RESEARCH ARTICLE

In-silico Assessment of Potent Immunomodulators from *Pimpinella anisum* and its Antioxidant Potential

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ABSTRACT

New targets, effective substances, and safety in pharmacological models are highlighted by recent drug development. The study's primary goal is to use an *in-silico* technique to determine the immunomodulatory potential of phytoconstituents from *Pimpinella anisum.* Several computer aided tool were utilized for *in-silico*. autodock vina is major free tool utilized to examine a drug discovery strategy based on the atomic-level screening of small compounds and proteins and protein-ligand interactions. Basic docking phases include ligand position, orientation, and binding affinities. The current methodology includes ligand choice, protein prep, target, ligand optimisation, target active binding site analysis, and binding affinity. SwissADME was used to assess the pharmacokinetic parameters, and Lipinski's "rules" were used to assess drug similarity. In the current research paper six ligands such as P-anisaldehyde, trans-anethole, cis-anethole, estragole, and linalool were dock against two proteins 1M48 and 1P9M. Molecular docking studies suggest strong binding affinity between -6.9 to -4.2 in case on 1M48 and -6.2 to -4.7 in case of 1P9M. Further antioxidant potential of *P. anisum* using several solvents was determined. *In-vitro* antioxidant study and *in-silico* screening suggested that *P. anisum* ethanolic extract can be contributed in immunomodulation.

Keywords: *In-silico, In-vitro* antioxidant**,** Autodock vina, immunomodulatory, Docking, *Pimpinella anisum*, DPPH.

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INTRODUCTION

The Latin word immunitas, which meant to be excused from military service, is where the word immunity originated. Immunity is a powerful defense system in the body that guards against infectious disease.[1] It consists of elements of both innate and adaptive immunity, the latter of which is more focused. Herbal plants have been used in traditional and alternative medicine to treat illnesses since the dawn of humanity.[2] Natural remedies are 'Gifted Gods' for healing, helping, and rehabilitating patients. The modulation of immune function may also be influenced by the main and secondary metabolites of plants. The ancient systems of medicinal play a vital role in medicine and healthcare sector. *Pimpinella anisum* a well known herb and spice in ayurvedic and unani, system of medicine.[3] Majorly this herb used as flavouring agent, carminative and stimulatant, besides that is used in asthma, coughs, flatulence, diuretic, and menopause as a remedial treatment.[4] *P. anisum* having several major phyto-constituents including *trans*-anethole, estragole, (E)-methyleugenol, α-cuparene, α-himachalene, β-bisabolene, *p*-anisaldehyde, phenylpropanoid, and *cis*-anethole.[5-7] In recent times, molecular docking has taken over as the primary computer-aided drug design tool for analyzing new ligands, their positions and orientations, binding affinities, and small molecule behavior at the target protein's binding site.[8-11] The current study aimed to scrutinize a *in-silico* interaction of chosen five compounds against protein 1M48 and 1P9M. Basides that the *in-vitro* antioxidant study of extract was carried out using DPPH assay.

MATERIAL AND METHOD

Molecular Docking

Software

For detail study of protein ligand interation Autodock_vina an computer aided software was employed (https://vina.scripps.edu).[12-13] The protein binding was visualized using BIOVIA Discovery Studio.[14] Open bable 3.1.1 software was employed for file conversion.[15]

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Selection of protein

1M48 and 1P9M were selected as protein and the 3D structured protein file in PDB format were downloaded from http://www.rcsb.org.

Selection of ligand

From *P. anisum* total five major constituents were selected as ligand on its phytochemical basis such as anisaldehyde, trans-anethole, cis-anethole, estragole, and linolool. Two dimenstional and three dimenstional structures of these ligands are excluded from pubchem.

Protein preparation

The binding pockets of protein was observed using CASTp tool. The whole development of protein was carried out using BIOVIA Discovery Studio.[14] Het-atoms deletion, water molecules removal are necessary steps to ultimate rise in resolution. Afterwards the ligand groups was deleted, polar hydrogen atoms, Kollman charges were added. The final file was saved in PDB format in BIOVIA Discovery Studio.[16-17]

Protein ligand interaction

Ligand was entrenched with protein file which as saved in PDBQT format utilizing Autodock Vina. Command prompt is an extremely important step in while docking process along with nine binding modes and eight set as exhaustiveness which was accepted universally. Detailed protein ligand interactions were observed using Biovia Discovery Studio.

