Research article (Award paper)

***In silico* docking analysis of selective bioactive compounds of *Phyllanthus acidus* aqueous fruit extract against MAPK1**Padmini E.¹, Kavitha M.²¹Former Principal, Presidency College, Chennai, Tamil Nadu, India²Assistant Professor, Department of Biochemistry, D.G. Vaishnav College, Arumbakkam, Chennai 600106, Tamil Nadu, IndiaCorresponding author: **Kavitha M.** Email: kavidharan76@gmail.com**ABSTRACT**

Introduction and Aim: *Phyllanthus acidus* L. Skeels (Family: *Phyllanthaceae*) or Star Gooseberry which bears small, edible, juicy, sour, yellow berries fruit is known as a “liver tonic” in ayurvedic medicine. However, the behavior of the plant fruit or its constituents in cell apoptosis/cell survival is unknown. Hence, the purpose of the present study was to perform an *in silico* docking of selective bioactive compounds of aqueous extract of fruit of *P. acidus* (PAFAE) against MAPK1. Mitogen activated protein kinase is a family of serine threonine specific protein kinases- MAPK1/ERK1/2, JNK1-3, p38MAPK and ERK5. Activation of MAPK1 promotes cell survival in certain tissues by inhibiting proapoptotic proteins and by stimulating anti apoptotic factors.

Methodology: *In silico* docking studies was carried out using bioinformatics tools. The active compounds (Trihomovitamin D3; 2Z,6Z,8Z,12E Hexadecatetraenoic acid, Methyl prednisolone, Hydroxysalmeterol and Tridesacetoxymivorin) of *P. acidus* aqueous fruit extract were docked against MAPK1 resulting in receptor-ligand complex.

Results: The binding energy is correlated with the probability of affinity and stable bound between ligand and its receptor.

Conclusion: The molecular docking study of selective bioactive compounds of PAFAE with MAPK1 protein revealed that Tridesacetoxymivorin and Methyl Prednisolone, is having good interaction in favorable pose with MAPK1 as shown from their effective binding energy (-7.79kcal/mol and -7.19 kcal/mol), strong bond length and interactions with active site of MAPK1.

Keywords: LCMS; docking; bioactive compounds; MAPK1; Autodock; PAFAE.

INTRODUCTION

Star gooseberry (*Phyllanthus acidus* L.) is a crisp, sour, juicy edible fruit. The plant is found throughout Asia, Caribbean, Central and South America. The plant has wide range of its use from culinary to medicinal. Conventionally, it is used in the treatment of liver disease, bronchitis, urinary tract disease, rheumatism, constipation, diabetes, etc., (1). Further, the plant is reported to possess antimicrobial, anti-inflammatory, anti-nematodal, hypolipidemic, hypoglycemic, antihypertensive, antioxidant and hepatoprotective properties (2-9). However, the antiapoptotic activity of the plant remains unexplored as per literature, which is indeed significant in the context of “oxidative stress induced cell death”, an implication of several chronic diseases.

Fig. 1: *Phyllanthus acidus* L.

Cellular signaling pathways that involve protein kinases play critical roles in determining the balance between cell death and survival (10). One such pathway is mitogen-activated protein kinase (MAPK) signaling pathway. MAPK are serine/threonine specific protein kinases that respond to varied extracellular stimuli like mitogens, osmotic stress, heat shock, proinflammatory cytokines, growth factors, etc. and regulate various cellular activities including development, differentiation, proliferation, inflammatory responses and apoptosis. The three well-known MAPK pathways in mammalian system are extracellular signal-regulated kinases (ERKs1/2), c-Jun N-terminal kinases (JNKs1/2/3), and p38-MAPKs. MAPK1 or ERK1/2, plays an “anti-apoptotic role” by the downregulation of pro-apoptotic proteins and upregulation of anti-apoptotic proteins through both transcriptional and post-translational mechanisms (11).

Hence, the aim of this work was to carry out an *in silico* docking of selective compounds of aqueous extract of fruit of *P. acidus* (PAFAE) against MAPK1, a prime protein target involved in cell survival and apoptosis; so as to find the best compound/phytochemical among the selected that could target MAPK1 and therefore will be useful for controlling oxidative stress induced cell death.

Nowadays, the use of bioinformatic tools, before doing wet lab activities to determine the binding of datasets of small molecules to known receptors is a major component of drug discovery as it is cost effective and time saving (12-14).

