

IN SILICO ANTI-DIABETIC EFFECT OF PHYTOCOMPOUNDS SYNTHESIZED FROM *MICROLEPIA SPELUNCAE* (L.) MOORE

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Abstract: *Microlepia speluncae* (L.) Moore is a medicinal and economically important fern belonging to the family Dennstaedtiaceae, distributed along the Western Ghats of India. Diabetes mellitus (DM) is recognized as a metabolic disorder which results from the defect in insulin secretion and action. Peroxisome proliferator-activated receptor gamma (PPAR- γ) agonists are beneficial in the treatment of diabetes by stimulating insulin sensitivity and antagonizing hepatic gluconeogenesis. *In silico* docking techniques are being used to investigate the complementarily at the molecular level of a ligand and a protein target. The aim of the present study was to investigate the PPAR- γ agonist property of phytocompounds from *Microlepia speluncae* (L.) Moore using GC-MS analysis and selected phytoconstituents binding affinity through *in silico* approach. Molecular docking of *Microlepia speluncae* on PPAR- γ protein was determined by Auto/Vina in Pymol 4.2. Four compounds were revealed through GC-MS analysis and screened using AutoDock/Vina against PPAR- γ . Docking studies recommended that *Microlepia speluncae* had the highest fitness score and a significant antidiabetic activity against PPAR- γ . The molecular binding interaction of *in silico* data demonstrated that PPAR- γ binding site and could be a potent antidiabetic compound.

Keywords: *Microlepia speluncae*, GC-MS, Molecular Docking, Antidiabetic.

I. INTRODUCTION

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 (Patil *et al.*, 2011). According to World Health Organization, an estimated 422 million adults are living with diabetes mellitus (WHO, 2016). Diabetes mellitus can be managed by diet, physical exercise, and insulin and/or oral hypoglycemic drugs (Koski, 2006). Several research studies have identified peroxisome proliferator activated receptor as key regulators of glucose and lipid metabolism (Elbrecht *et al.*, 1996; Molle, 2001), because they act as transcription factors activating protein synthesis in a wide variety of processes.

Molecular docking is one of the systems for molecular modelling, which allows delivering agonism/antagonism to the biological target selected. The most favourable orientation for formation of a stable complex and the location of one molecule in relation to another molecule (Sousa *et al.*, 2013; Kahraman *et al.*, 2007). The detection of ligand-binding sites is often the starting point for protein function identification and drug discovery (Srivastava *et al.*, 2010) for which molecular docking plays a pivotal role. It is a frequently used tool in computer-aided structure-based rational drug design. It evaluates how small molecules called ligands (substrates, inhibitors, drugs or drug candidates) and the target macromolecule (receptor, enzyme or nucleic acid) fit together (Sahu *et al.*, 2012).

The medicinal plants as sources of bioactive compounds continue to play a dominant role in the maintenance of human health. Reports available on green plants represent a reservoir of effective chemotherapeutants; these are nonphototoxic, more systemic and easily biodegradable. Different extracts from medicinal plants have also been used traditionally to manage diabetes globally, and these are considered as relatively inexpensive, less toxic and with relatively little or no side effects (Vyas, 1999; Kaushik *et al.*, 2002; Chamanlal and Verma, 2006; Gupta *et al.*, 2008). There are also medicinal plants that contain some toxic constituents such as the cytotoxic anti-cancer plant derived drugs, digitalis; however, the side effects of the phytotherapeutic agents are less common compared with synthetic drugs (Calixto, 2000). The aim of this present study is to investigate the antidiabetic constituents present in the ethanol extracts of *Microlepia speluncae* (L.) Moore using GC-MS analysis and molecular docking studies to determine the potent compound which can be used in the treatment of diabetics.

II. MATERIALS AND METHODS

2.1. Collection of Plant Sample

The plant material used in the present study was *Microlepia speluncae* (L.) Moore belonging to the family Dennstaedtiaceae. The plant material for the present study were collected from Ponnudi and identified and confirmed by Pteridophyte flora of the Western Ghats - South India (Manickam and Irudayaraj, 1991).

2.2. Preparation of extracts

For the preparation of different extracts, the plant specimens were washed thoroughly and placed on blotting paper and spread out at room temperature in the shade condition for drying. The shade dried samples were grounded to fine powder using a tissue blender. The powdered samples were then stored in the refrigerator for further use. 30g powdered samples were packed in Soxhlet apparatus and extracted with ethanol for 8h separately (John Peter Paul, 2012; John Peter Paul and Iniya Udhaya, 2017).