In-silico ADME Profiling

For pharmacokinetic profiling of selected ligands Swiss ADME (http://www.swissadme.ch/index.php) software was employed.[18,19] Ligands were targeted for ADME prediction where conical smiles serve as key. Furthermore Lipinski rule of five predict drug likeness of any substance.[20]

In-vitro Antioxidant Study

Preparation of extracts

For the determination of antioxidant profile of *P. anisum* the extraction of crude drug along with several solvants were carried out. Cold maceration method of extraction is chosen for extraction while hexane, ethyl acetate, chloroform, ethanol, methanol and water were selected as solvants. After 7 days, the extracts i.e PAH (P. *anisum* hexane extract), PAEA (P. *anisum* ethyl acetate extract), PAC (*P. anisum* chloroform extract), PAE (P. *anisum* ethanolic extract), and PAM (*P. anisum* methanolic extract) were collected and stored in cool condition until its further use.

DPPH assay

In 96 well method was employed to examine *P. anisum* antioxidant potential. 100 µL of PAH, PAEA, PAC, PAE, and PAM were screened in the microtiter plate, 100 µL of 0.1% methanolic DPPH was mixed with test solution and incubated for 30 minutes in dark ascorbic acid was served as standard, plates were read on ELIZA's plate reader at 490 nm.

RESULTS

Several major protein were avaliable at RCSB for screening of immunomodulatory compounds. PDB ID: 1M48 **(**Crystal Structure of Human IL-2 Complexed with (R)-N-[2-[1-(Aminoiminomethyl)-3-piperidinyl]-1-oxoethyl]-4-(phenylethynyl)-L-phenylalanine methyl ester) [area (SA) 4126.492 and (SA) Å3 volume 4577.551] while PDB ID:1P9M (Crystal structure of the hexameric human IL-6/IL-6 alpha receptor/gp130 complex [area (SA) 1562.214 and (SA) Å3 volume 917.876] were considered to screen its immunomodulatory effect. The active binding pockets were observed Figures 1 and 2, respectively

Recognize how tiny molecules and pharmacophores interact for logical drug discovery and design. Put the ligand into the preferential binding areas of the target protein, where it may combine with the receptor to create a complex. While performing the molecular docking study basic information was mandatory. The constituents selected as ligand and chemical databases were described in Table 1.

Tables 2 and 3 indicate 2D and 3D representation of five constituents against 1M48 and 1P9M, respectively. 1M48 represents IL-2 and shows interaction with several amino acid residues including LEU72, ALA73, LYS35, ARG38, MET39, PHE42, and PRO65 at the chain A position of protein. Amongst all the compounds, estragole shows the highest binding affinity -6.9 kcal/mol. The detail amino acid residues and binding affinity for each compound was specified in Tables 4 and 5 for PDB ID: 1M48 and 1P9M respectively.

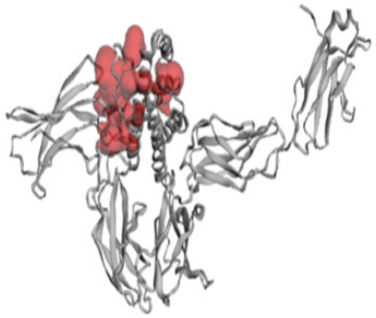


Figure 1: 1M48 binding pocket

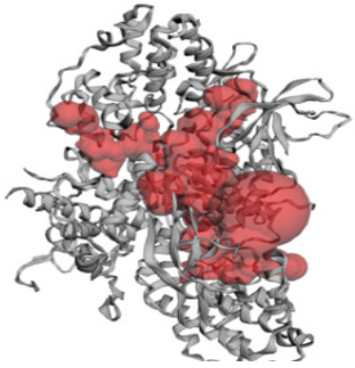


Figure 2: 1P9M binding pocket

Table 1: Chemical database of selected immunomodulatory constituents from *P. anisum*

