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Analysis of anti-diabetic properties of *Phyllanthus urinaria* by docking studies

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ABSTRACT

The objective of this research was to analyze the anti-diabetic properties of the bioactive compounds extracted from P. urinaria using molecular docking servers. Two extracts were prepared using distilled water and chloroform as solvents by optimized maceration procedure. Determination of phytochemical constituents of these extracts was then carried out by using standard set of protocols. GC-MS analysis was carried out to obtain the physical properties and the structural analogs of the phytochemicals. Docking of the respective phytochemicals with TXNIP using metformin as the reference drug was performed using Patch dock. Upon phytochemical screening it was found that both the chloroform and aqueous extract contain bioactive compounds. Comparatively the aqueous extract was found to be richer in phytochemicals than the chloroform extract. The GC-MS study provided the structural analog and the physical properties of six phytochemicals present in the chloroform extract. Docking study confirmed the fact that five out of the six phytochemicals have better binding efficiency to TXNIP as compared to the conventional drug Metformin. The phytochemicals so obtained have better efficiency in lowering blood glucose level as compared to metformin. The present study confirms the fact that the bioactive so obtained possess efficient anti-diabetic properties. The molecular docking study confirms the fact that the phytochemicals have better efficiency in lowering blood glucose level. Hence the phytochemicals from P. urinaria can be a substitute for the more toxic drug Metformin for diabetes treatment.

Keywords: Anti-diabetic, Phytochemicals, GC-MS, TXNIP, Docking.

INTRODUCTION

Diabetes mellitus is a common and very prevalent disease affecting the citizens of both developed and developing nations [1]. Diabetes mellitus is a chronic endocrine disorder caused by an absolute or relative lack of insulin and/or reduced insulin activity that results in hyperglycemia and abnormalities in carbohydrate, fat and protein metabolism [2]. TXNIP (Thioredoxin Interacting Protein) plays a major physiological role in glucose metabolism and cell differentiation [3]. A single mutation, C247S of TXNIP inhibits the binding of glucose with thioredoxin leading to abnormality in glucose metabolism [4]. Glucose and diabetes up regulate beta-cell TXNIP expression and TXNIP overexpression induces beta-cell apoptosis [5].

Since the hypoglycemic drugs for a long term therapy currently have various toxicities, there has been a growing focus on searching and discovering new anti-diabetic drugs with high safety, and it is all the while deemed to be a good idea to find drugs from natural plant source [6]. Current mode of treatment available for diabetes patient is oral administration of anti-diabetic drugs and parental insulin delivery. With

the former indicating serious side effects it was rendered mandatory to search for alternative drugs with fewer side effects. The current study is focused on screening of phytochemicals from *Phyllanthus urinaria* that has substantial anti-diabetic effects. It has its origin in Asia and is widely distributed in the tropical regions of the world [7]. All parts of the plants are used extensively for therapeutic purpose. The most important bioactive compounds used for therapeutic purpose are alkaloids, tannins, flavonoids and phenolic compounds [8]. Tannins are the group of compounds responsible for glucose transport and adipose differentiation inhibitory activities [9]. The optimal effectiveness of a medicinal plant may not be due to one active constituent, but due to the combined action of all the different compounds present originally in the plant [10].

The characterization of bioactive compounds in terms of their physical and chemical properties is of utmost importance prior to studying their biological activities. The combination of an ideal separating technique i.e. Gas chromatography with the best identification technique i.e. Mass spectroscopy makes GC-MS an ideal technique for qualitative and quantitative analysis of volatile and semi-volatile compounds [11]. The effectiveness of the phytochemicals in treating diabetes can be analyzed by monitoring the extent to which it interacts with the target protein.

The current study is primarily focused on the comparative analysis of phytochemicals with the conventional chemical drug Metformin in the treatment of diabetes.

MATERIALS AND METHODS

Plant Material

Phyllanthus urinaria stems were obtained from the local market in Vellore, Tamil Nadu, India. The stems were dried in shade for 48 hours followed by which it was grinded to fine powdered form for further analysis.

