INTERNATIONAL JOURNAL OF PHARMACEUTICS & DRUG ANALYSIS

VOL.6 ISSUE 2, 2018; 271 – 278 ; http://ijpda.com; ISSN: 2348-8948

MEDFOOD'18 [1st February 2018]

National Conference on Phytochemicals in Medicinal Plants and Food

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Research Article

Molecular Docking Of Bioactive Compounds From *Syzygium Aqueum* Against Type 2 Diabetes Susceptibility Gene TCF7L2

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Date Received: 23rd January 2018; Date accepted: 29th January 2018; Date Published: 21st February 2018

Abstract

Syzygium aqueum is well known medicinal plant and the bioactive compound of this plant is best source for curing diabetes mellitus (DM). Untreated Type 2 DM leads to several complications and need continuous medical treatment. In addition, there is an immediate need to find natural therapy using bioactive compounds for DM to reduce side effects. However, there is a lacking of in silico studies to develop drug molecule using *S. aqueum*. In the present study, 14 bioactive compounds isolated from the *S.aqueum* leaf extracts and identified using GC-MS analysis. The identified compound tested against TCF7L2 gene, which is potential indicator gene for type 2 DM through molecular docking. Among 14 compounds tested, 1-Monolinoleoylglycerol trimethylsilyl ether showed high-energy values and considered as good bioactive compound against type 2 diabetes susceptibility gene TCF7L2.

Keywords: Diabetes mellitus, TCF7L2 gene, *Syzygium aqueum*, bioactive compound, molecular docking

INTRODUCTION

Syzygium aqueum (*Eugenia aquea*) is a known medicinal plant and widely found in tropical regions such as India, Malaysia and Indonesia. *S.aqueum*, known as jambul, jambolan, jamblang or jamun, is an evergreen tropical tree in the flowering plant family Myrtaceae [1]. It has been used for treatment and in prevention of different diseases in homoeopathy practice for more than five decades in different countries. Various parts of this plant are used in traditional medicine; while the leaves have been shown to possess antibiotic activity and relieving child birth pains. Therefore, scientists are curious to prove the pharmacological and phytochemical actions [2]. Recently, the *S.aqueum* was shown to have anti hyperglycemic properties and its compounds enhance adipogenesis, stimulated glucose uptake, and increased adiponectin secretion [3]. Another reports showed that *S.aqueum* leaf extracts can be used for treatment of diabetes mellitus (DM) [4]. Because DM is fast gaining the status of a potential epidemic in India with more than 62 million diabetic individuals currently diagnosed with

the disease[5]. Among three types of DM, Type 2 DM associated with impaired insulin secretion and constituting 80%–90% of all reported diabetes cases. If left untreated, diabetes can cause many complications such as cardiovascular disease, stroke, chronic kidney failure, foot ulcers, and diabetic retinopathy[6].Hence, there is an immediate need to find herbal medicine to treat and reduce the negative impacts of DM and its severe complications. However, recent studies have shown that variants of the Transcription factor 7-like 2 (T-cell specific, HMG-box) also known as TCF7L2 gene increase susceptibility to type 2 diabetes [7]. TCF7L2 is implicated in a large variety of diseases and it was found to be a major determinant of type 2 risk in European populations [8].

In the field of bioinformatics, drug design is the inventive process of finding a new method based on the knowledge of a biological target[9]. The drug is most commonly an organic small molecule that activates or inhibits the function of a biomolecule such as a protein, which in turn results in a therapeutic benefit to the patient. In the most basic sense, drug design involves the design of small molecules that are complementary in shape and charge to the biomolecular target with which they interact and therefore will bind to it [10]. This type of modeling is often referred to as computer-aided drug design. Finally, drug design that relies on the knowledge of the three-dimensional structure of the biomolecular target is known as structure-based drug design. Thus the drug design is the design of a small molecule that will bind tightly to its target, receptor protein molecule [11].Despite the increasing number of reports on the medicinal benefits of the *S. aqueum*, the *in silico* study of the plant extract of antidiabetic property has yet to be reported. By considering these factors, the aim of the present study is to isolate bioactive compounds from *S.aqueum* leaves and tested against genes which are possible cause of type2 diabetes.