Name	*Pubchem CID*	*Molecular wt (g/mol)*	*Molecular formula*	*Canonical smiles*
P-Anisaldehyde	31244	136.15	$C_8H_8O_2$	COC1=CC=C(C=C1)C=O
Cis-anethole	1549040	148.20	$C_{10}H_{12}O$	CC=CC1=CC=C(C=C1)OC
Trans-anethole	637563	148.20	$C_{10}H_{12}O$	CC=CC1=CC=C(C=C1)OC
Estragole	8815	148.20	$C_{10}H_{12}O$	COC1=CC=C(C=C1)CC=C
Linolool	6549	154.25	$C_{10}H_{18}O$	CC(=CCCC(C)(C=C)O)C

Table 2: Protein ligand interation dababase of selected ligand against protein 1M48

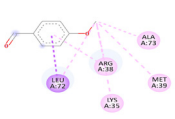
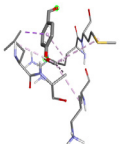
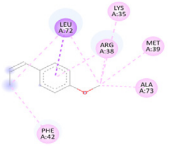
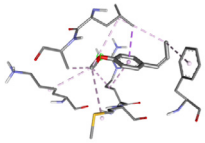
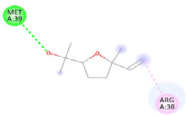
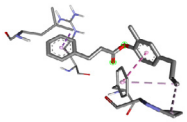
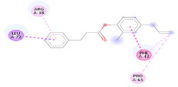
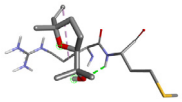
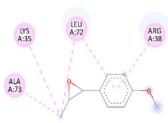
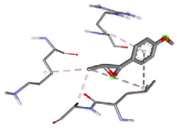
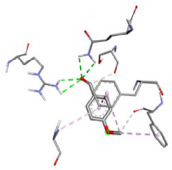
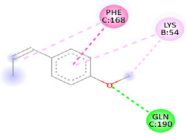
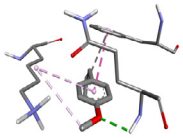
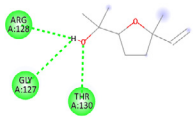
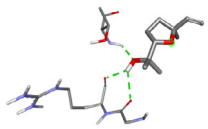
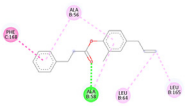
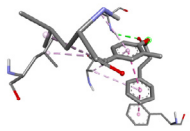
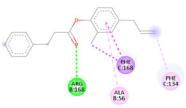
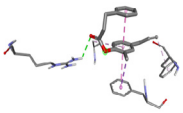
Compound	*2D Structure*	*3D Structure*
P- Anisaldehyde		
Cis-anethole		
Linolool		
Estragole		
Trans-anethole		

Table 3: Protein ligand interation dababase of selected ligand against protein 1P9M

Compound	*2D Structure*	*3D Structure*
P-Anisaldehyde		
Cis-anethole		
Linolool		
Estragole		
Trans-anethole		

Additionally, 1P9M represents IL-6 and shows remarkable binding interation with amino acid residues such as ARG168, GLN190, PHE134, SER166, PHE168, ALA56, LYS54, THR130, GLY127, ARG128, ALA58, PHE168, LEU64, and LEU165 at the chain B position of protein. From all the molecular docking data for protein 1P9M estragole shows the highest binding affinity -6.2 kcal/mol. The detailed amino acid residues and binding affinity for each compound was specified in table

A bar diagram is drawn for better understating of binding affinities between 1M48 and 1P9M protein in Figure 3. This diagram represents the suitable phytoconstituents with promising binding affinity towards IL2 and IL6. IL-2 is known for its T-cell activation in immune functioning. On