Chemicals

The organic solvent, chloroform used for preparing plant extract was of analytical grade and was procured locally.

Extract preparation

Two different solvents, chloroform and water based on their polarity were used to prepare the extract. The aqueous extract was prepared by mixing 50 grams of stem powder with 500 mL of distilled water. On the other hand organic extract was prepared by mixing 50 grams of seed extract with 250 mL of chloroform. Double the amount of water is used in case of aqueous extract because stem powder forms gelatinous mass when mixed with lower quantity of water. The mixtures were placed on orbital shaker (The I L E Company, Chennai, Tamil Nadu, India) for 72 hours. The solvents were then filtered through Whatman filter paper and the filtrates were dried to yield extracts for experimental studies.

Phytochemicals screening

Phytochemical screening was performed using standard set of procedures to identify the respective phytochemicals that were present in the plant sample [12].

Gas Spectroscopy-Mass Spectroscopy Analysis

GC-MS of the plant extract was performed using Perkin Elmer GC system comprising of an auto sampler and a gas chromatograph interfaced to a mass spectroscopy. The diameter of the capillary column used to run the sample was 5 micro meters. For GC-MS detection, an electron impact mode was operated with an ionizing energy of 70eV. Helium was used as the carrier gas for the experiment. The injector temperature was maintained at 250°C for the entire run. The run time of the sample was 36 minutes.

Protein-Ligand Docking

After having successfully received the structures of the phytochemicals from the GC-MS analysis the compounds were incorporated into pubchem to get the smiles format. The smiles format was fed into the CORINA software which gave the pdb form of the compound.

Patch dock algorithm works by object recognition and image segmentation techniques used in computer vision. During molecular shape representation the entire surface of the molecule is analyzed i.e. the concave, convex and flat surfaces are monitored and only the relevant surfaces termed as "Hot Spots" remain on the molecule. After the complementary complex has been formed the candidates are ranked according to their complementary score. The ligand-receptor interaction was visualized using RasWin software.

RESULTS AND DISCUSSION

Phytochemical Screening

The outcome of phytochemical screening is depicted in Table 1. Aqueous extract has more phytochemicals than the chloroform extract. This can be attributed to the high dielectric constant of water and the structural aspects of the phytochemicals. The most important phytochemicals required in the treatment of diabetes namely Flavonoids, Cardiac glycosides and Phenolic compounds were present in both the extract. Tannins were found only in chloroform extract. This could be attributed to the non-polar nature of Tannin compounds. Whereas Saponins, Cardenolides and Phlobatannins were present only in aqueous extract. Anthraquinones were found to be absent in both the extract.

Table 1. It depicts the phytochemicals that were screened from the plant extract. A +sign indicates the presence of it while the –sign conveys its absence

Phytochemicals	Chloroform	Aqueous
Tannins	+	-
Saponins	-	+
Flavonoids	+	+
Cardenolides	-	+
Phlobatannins	-	+
Anthroquinones	-	-
Cardiac glycosides	+	+
Phenolic compounds	+	+

Gas Chromatography – Mass Spectroscopy Analysis

As depicted in the chromatogram shown in Table 2. Six subclasses of phytochemicals were obtained along with their structure and physico-chemical properties. The chromatogram so generated was used to determine the respective compounds by comparing with the standard library available. The analysis was done in two phase: initially the extract was made to run on a chromatographic column followed by mass spectroscopy of the extract based on charge to mass ratio. There were peaks generated at different time intervals as depicted in Figure 1. during the GC-MS run corresponding to the specific compound present in the extract. GC-MS is the preferred analytical technique for quick screening of volatile compounds as it is less time consuming and uses only minute quantity of substance [13].

Table 2. It depicts the specific bioactive compounds that were screened from the plant extract at different time intervals during the chromatographic run.