MATERIALS AND METHODS

Receptor preparation

Ligand molecules bind to the active site of the enzyme through hydrogen bonds, hydrophobic interactions, temporary covalent interactions (van der Waals) or a combination of all of these to form the receptor-ligand complex. Residues of the active site will act as donors or acceptors of protons or other groups on the drug (ligand) to facilitate the reaction. In other words, the active site modifies the reaction mechanism in order to change the activation energy of the reaction. Active sites can be mapped to aid design of new drugs such as enzyme inhibitors. This involves description of the size of an active site and the number and properties of sub-sites, such as details of the binding interaction [12]. An emerging challenge in drug discovery concerns the identification of allosteric ligand binding sites, through which drugs can modulate the effects of ligands that bind at the primary site. The simulated ligands circled the target protein extensively before finding the binding site. More generally, an important limitation of traditional virtual drug screening is that it must start with a well-defined binding site [13,14]. By allowing the identification of previously unknown binding sites, molecular dynamics simulations of protein– ligand binding, such as those presented here, may substantially broaden the applicability of computational techniques to drug development. The ligand correctly identifies its target binding site, forming a complex virtually identical to the crystallographically determined bound structure.

Identification of active sites is crucial in the process of target based drug design. The 3-D structure of the enzyme is analyzed to identify active sites and design drugs which can fit into them. In the present study TCF7L2 is prepared and active site identified.

Bioactive compound preparation

30 g powdered sample of *S. aqueum* leaves was soaked and dissolved in 75 ml of methanol for 24 hrs. Then the filtrates were collected by evaporating under liquid nitrogen. The GC-MS analysis was carried out using a Clarus 500 Perkin- Elmer (Auto System XL) Gas Chromatograph equipped and coupled to a mass detector Turbo mass gold – Perking ElmerTurbomas 5.2 spectrometer with an Elite-1 (100% Dimethyl ply siloxane), 300 m x 0.25 mm x 1µm df capillary column. The instrument was set to an initial temperature of 110°C, and maintained at this temperature for 2 min. At the end of this period, the oven temperature was raised upto 280°C, at the rate of an increase of 5°C/min, and maintained for 9 min. Injection port temperature was ensured as 250°C and Helium

flow rate as 1 ml/min. The ionization voltage was 70 eV. The samples were injected in split mode as 10:1. Mass Spectral scan range was set at 45- 450 (mhz). The chemical constituents were identified by GC-MS. The fragmentation patterns of mass spectra were compared with those stored in the spectrometer database using National Institute of Standards and Technology Mass Spectral database (NIST-MS). The percentage of each component was calculated from relative peak area of each component in the chromatogram.

Docking simulations

Typically a drug target is a key molecule involved in a particular metabolic or signaling pathway that is specific to a disease condition or pathology. Some approaches attempt to inhibit the functioning of the pathway in the diseased state by causing a key molecule to stop functioning. Drugs may be designed that bind to the active region and inhibit this key molecule. In the potential target, the active site on the receptor molecule can be identified *in silico* by establishing the structure activity relationship of small molecules, ligands [15]. In the present investigation, bioactive compounds were tried against TCF7L2 gene.

Drug design broadly divided into two categories. The first one is the structure-based drug design which is usually referred to as receptor-based drug design. In this case, ligand molecules are shaped within the constraints of the binding pocket by assembling small pieces in a stepwise manner[16,17,18]. Another category is finding a suitable ligand molecule for a given receptor. In this case, a large number of potential ligand molecules are screened to find those ligands fitting with the binding pocket of the receptor. This method is usually referred to as ligand-based drug design [19]. In the present study, ligand-based drug design has been applied in which large number of ligands for the given receptor were screened and identified.