2.3. Instrumentation

The GC-MS analysis of ethanol extracts of *Microlepidia speluncae* (L.) Moore were carried out using GC model Clarus 680, Mass Spectrometer Clarus 600 (EI) Perkin Elmer, Gas Chromatograph equipped and coupled to a mass detector TurboMass 5.4.2 spectrometer with an Elite-5MS, (100% Dimethyl ply siloxane), 30.0m × 250µm df capillary column. The instrument was set to an initial temperature of 60°C and maintained at this temperature for 2min. At the end of this period, the oven temperature was raised upto 300°C, at the rate of an increase of 10°C/min and maintained for 6min. Injection port temperature was ensured as 250°C and Helium flow rate as 1ml/min. The ionization voltage was 70eV. The samples were injected in split mode as 10:1. Mass Spectral condition solvent delay 2min, transfer temperature 240°C, source temperature 240°C and scanning range was set at 50-600Da. The chemical constituents were identified by GC-MS.

2.4. Identification of phytochemical constituents

Interpretation of mass spectrum of GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the known component was compared with the spectrum of the known components stored in the NIST library. The name, retention time, molecular weight and molecular formula of the components of the test materials was ascertained.

2.5. Docking software's

Different types of software's like Open babel, PyRx and PyMol were used for the Molecular Docking analysis of antidiabetic activity.

2.6. Phytoconstituents/Ligands

Phytoconstituents/ligands like Oxalic acid, allyl octadecyl ester, .beta.-Ocimene, D-Limonene and Oxalic acid, allyl hexadecyl ester were used for Molecular Docking studies.

2.7. Potential targets and Binding site

2.7.1. Protein preparation

The 3D structure of PPAR gamma (PDB ID: 4EM9) was downloaded from the Protein Data Bank (PDB) (<http://www.pdb.org/pdb/home/home.do>) before initiating the docking simulations. All non-protein molecules were removed from 4EM9; for any alternative atom locations, only the first location was retained. PPAR gamma was modified by adding polar hydrogen and then kept rigid in the docking process. The interactions and the affinities between the phytochemical constituents and receptor were predicted by using Auto/Vina in Pymol 4.2 program (Rarey *et al.*, 1996).

2.7.2. Ligand generation

The 2D structures of phytochemical constituents from the ethanol extracts of *Microlepidia speluncae* (L.) Moore were retrieved from Pub Chem database in structure data format [SDF] format. The 3D structures were obtained and converted into SDF files by using Open babel tool (Weininger, 1988).

2.8. Molecular Docking analysis

Binding mode and interaction of Peroxisome proliferator-activated receptor gamma (PPAR-γ) agonists with individual chemical constituents of *Microlepidia speluncae* (L.) Moore were performed using Auto/Vina in Pymol 4.2 software. Docking was performed to obtain a population of possible conformations and orientations for the ligand at the binding site. The protein was loaded in PyRx software, creating a PDBQT file that contains a protein structure with hydrogens in all polar residues. All bonds of ligands were set to be rotatable. The docking site on protein target was defined by establishing a grid box with the dimensions of X: 69.5816, Y: 68.1907, Z: 70.8677Å, with a grid spacing of 0.3750 Å and number of points on X: 20, Y: 12 and Z: 25. The best conformation was chosen with the lowest docked energy, after the docking search was completed.

III. RESULTS AND DISCUSSION

3.1. GC-MS analysis of ethanol extract of *Microlepidia speluncae*

The ethanol extract of *Microlepidia speluncae* (L.) Moore was a complex mixture of four compounds which has been identified by GC-MS analysis. Phytoconstituents such as Oxalic acid, allyl octadecyl ester, .beta.-Ocimene, D-Limonene and Oxalic acid, allyl hexadecyl ester. The spectrum profile of GC-MS confirmed the presence of four major components with retention time 2.153min, 2.295min, 2.390min, and 2.777min respectively (Figure-1 and Table-1).