Bioactive compounds	Run time (minutes)
Methylene chloride	02.800
2-(3- Oxo- 1,3-Dihydroisobenzenefuran-1- ylmethyl) Benzoic acid	25.052
Carissanol dimethyl ether	25.162
Phenethylamine, 2-Methoxy α,-Methyl-4,5-(Methylenedioxy)	26.258
16 - Heptadecanal	27.268
Cyclohexane, 1-(1,5- Dimethylhexyl)-4-(4- Methylpentyl)	29.169

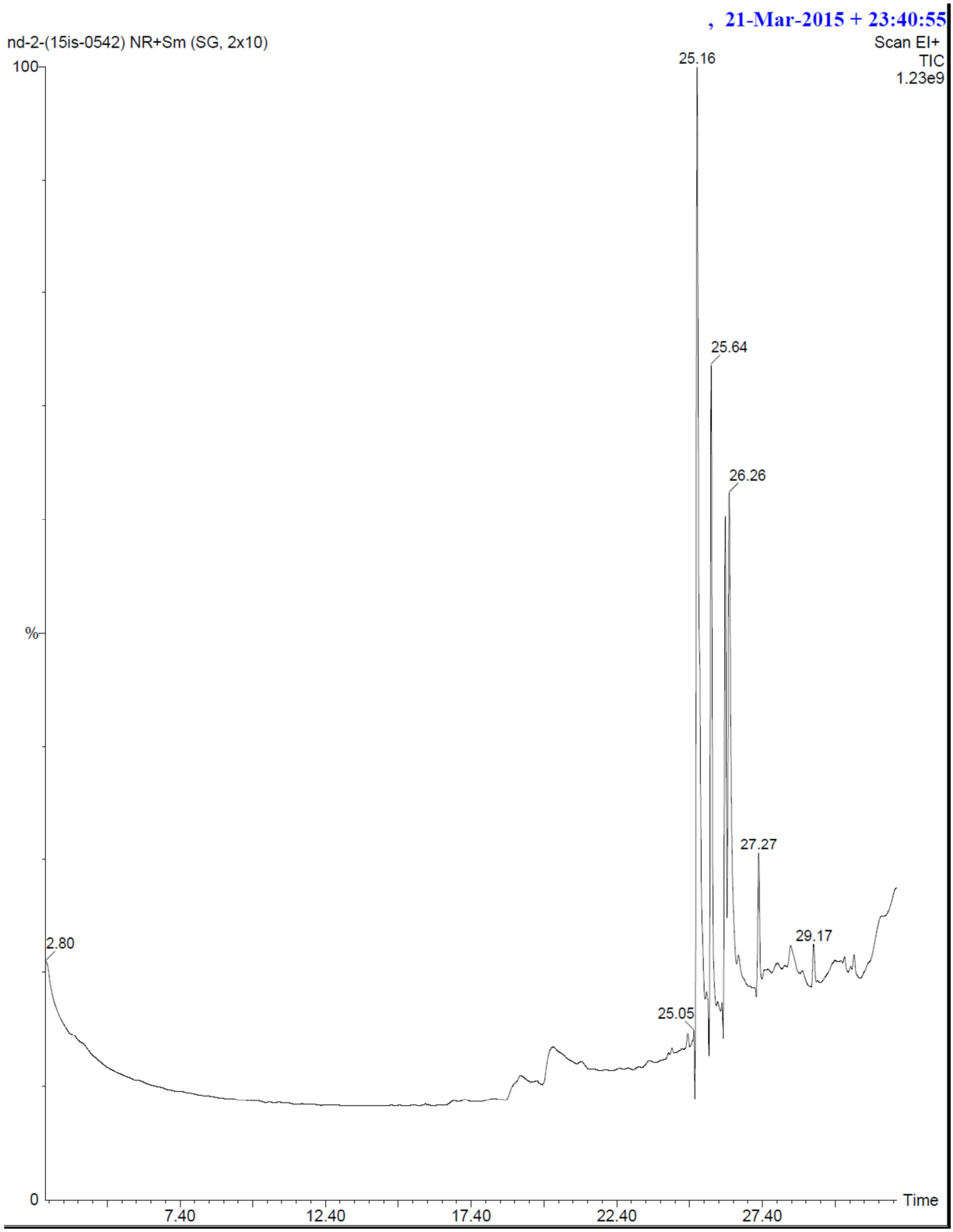

Figure 1. It depicts the time period at which the respective peaks were obtained for the phytochemicals during the GC-MS analysis