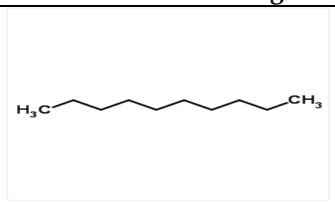
9-Octadecenamide,(Z)-,17.alfa.,21á-28,30-Bisnorhopane,Spirost-8-en-11-one,3-hydroxy-,(3á,5à,14á,20á,22á,25R)-, 13-Docosenamide, (Z)-, Bis(cis-13-docosenamido)methane, Decane, Undecane, Undecane, 4,6-dimethyl-,1-Monolinoleoylglycerol trimethylsilyl ether, 9,12,15-Octadecatrienoic acid, 2,3-bis[(trimethylsilyl)oxy]propylester, (Z,Z,Z)-, Dasycarpidan-1-methanol, acetate(ester), Trilinolein,8,14-Seco-3,19-epoxyandrostane-8,14-dione,17-acetoxy-3á-methoxy-4,4-dimethyl-, 1,8-Dioxa-5-thiaoctane and 8-(9-borabicyclo [3.3.1]non-9-yl)-3-(9-borabicyclo[3.3.1] non-9-yloxy) -1-phenyl bioactive compounds from *S. aqueum* were retrieved from Chemspider database[20]. The above said 2D structures are converted into 3D structure using swisspdb viewer [21]. TCF7L2 is retrieved from UniProtKB/Swiss-Prot database [22]. TCF7L2 is treated with these bioactive compounds using Schrodinger docking program [23].

Results And Discussion

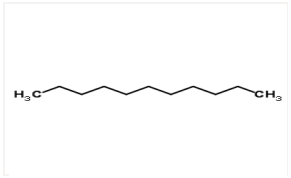
A total of 14 bioactive compounds from *S.aqueum* were taken for the molecular docking analysis. The selected 14 bioactive compounds are 1-13-Docosenamide, (Z)-, 9-Octadecenamide, (Z)-, Bis(cis-13-docosenamido)methane, Decane, Undecane, Undecane 4,6-dimethyl-, 1-Monolinoleoylglycerol trimethylsilyl ether, 9,12,15-Octadecatrienoic acid,2,3-bis [(trimethylsilyl)oxy]propyl ester, (Z,Z,Z)-, Dasycarpidan-1-methanol,acetate(ester), Trilinolein, 8,14-Seco-3,19-epoxy androstane-8,14-dione, 17-acetoxy-3á-methoxy-4,4-dimethyl-, 17.alfa.,21á-28,30- Bisnorhopane, Spirost-8-en-11-one, 3-hydroxy-,(3á,5à,14á,20á,22á,25R)-, 1,8-Dioxa-5-thiaoctane and 8-(9-borabicyclo [3.3.1] non-9-yl) -3-(9-borabicyclo [3.3.1] non-9-yloxy)-1-phenyl bioactive compounds (Fig.1).These ligands have been used to target against TCF7L2 which bound to the receptor to inhibit its function. The length of the receptor is 529 amino acids (Fig.2). The nature of the complex between the drug and the receptor molecule was identified via docking and the inhibition nature of the ligands and their binding affinities were calculated using free energy simulations. Docking results between TCF7L2 receptor and phytochemical drugs were tabulated (Table.1).

In this study, 1-Monolinoleoylglycerol trimethyl silylether from *S. aqueum* showed a maximum e-value --11.601 (Fig.3). 1-Monolinoleoylglycerol trimethyl silyl ether is strongly interacted active site of receptor. This site have more hydrophobic amino acids indicates good binding (Fig.4).