MATERIALS AND METHODS

Selection of Ligands

In the present study, five bioactive compounds- Methyl Prednisolone, Tridesacetoxykhivirin, Hydroxy salmeterol, 2Z,6Z,8Z,12E Hexadecatetraenoic acid and Trihomovitamin D3 identified in PAFAE using Liquid Chromatography Mass Spectroscopy (LCMS) served as ligands (15).

They were further docked with MAPK1 using automated docking software (16). Before docking, the 3D structure of both ligands and the target protein in pdb format were retrieved using the following bioinformatics tools:

UniProt

UniProt is a comprehensive, high-quality, freely accessible resource of protein sequence and functional information. (<http://www.uniprot.org/>). The sequence of the target protein (MAPK1) was retrieved from UNIPROT database.

PDB

The Protein Data Bank (PDB) is a repository for the 3-D structural data of large biological molecules, such as proteins and nucleic acids (<http://www.rcsb.org/pdb/home/home.do>). The crystal structure of ERK2 (MAPK1) was retrieved from PDB database.

PubChem

PubChem is the world's largest collection of freely accessible chemical information. (<http://pubchem.ncbi.nlm.nih.gov/>). The three-dimensional structure of the bioactive compounds

selected for the present study were retrieved from PubChem database in SDF and MOL formats.

ACD/ChemSketch

ACD/ChemSketch is an easy-to-use, molecular structure drawing application used worldwide. (<https://www.acdlabs.com>). The structure of some of the compounds/ligands under study which were not found in PubChem, were drawn using ACD Chems sketch.

Open Babel Converter

Open Babel converter is used for converting various chemical file formats. (<http://openbabel.org/docs/current/Introduction/goals.html>). In the present study, the compound structure saved in.mol format are converted to .pdb format using Open Babel molecular converter tool.

Autodock

AutoDock is molecular modeling simulation software used especially for protein-ligand docking studies (<http://autodock.scripps.edu/resources/tools>). In the present study, the binding efficiency between the ligands and the target protein was identified using Autodock.

Discovery Studio Visualizer

Molecular visualization is a key aspect of the analysis and communication of modeling studies. The hydrogen bond interaction between compound and MAPK1 were visualized using Discovery Studio Visualizer. (<http://accelrys.com/products/discovery-studio/visualization.html>).

RESULTS

Protein sequence retrieval: MAPK1

The sequence of the target protein MAPK1 was retrieved from UNIPROT database and its ID is P28482. Gene name is MAPK1 from organism Homo sapiens (Human).

UniProtKB - P28482 (MK01_HUMAN)

Protein | Mitogen-activated protein kinase 1
Gene | MAPK1
Organism | Homo sapiens (Human)
Sequence features | View only features (sites, domains, PTMs ...)
Status | Reviewed - Annotation score: ●●●●● - Experimental evidence at protein level¹

Display | None | BLAST | Align | Format | Add to basket | History

Function¹

Serine/threonine kinase which acts as an essential component of the MAP kinase signal transduction pathway. MAPK1/ERK2 and MAPK3/ERK1 are the 2 MAPKs which play an important role in the MAPK/ERK cascade. They participate also in a signaling cascade initiated by activated KIT and KITLG/SCF. Depending on the cellular context, the MAPK/ERK cascade mediates diverse biological functions such as cell growth, adhesion, survival and differentiation through the regulation of transcription, translation, cytoskeletal rearrangements. The MAPK/ERK cascade plays also a role in initiation and regulation of meiosis, mitosis, and postmitotic functions in differentiated cells by phosphorylating a number of transcription factors. About 160 substrates have already been discovered for ERKs. Many of these substrates are localized in the nucleus, and seem to participate in the regulation of transcription upon stimulation. However, other substrates are found in the cytosol as well as in other cellular organelles, and those are responsible for processes such as translation, mitosis and apoptosis. Moreover, the MAPK/ERK cascade is also involved in the regulation of the endosomal dynamics, including lysosome processing and endosome cycling through the perinuclear recycling compartment (PNRC); as well as in the fragmentation of the Golgi apparatus during mitosis. The substrates include transcription factors (such as ATF2, BCL6, ELK1, ERF, FOS, HSF4 or SPZ1), cytoskeletal elements (such as CANX, CTTN, GJA1, MAP2, MAPT, PKN, SORBS3 or STMN1), regulators of apoptosis (such as BAD, BTG2, CASP9, DAPK1, IER3, MCL1 or PPARG), regulators of translation (such as EIF4EBP1) and a variety of other signaling-related molecules (like ARHGEF2, DCC, FRS2 or GRB10). Protein kinases (such as RAF1, RPS6KA1/RSK1, RPS6KA2/RSK2, RPS6KA3/RSK3, RPS6KA6/RSK4, SYK, MKNK1/MNK1, MKNK2/MNK2,