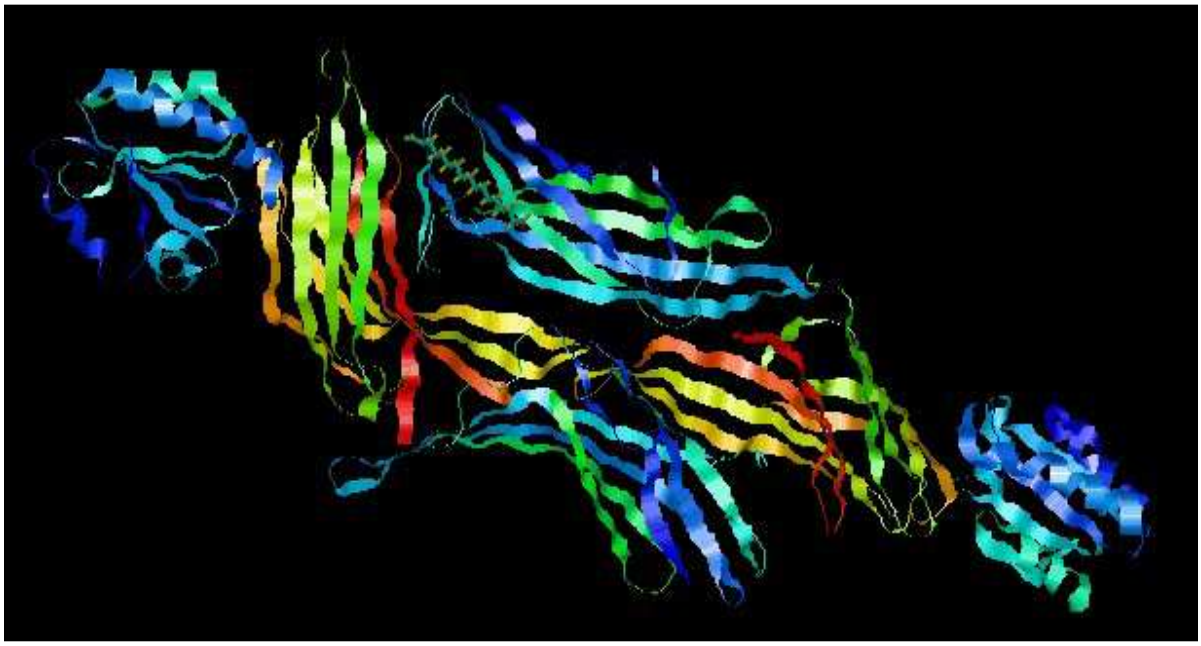

Figure 2. The figure as obtained from RasWin server depicts the binding site of the phytochemical, Phenethylamine, 2-Methoxy α,- Methyl -4,5-(Methylenedioxy) with TXNIP

