Compare to my results,

Only a few of the bio compounds present have known therapeutic properties. Ethyl acetate, 3-Trifluoroacetoxypentadecane, Dodecane, 1,2-dibromo-, 4-Trifluoroacetoxypentadecane, Trichloroacetic acid, pentadecyl ester, Trichloroacetic acid hexadecyl ester, E14 Hexadecenal, E-11,13-Tetradecadien-1-ol There are a few additional molecules that are present in little amounts but could play a large or minor role in medicine. More research is being done to assess these compounds and correlate their activities to Katakakhadiradi Kashayam in order to better understand the medicine's mechanism of action as well as other pharmacological aspects.

The molecular docking was done using the proteins (6Y6C & 6YYE)and the ligands of GC-MS results by the PyRx software.

Table 3: Binding affinity analysis of 6Y6C Protein

PROTEIN	PUBCHEM ID	PROBABLE COMPOUND	BINDING AFFINITY
6Y6C	536772	Imidazole, 2-fluoro-1-triacetylribofuranosyl	-6.6
	574859	Dispiro[1,3-dioxolane-2,2'-bicyclo[2.2.1]heptane-3',2"-(1",3"-dioxolane)], 4',7',7'-trimethyl	-6.2
	590903	Phosphine, (myrtenoyl)diphenyl	-7
	45382147	2-Pyridinecarbonitrile, 3-nitro	-6.1
	102070384	Bicyclo[2.2.1]heptan-2-ol, 1,3,3-trimethyl	-6.4
	66831472	Thiophene, 2,5-dihydro-	-7.7

The docking studies against 6Y6C revealed in GC-MS analysis of bioactiveCompounds such as Imidazole, 2-fluoro-1-triacetyl ribofuranosyl, Dispiro [1,3-dioxolane-2, 2'-bicyclo[2.2.1] heptane-3',2"- (1",3"-dioxolane)], 4',7',7'-trimethyl, Phosphine, (myrtenoyl) diphenyl,2-Pyridine carbonitrile, 3-nitro, Bicyclo [2.2.1] heptan-2-ol, 1,3,3-trimethyl, Thiophene, 2,5-dihydro-, showed the docking binding energy of -6.6,-6.2,-7,-6.1,-6.4,-7.7 Kcal/mol respectively. Thiophene, 2, 5-dihydro-have good anti-diabetic activity against 6Y6C protein. This study identified anti-diabetic compounds from the ethanol extract of *S.Potatorum* seeds against 6Y6C protein, which is considered as a new research task in the technology of current docking studies.

Table 3: Binding affinity analysis of 6YYE protein:

PROTEIN	PUBCHEM ID	PROBABLE COMPOUNDS	BINDING AFFINITY
6YYE	28813	1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester	-6.2
	536772	Imidazole, 2-fluoro-1-triacetylribofuranosyl	-6.5
	538744	2,3;5,6-Diacetone-4-O-methylmannitol	-6.7
	574859	Dispiro[1,3-dioxolane-2,2'-bicyclo[2.2.1]heptane-3',2"-(1",3"-dioxolane)], 4',7',7'-trimethyl	-7.4
	590903	Phosphine, (myrtenoyl)diphenyl	-8.3
	638072	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-	-6.5
	23571816	Bicyclo[2.2.1]hept-2-ene, 2-methyl	-7.2
	45276392	1,4-Hexadiene, 4-methyl	-6.1
	45382147	2-Pyridinecarbonitrile, 3-nitro	-6.6
	66831472	Thiophene, 2,5-dihydro-	-8.4
	91720425	Phthalic acid, cycloheptyl isohexyl ester	-6.8
	102070384	Bicyclo[2.2.1]heptan-2-ol, 1,3,3-trimethyl	-8.2

The docking studies against 6YYE revealed in GC-MS analysis of bioactiveCompounds such as1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester,Imidazole, 2-fluoro-1-triacetylribofuranosyl,2,3;5,6-Diacetone-4-O-methyl mannitol, Dispiro [1,3-dioxolane-2, 2'-bicyclo[2.2.1] heptane-3',2"-(1",3"-dioxolane)], 4',7',7'-trimethyl, Phosphine, (myrtenoyl)diphenyl,2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-, Bicyclo [2.2.1]hept-2-ene, 2-methyl,1,4-Hexadiene, 4-methyl,2-Pyridine carbonitrile, 3-nitro,Thiophene, 2,5-dihydro-,Phthalic acid, cycloheptyl isohexyl ester, Bicyclo[2.2.1]heptan-2-ol, 1,3,3-trimethyl,showed the dockingbinding energy of -6.2,-6.5,-6.7,-7.4,-8.3,-6.5,-7.2,-6.1,-6.6,-8.4,-6.8,-8.2 Kcal/mol respectively. Thiophene, 2,5-dihydro-have goodanti-diabetic activity against 6YYE protein. This study identified anti-diabetic compounds from the ethanol extract of *S.Potatorum* seeds against 6YYE protein, which is considered as a new research task in the technology of current docking studies.