Fig. 2: UNIPROT result page of MAPK1

Structure retrieval

The Crystal structure of ERK2 (MAPK1) was retrieved from PDB database and its PDB ID is 2OJJ. The structure of MAPK1 visualized using RASMOL

tool (Figure 4). Pink Color coil indicates α -helix; Yellow color arrow indicates β -sheets; White color indicates turns.

Fig. 3: PDB result page of MAPK1

Fig. 4: Three-dimensional structure visualization of MAPK1 using RASMOL

Preparation of ligands

Five bioactive compounds/ligands to be used for the present docking study were identified via LCMS from PAFAE and were selected based on their

pharmacological property. The structure and molecular formula of the compounds of PAFAE is shown in Table 1.

Table 1: 2D and 3D structure of bioactive compounds extracted from *P. acidus* fruits

Sl. No	Compound name	Molecular formula	Molecular weight	2D	3D
1	Methyl Prednisolone	C ₂₄ H ₃₆ O ₆	416.507
2	Tridesacetoxykhivorin	C ₂₆ H ₃₆ O ₇	460.559
3	Hydroxy salmeterol	C ₂₅ H ₃₇ NO ₅	431.564
4	2Z,6Z,8Z,12Ehexadeca tetraenoic acid	C ₁₆ H ₂₄ O ₂	248.360
5	Trihomovitamin D3	C ₃₂ H ₅₀ O ₂	498.737

Molecular docking analysis

Using AutoDock 4.2, molecular docking analysis was performed so as to ascertain the binding conformation of the protein–ligand complex. The binding

conformation would in turn help to reveal the binding energy of the MAPK1 and the selected ligands (17).

Table 2: The docking interactions between MAPK1 and Trihomovitamin D3

MAPK1		TRIHOMOVITAMIN	Distance (Å)	Docking Energy
Residue	Atom	D3		(Kcal/Mol)
MET106	O	H	2.09	-6.15
ASP104	O	H	1.94

Fig. 6: (a) Docking score (b) Interaction between MAPK1 and 2Z,6Z,8Z,12E Hexadecatetraenoic acid as visualized using Autodock (c) Visualization of Hydrogen interaction between MAPK1 and 2Z,6Z,8Z,12E Hexadecatetraenoic acid using Acceryl Discovery Studio Visualizer (MAPK1-blue colour; Ligand-Purple colour; Hydrogen bonding- green colour dotted lines)

Table 3: The docking interactions between MAPK1 and 2Z,6Z,8Z,12E Hexadecatetraenoic acid

MAPK1		2Z,6Z,8Z,12E Hexadecatetraenoic acid	Distance (Å)	Docking Energy (Kcal/Mol)
Residue	Atom			
LYS229	NZ	O	2.59	-4.75
LYS229	NZ	O	2.84

Fig. 7: (a) Docking score (b) Interaction between MAPK1 and Methyl Prednisolone as visualized using Autodock (c) Visualization of Hydrogen interaction between MAPK1 and Methyl Prednisolone using Acceryl Discovery Studio Visualizer (MAPK1-blue colour; Ligand-Purple colour; Hydrogen bonding- green colour dotted lines)

Table 4: The docking interactions between MAPK1 and Methyl Prednisolone

MAPK1		Methyl Prednisolone	Distance (Å)	Docking Energy (Kcal/Mol)
Residue	Atom			
ASP165	OD1	H	2.00	-7.18
MET106	N	O	2.65	
ASN152	ND2	O	2.75

Fig. 8: (a) Docking score (b) Interaction between MAPK1 and Hydroxy Salmeterol as visualized using Autodock (c) Visualization of Hydrogen interaction between MAPK1 and Hydroxy Salmeterol using Acceryl Discovery Studio Visualizer (MAPK1-blue colour; Ligand-Purple colour; Hydrogen bonding- green colour dotted lines)