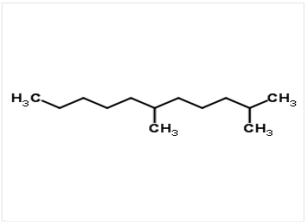
Fig. 1 – Molecular structures of isolated bioactive compounds



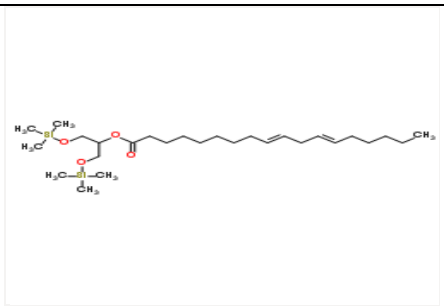
Decane



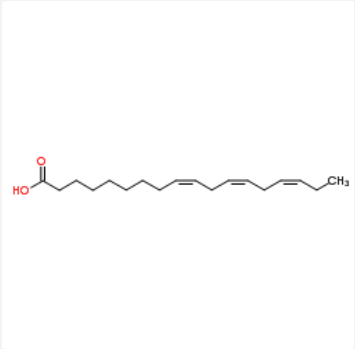
Undecane



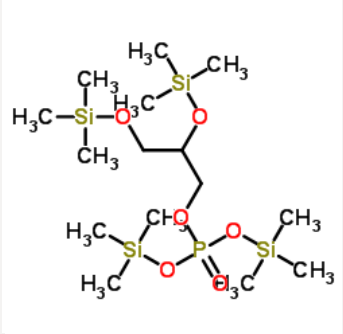
Undecane, 4,6-dimethyl-



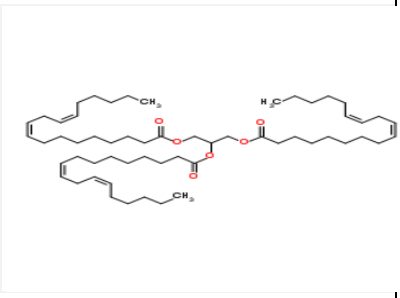
1-Monolinoleoylglycerol trimethylsilyl ether



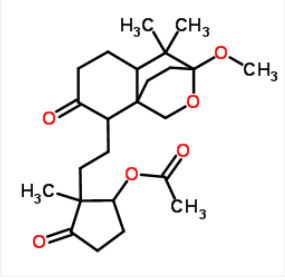
9,12,15-Octadecatrienoic acid



2,3-bis[(trimethylsilyl)oxy]propylester, Z,Z,Z)- Dasycarpidan-1-methanol, acetate (ester)



Trilinolein



8,14-Seco-3,19-epoxyandrostane-8,14-dione, 17-acetoxy-3á-methoxy-4,4-dimethyl-

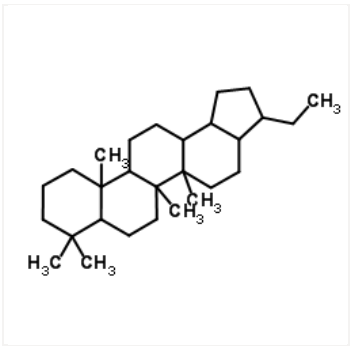
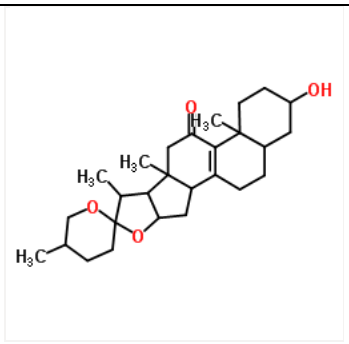
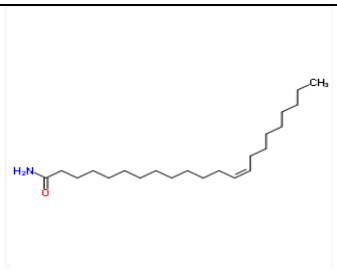
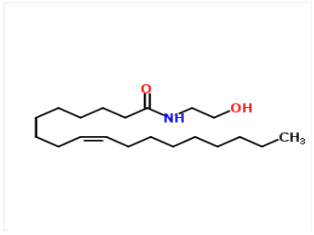
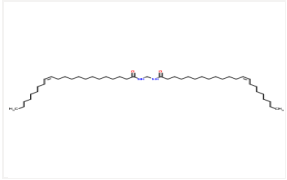
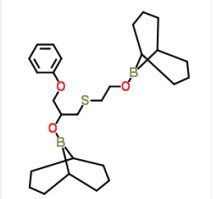
 **17.alfa.,21á-28,30-Bisnorhopane**	 **Spirost-8-en-11-one, 3-hydroxy-, (3á,5à,14á,20á,22á,25R)-**
 **13-Docosenamide, (Z)-**	 **9-Octadecenamide, (Z)-**
 **Bis(cis-13-docosenamido) methane**	 **1,8-Dioxa-5-thiaoctane,8-(9-borabicyclo[3.3.1]non-9-yl)-3-(9-borabicyclo[3.3.1]non-9-yloxy)-1-phenyl-**