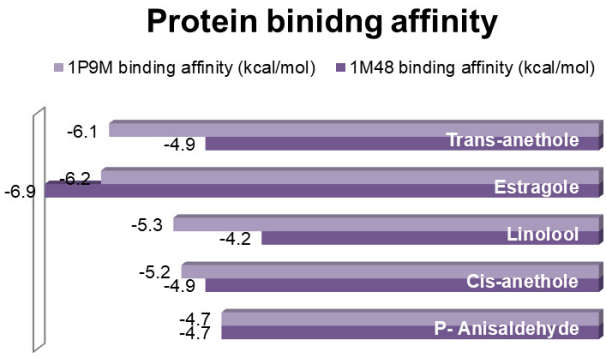


Figure 3: Protein binding affinity of selected compounds against 1M48 and 1P9M

Table 4: Involvement of amino acid residue and binding affinity for protein 1M48

Compound Name	*Binding energy (Kcal/mol)*	*Bond*	*Amino acid residues*
P- Anisaldehyde	-4.7	Hydrophobic	LEU72, ALA73, LYS35, ARG38, MET39
Cis-anethole	-4.9	Hydrophobic	LEU72, ALA73, LYS35, ARG38, MET39, PHE42
Linolool	-4.2	Hydrogen Bond Hydrophobic	MET39, ARG38
Estragole	-6.9	Hydrophobic	A:LEU72, PHE42, PRO65, ARG38
Trans-anethole	-4.9	Hydrophobic	ALA73, LYS35, LEU72, ARG38

Table 5: Involvement of amino acid residue and binding affinity for protein 1P9M

Compound name	*Binding energy (Kcal/mol)*	*Bond*	*Amino acid residues*
P- Anisaldehyde	-4.7	Hydrogen Bond Hydrophobic	ARG168, GLN190, PHE134, SER166, PHE168, ALA56
Cis-anethole	-5.2	Hydrogen Bond Hydrophobic	GLN190, PHE168, LYS54
Linolool	-5.3	Hydrogen Bond	THR130, GLY127, ARG128
Estragole	-6.2	Hydrogen Bond Hydrophobic	ALA58, PHE168, LEU64, LEU165, ALA56
Trans-anethole	-6.1	Hydrogen Bond Hydrophobic	ARG168, PHE168, PHE134, ALA56

Table 6: Percentage of DPPH radical scavenging for *Pimpinella anisum* extracts

Extract	*Concentration (µg/mL)*	*Percentage of DPPH radical scavenging*
P. anisum hexane (PAH)	1000	44.72
P. anisum ethyl acetate (PAEA)	1000	39.40
P. anisum chloroform (PAC)	1000	42.18
P. anisum ethanol (PAE)	1000	56.04
P. anisum methanol (PAM)	1000	50.78
P. anisum aqueous (PAA)	1000	55.01
Ascorbic acid	1000	61.16

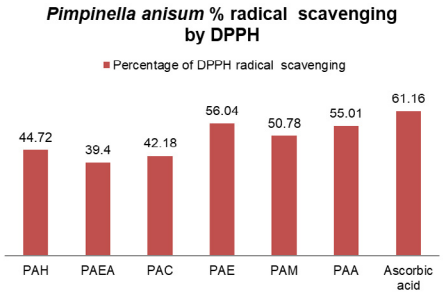


Figure 4: Percentage of radical scavenging using DPPH assay

contrary IL-6 act as pro-inflammatory and anti-inflammatory cytokine.[21-23]

Besides *in-silico* molecular docking study the pharmacokinetic study were carried out utilizing a software naimg swiss ADME. All the five major constituents of *P. anisum* were screened for its absorption, distribution, metabolism, and elimination and results concluded that not a single drug shows toxicity nor interference in metabolism and all passes Lipinski rule. Due to this prediction based on *in-silico* study, the entire compounds can serve as a candidates for further pharmacological evaluation for screening its immunomodulatory potential.

The antioxidant potential of several extracts was estimated using DPPH assay and results shows that ethanolic extract of *P. anisum* shows good antioxidant potential. In this study, ascorbic acid serve as standard; the final concentration was 1000 µg/mL. the detail radical scavenging data was expressed in Table 6, while the comparison between different extracts was collectively represented in Figure 4 as a chart.

CONCLUSION

The research work emphasized on the immunomodulatory potential of *P. anisum* and its major phytoconstituents. To screen its immunomodulatory potential *in-silico* molecular docking, the study was performed using two proteins naming 1M48 and 1P9M. PDB ID: 1M48 represents IL2 and PDB ID: 1P9M represents IL-6 both are secreted during immunomodulatory activity. In conclusion, in molecular docking study estragole shows remarkable binding affinity against both proteins. Furthermore, the *in-vitro* antioxidant study was carried out using DPPH assay and results concluded that ethanolic extract of *P. anisum* has the highest antioxidant potential and that the aqueous extract also possesses remarkable antioxidant potential. Co-relating both the data, the ethanolic extract of *P. anisum* can be further screen for its *in-vitro* and *in-vivo* immunomodulatory potential targeting its effect on IL2.

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