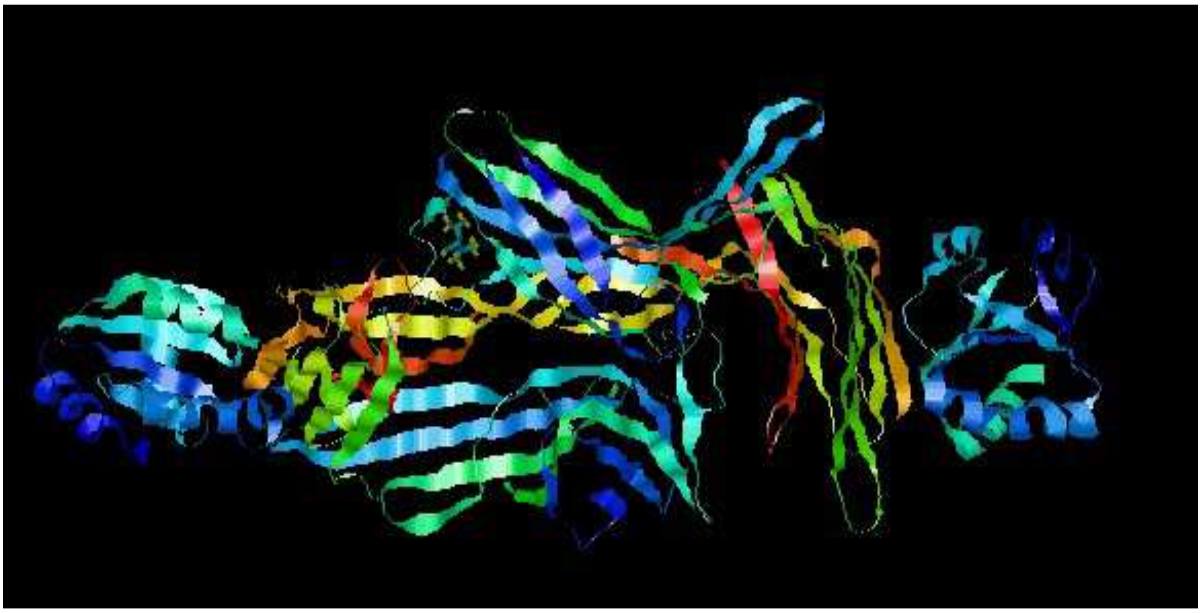

Figure 3. The figure as obtained from RasWin server depicts the binding site of the conventional drug Metformin with TXNIP

Ligand – Receptor Docking Analysis

TXNIP plays a significant role in β cell apoptosis which in turn leads to alarming rise in blood glucose level. Hence it was rendered mandatory to suppress the overexpression of TXNIP. Metformin, a chemical analog along with the six phytochemicals obtained from *P. urinaria*were docked to TXNIP using Patch dock – an online server to check for their binding efficiency. As per the docking score depicted in Table 3. Five out of the six phytochemical constituents were found to bind more efficiently than metformin to TXNIP. The binding of ligand molecule to the receptor is primarily due to hydrogen bonding or Vander Waal's force of interaction [14]. The three dimensional interaction of TXNIP with the Metformin is shown in Figure 3 and with Phenethylamine, 2- Methoxy α,- Methyl- 4,5-(Methylenedioxy) in Figure 2. It can be

inferred from the figure that only certain amino acid residues and proper orientation of the molecule are responsible for the interaction.

Table 3. The tabular representation of the docking scores suggests that the phytochemicals extracted from P.urinaria are having good potential in binding to TXNIP thereby suppressing its overexpression and regulating the glucose metabolism to optimum level.

Ligands	Protein	Docking Score
Metformin	TXNIP	2882
Methylene chloride	TXNIP	5274
2-(3-Oxo-1,3- Dihydroisobenzenefuran -1- ylmethyl) Benzoic acid	TXNIP	2054
Carissanol dimethyl ether	TXNIP	4064
Phenethylamine, 2- Methoxy α,-Methyl- 4,5- (Methylenedioxy)	TXNIP	4916
16 - Heptadecanal	TXNIP	5270
Cyclohexane, 1-(1,5- Dimethylhexyl)- 4 -(4-Methylpentyl)	TXNIP	3578

CONCLUSION

Based on the phytochemical screening it was found that *P. urinaria* contains many bioactive compounds like flavonoids, tannins, saponins, etc. that are potent source of therapeutic agents. Flavonoids are an important class of phytochemicals as they act on various molecular targets and regulate different signaling pathways in pancreatic β-cells, hepatocytes, adipocytes and skeletal myofibres [15]. However it should be noted to take into account the solvation property of phytochemicals as aqueous extrac t outnumbered chloroform extract in the number of phytochemicals isolated. Further analysis by GC-MS technique provided the physical and chemical properties of the sub classes of phytochemicals present in the extracts. This paved way for us to conduct Docking studies which went on to yield positive results in binding to TXNIP than the chemical drug Metformin. Hence it can be safely concluded that our plant contains phytochemicals that shows good promise in binding to TXNIP and regulating blood glucose to optimum level.

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