Table.1.showing molecular interaction results of TCF7L2 with bioactive compounds

S.No	Bioactive compounds	Molecular formula	Molecular mass	Energy value
1	Decane	$C_{10}H_{22}$	142.282 Da	-6.116
2	Undecane	$C_{11}H_{24}$	156.308 Da	-6.700
3	Undecane, 4,6-dimethyl-	$C_{13}H_{28}$	184.361 Da	-7.865
4	1-Monolinoleoylglycerol trimethylsilyl ether	$C_{27}H_{54}O_4Si_2$	498.886 Da	**-11.601**
5	9,12,15-Octadecatrienoic acid,	$C_{18}H_{30}O_2$	278.430 Da	-8.118
6	2,3-bis[(trimethylsilyl)oxy]propyl ester, (Z,Z,Z)- Dasycarpidan-1-methanol, acetate (ester)	$C_{15}H_{41}O_6\psi 4$	460.798 Da	-8.154
7	Trilinolein	$C_{57}H_{98}O_6$	879.384 Da	-8.009
8	8,14-Seco-3,19-epoxyandrostane-8,14-dione, 17-acetoxy-3á-methoxy-4,4-dimethyl-	$C_{24}H_{36}O_6$	420.539 Da	-7.001
9	17.alfa.,21á-28,30-Bisnorhopane	$C_{28}H_{48}$	384.681 Da	-6.715
10	Spirost-8-en-11-one, 3-hydroxy-, (3á,5à,14á,20á,22á,25R)-	$C_{27}H_{40}O_4$	428.604 Da	-6.129
11	13-Docosenamide, (Z)-	$C_{22}H_{43}NO$	337.583 Da	-6.474
12	9-Octadecenamide, (Z)-	$C_{20}H_{39}NO_2$	325.529 Da	-6.319
13	Bis(cis-13-docosenamido)methane	$C_{45}H_{86}N_2O_2$	687.177 Da	-6.214
14	1,8-Dioxa-5-thiaoctane, 8-(9-borabicyclo [3.3.1]non-9-yl)-3-(9- borabicyclo [3.3.1]non-9-yloxy)-1-phenyl-	$C_{27}H_{42}B_2O_3S$	468.308 Da	-6.010

Fig.2. 3D structure of TCF7L2

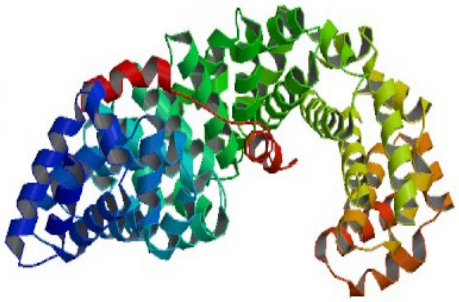
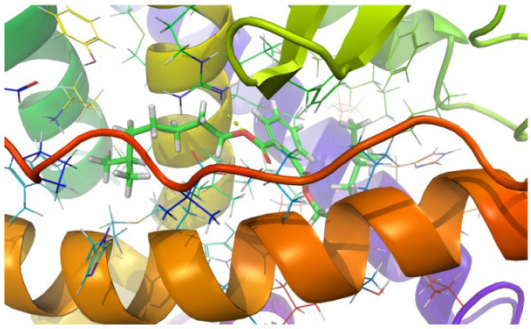
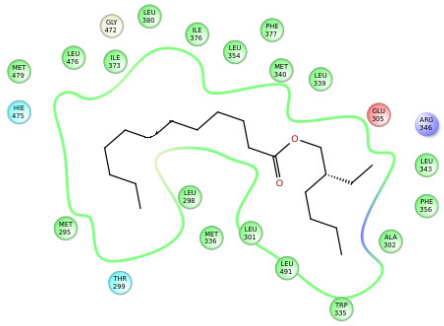