3.2. Molecular Docking results

The phytoconstituents/ligands were docked against target protein receptor Peroxisome proliferator-activated receptor gamma (PPAR-γ) [4EM9]. Regarding the interaction with 4EM9, compound D-Limonene showed an intense binding affinity due to the existence of higher number of active sites and the binding energy value was found to be -6.5. A significant variance in the binding energy value (-5.3) was observed in .beta.-Ocimene. Due to the limited interactions with active sites, a further reduction in the binding energy values (-5.1 & -4.0) were found for Decane and Tridecane (Table 2 and Figure 2).

Table 1: Compounds Identified in Ethanol Extract of *Microlepidia speluncae* (L.) Moore

S. No.	RT	Name of compound	M/F	M/W	Area %	Structure
1.	2.153	Decane	C ₁₀ H ₂₂	142.286	16.06	
2.	2.295	.beta.-Ocimene	C ₁₀ H ₁₆	136.238	51.41	
3.	2.390	D-Limonene	C ₁₀ H ₁₆	136.238	19.64	
4.	2.777	Tridecane	C ₁₃ H ₂₈	184.367	12.89	

Table 2: Binding analysis of phytochemical isolated from the *Microlepidia speluncae* (L.) Moore with PPAR gamma.

S/N	Compounds	Affinity (kcal/mol)
1.	Decane	-5.1
2.	.beta.-Ocimene	-5.3
3.	D-Limonene	-6.5
4.	Tridecane	-4.0

Figure 1: GC MS of ethanol extract of *Microlepidia speluncae* (L.) Moore

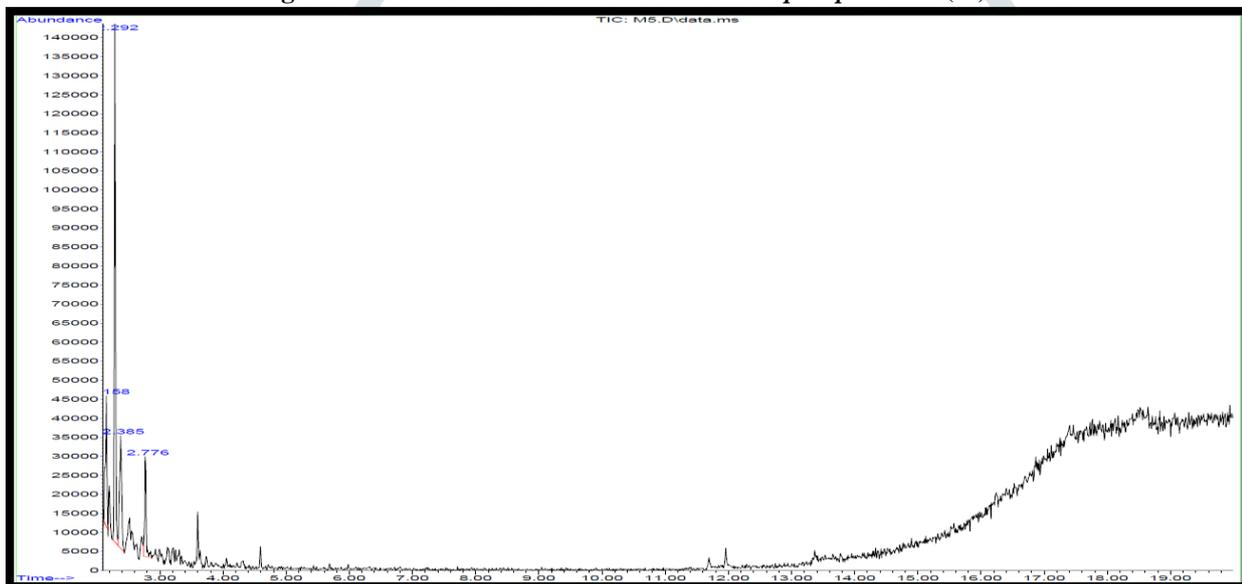
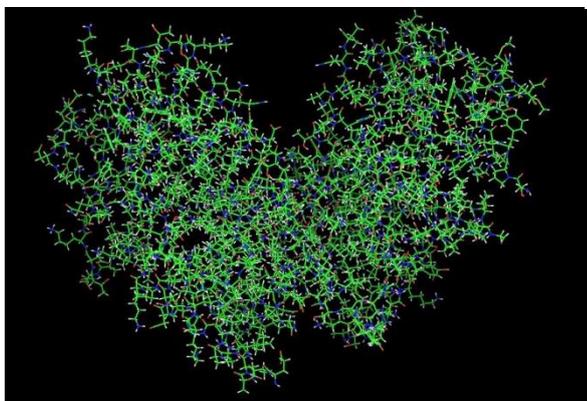
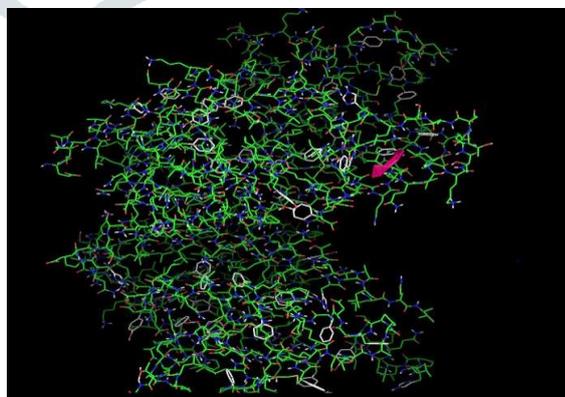