Table 5: The docking interactions between MAPK1 and Hydroxy Salmeterol

MAPK1		Hydroxy Salmeterol	Distance (Å)	Docking Energy (Kcal/Mol)
Residue	Atom			
ASP109	OD2	H	2.16	-3.16
MET106	N	O	2.83

Fig. 9: (a) Docking score (b) Interaction between MAPK1 and Tridesacetoxykhivorin as visualized using Autodock (c) Visualization of Hydrogen interaction between MAPK1 and Tridesacetoxykhivorin using Acceryl Discovery Studio Visualizer (MAPK1-blue colour; Ligand-Purple colour; Hydrogen bonding- green colour dotted lines)

Table 6: The docking interactions between MAPK1 and Tridesacetoxykhivorin

MAPK1		Tridesacetoxykhivorin	Distance (Å)	Docking Energy (Kcal/Mol)
Residue	Atom			
LYS52	NZ	O	3.15	-7.79
CYS164	SG	O	3.20	
ASP109	OD2	H	2.20	
MET106	N	O	2.82	
MET106	O	H	1.78	

Table 7: Overall Docking results of MAPK1 with bioactive compounds of *P. acidus* aqueous fruit extract

Sl. No.	Compound names	No. of Hydrogen bonds formed	Docking Energy	Key atoms of MAPK1
1	Methyl Prednisolone	3	-7.18	ASP165; MET106; ASN152
2	Tridesacetoxykhivorin	5	-7.79	LYS52; CYS164; ASP109; MET106
3	Hydroxy salmeterol	2	-3.16	ASP109, MET106
4	2Z,6Z,8Z,12Ehexadecatetraenoic acid	2	-4.75	LYS229
5	Trihomovitamin D3	2	-6.15	MET106, ASP104

DISCUSSION

After successful docking of the bioactive compounds one by one with the target protein, the receptor/ligand complex models generated was analyzed based on hydrogen bond interactions, binding energy and orientation of the docked compound within the active site of the target protein, MAPK1 (18, 19, 20).

The range of binding affinities of the five selected bioactive principles from PAFAE with MAPK1 was found to be: - 7.18 kcal/mol for Methyl Prednisolone; - 7.79 kcal/mol for Tridesacetoxykhivorin; -3.16 kcal/mol for Hydroxy Salmeterol; -4.75 kcal/mol for 2Z,6Z,8Z,12Ehexadecatetraenoic acid and -6.15 kcal/mol for - Trihomovitamin D3. Among these, Tridesacetoxykhivorin with maximum binding affinity of - 7.79 kcal/mol has been found to form five hydrogen bonds with LYS52; CYS164; ASP109; MET106 amino acid residues of MAPK1 protein. In the present study, Methyl Prednisolone (- 7.18 kcal/mol) and Trihomovitamin D3(- 6.15 kcal/mol) from PAFAE is also found to have higher binding affinity to MAPK1. However, the dock score of two compounds from aqueous fruit extract of *P. acidus* was found to be less favourable; - 3.16 kcal/mol for Hydroxy salmeterol and

-4.75 kcal/mol for 2Z,6Z,8Z,12Ehexadecatetraenoic acid.

Thus, the binding energy of Tridesacetoxykhivorin and Methyl Prednisolone with MAPK1, as obtained from analysis of molecular docking supports a possible antiapoptotic role of PAFAE compounds. It is suggested so, based on the finding that MAPK1 inhibits apoptosis upon activation although the mechanism varies with cell types. MAPK1 promotes cell survival by inhibiting pro apoptotic proteins (BAD) and by activating anti apoptotic factors like BCL2 and MCL1 (21).

CONCLUSION

In conclusion, the results of the present study clearly demonstrated the *in silico* molecular docking studies of Methyl Prednisolone, Tridesacetoxykhivorin, Hydroxy salmeterol, 2Z,6Z,8Z,12E Hexadecatetraenoic acid and Trihomovitamin D3 from PAFAE with MAPK1 and their binding interactions. From the present *in silico* analysis, it could be inferred that the secondary metabolites, Tridesacetoxykhivorin and Methyl Prednisolone of *Phyllanthus acidus* aqueous fruit extract (PAFAE) are capable of interacting with active binding site of the

target protein MAPK1 with maximum energy and may serve as a natural therapeutic agent in treating oxidative stress induced cell death in chronic diseases. However, these results are only preliminary screening just to facilitate subsequent *in vitro* and *in vivo* studies and thus warrants further investigation.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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