LIPINSKI RULE:

S.NO	COMPOUNDS	Molecular Weight (g/mol)	Log p<5	H-bond Donor <5	H-bond Acceptor <10	No. of vio-lation
1.	Imidazole, 2-fluoro-1-triacetylribofuranosyl	344.29	0.8	0	9	0
2.	Dispiro[1,3-dioxolane-2,2'-bicyclo[2.2.1]heptane-3',2"-(1",3"-dioxolane)], 4',7',7'-trimethyl	254.32	1.5	0	4	0

3.	Phosphine, (myrtenoyl)diphenyl	334.4	5.2	0	1	0
4.	2-Pyridinecarbonitrile, 3-nitro	217.10	1.6	0	7	0
5.	Bicyclo[2.2.1]heptan-2-ol, 1,3,3-trimethyl	330.5	4.6	1	3	0
6.	Thiophene, 2,5-dihydro-	86.16	1.1	0	1	0
7.	1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester	278.34	4.8	0	4	0
8.	2,3;5,6-Diacetone-4-O-methylmannitol	276.33	0	1	6	0
9.	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-	410.7	11.6	0	0	1
10.	Bicyclo[2.2.1]hept-2-ene, 2-methyl	272.23	5.3	0	6	1
11.	1,4-Hexadiene, 4-methyl	186.25	3.2	0	1	0
12.	2-Pyridinecarbonitrile, 3-nitro	217.10	1.6	0	7	0
13.	Phthalic acid, cycloheptyl isohexyl ester	346.5	6.6	0	4	0

From the GC-MS results received 13 best compounds from the *S.potatorum* seed ethanolic extract based on their binding affinity. For these 13 compounds have checked the following rules.

- ✓ Molecular mass < 500.
- ✓ Calculated octanol/water partition coefficient (CLogP) < 5.
- ✓ Number of hydrogen bond donors < 5.
- ✓ Number of hydrogen bond acceptors < 10(Walters *et al*.,2012).

In this study, identified anti-diabetic compounds from the ethanol extract of *S.Potatorum* seeds against 6Y6C & 6YYE proteins by docking studies. Present study checked the compounds that obey to the Lipinski rule which is considered as a new research task in the technology of current docking studies.

4. SUMMARY AND CONCLUSION:

Phytochemical study of *S. potatorum* ethanol extracts revealed the existence of bioactive substances.The TLC (thin layer chromatography) results revealed the presence of several bioactive substances. Medicinal plants are used to find and screen phytochemical ingredients and play an important role in illness prevention, such as antibacterial, anti-diabetic, and antioxidant properties. Preliminary phytochemical examination of *S. potatorum* seed ethanol extract reveals primary and secondary metabolites that are of commercial relevance to both research institutes and pharmaceutical corporations for the development of new medications

to cure a variety of ailments. Phytochemicals with varied activity are found in the therapeutic seeds chosen. We concentrated on anti-diabetic activity in this study.

The ethanol extract of *S.potatorum* seeds revealed good bactericidal activities against *E.coli* and it has the 275 µg/TAE mL^{-1} extract total anti-oxidant capacity. The docking studies against 6Y6C revealed in GC-MS analysis of bioactive Compounds such as Imidazole, 2-fluoro-1-triacetylribofuranosyl,Dispiro[1,3-dioxolane-2,2'-bicyclo[2.2.1] heptane-3',2"-(1",3"-dioxolane)], 4',7',7'-trimethyl,Phosphine, (myrtenoyl)diphenyl,2-Pyridinecarbonitrile, 3-nitro,Bicyclo[2.2.1]heptan-2-ol, 1,3,3-trimethyl,Thiophene, 2,5-dihydro-,showed the dockingbinding energy of -6.6,-6.2,-7,-6.1,-6.4,-7.7 Kcal/mol respectively. Thiophene, 2,5-dihydro-have goodanti-diabetic activity against 6Y6C protein.

In this study, identified anti-diabetic compounds of the ethanolic extraction of *S. potatorum* seeds. So, it has been considered as a novel research work in the current technology of docking studies. Now a day's drug designing plays a major role in the competitiveworld. In future studies, myresearch work is most useful and essential for the drug designing.

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