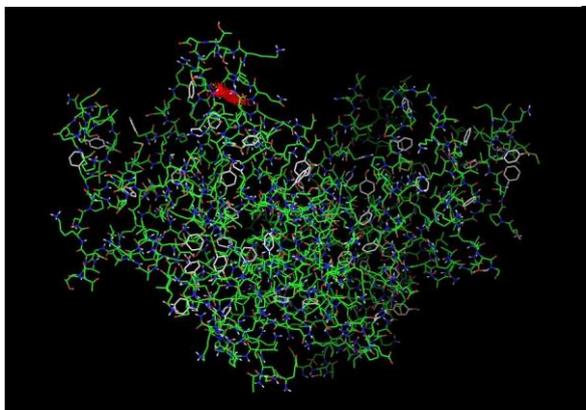
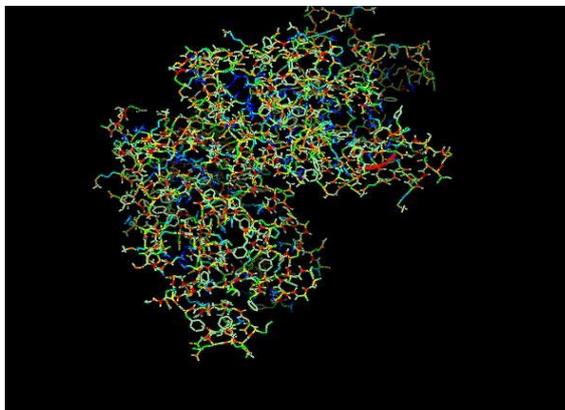
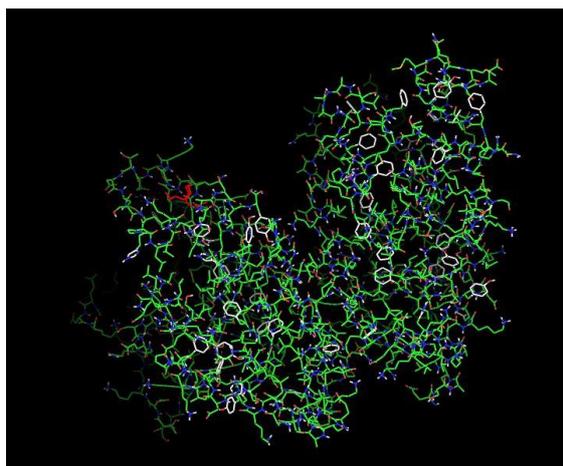
Figure 2: The Molecular binding pose of phytoconstituents/ligands with PPAR-γ.



PPAR-γ



.beta.-Ocimene with PPAR-γ

D-Limonene with PPAR- γ Decane with PPAR- γ Tridecane with PPAR- γ

IV. CONCLUSION

The molecular docking of PPAR- γ with the phytochemicals of *Microlepidia speluncae* (L.) Moore revealed that these identified phytochemicals would be used for antidiabetic agent. All the four compounds have favourable binding potential with the active site residues. The phytochemicals D-Limonene and .beta.-Ocimen shows the highest binding score than other two compounds. These results of docking study can be useful to identify inhibitors for specific diabetic target protein and thus this is useful to design new drugs. There is need for further studies in order to isolate, identify, characterize and elucidate the structure of these phytochemicals.

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