Fig.3. Molecular docking ofTCF7L2 with 1-Monolinoleoylglycerol trimethylsilyl ether



Castrop *et al*., [7] have reported that variants of the TCF7L2 gene increase susceptibility to type 2 diabetes. For people who inherit two copies of the variants, the risk of developing type 2 diabetes is about 80 percent higher than for those who do not carry the gene variant. Transcription factor 7-like 2 (T-cell specific, HMG-box) also known as TCF7L2 or TCF4 is a protein acting as a transcription factor. In humans this protein is encoded by the *TCF7L2* gene. The single nucleotide polymorphism (SNP) within the TCF7L2 gene, rs7903146, is, to date, the most significant genetic marker associated with Type 2 DM risk [24]. SNPs in this gene are linked to higher risk to develop type 2 diabetes as well as gestational diabetes [25]. TCF7L2 is a transcription factor influencing the transcription of several genes thereby exerting a large variety of functions within the cell. It is a member of signaling pathway [26].

Fig.4. Molecular interaction ofTCF7L2 with 1-Monolinoleoylglycerol trimethylsilyl ether



Stimulation of the pathway leads to the association of β-catenin with BCL9, translocation to the nucleus, and association with TCF7L2, which in turn results in the activation of target genes, specifically repressing proglucagon synthesis in enteroendocrine cells.TCF7L2 is implicated in a large variety of diseases. Several single nucleotide polymorphisms are associated with type 2 diabetes. In European populations it was found to be a major determinant of type 2 risk. A frame shift mutation of TCF7L2 is implicated in colorectal cancer. Variants of the gene are most likely involved in many other cancer types [27]. In this study TCF7L2 is taken into consideration as a disease causing receptor.1-Monolinoleoylglycerol trimethylsilyl ether with molecular formula $C_{27}H_{54}O_4Si_2$ has many biological activities such as antimicrobial, antioxidant, anti-arthritic, anti-inflammatory, antiasthma, diuretic and antidiabetic [28]. The identified steroids, ethyl iso-allocholate and 1-monolinoleoylglycerol trimethylsilyl ether were also shown previously in various reports to have antimicrobial and antibacterial potential [29]. *S. aqueum* phytochemicals helps to bring human beings from disease to health status (Vital Force) without any side effects. In this plant, leaves have myricitrin, myricetin, noctacosanol, mycaminose and maslinic acid [30,31].

Docking is frequently used to predict the binding orientation of small drug molecules to their protein targets in order to predict the affinity and activity of the small molecule. Hence docking plays an important role in the rational design of drugs. Given the biological and pharmaceutical significance of molecular docking, considerable efforts have been directed towards improving the methods used to

predict docking [32]. The focus of molecular docking is to computationally simulate the molecular recognition process. Overall analysis of 14 bioactive compounds, 1-Monolinoleoylglycerol trimethylsilyl ether showed a maximum e-value -11.601against diabetes causing receptor TCF7L2.

Conclusion

In conclusion, from the list of 14 bioactive compounds from the leaf extracts of *S.aqueum,* one compound named 1-Monolinoleoylglycerol trimethylsilyl ether was chosen due to high absolute value of binding energy to all receptors. Based on that, the binding affinity of this compound also suggested that this is good prospect for the treatment of Type 2 DM.To the best of our knowledge, this is the first report from the herbal plant *S.aqueum* and further in vitro studies are required to confirm these results.

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