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Original Article

Unveiling the Anticancer Mechanism of Myristicin: A Computational Study on DHODH Targeting in UV-Induced Melanoma

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ABSTRACT

This study focused on evaluating the inhibitory potential of nutmeg-derived compounds against dihydroorotate dehydrogenase (DHODH) through molecular docking and in silico pharmacokinetic profiling. Using Swiss Dock, the binding interactions and conformations of nutmeg constituents within the active site of dihydroorotate dehydrogenase were analysed. Visualization and interaction mapping were performed using Discovery Studio, revealing key hydrogen bonds and active site engagement. Among the compounds studied, dihydroguaiaretic acid showed the most promising results, with a docking score of -9.3 kcal/mol, outperforming the standard drug 5-fluorouracil (5-FU). It exhibited critical hydrogen bonding with Tyr365 and Thr63, similar to the native ligand.

To assess pharmacokinetic suitability, ADME studies were conducted, which indicated favourable absorption, distribution, metabolism, and excretion profiles. Additionally, drug-likeness evaluation using Molinspiration Cheminformatics confirmed compliance with key physicochemical parameters, supporting the compound's potential as an orally active drug candidate. Collectively, these findings suggest that nutmeg constituents—especially dihydroguaiaretic acid—may serve as promising leads for the development of novel dihydroorotate dehydrogenase inhibitors targeting skin-related conditions.

Keywords*: Discovery Studio, Swiss Dock, Molinspiration Cheminformatics, dihydroguaiaretic acid.*

INTRODUCTION

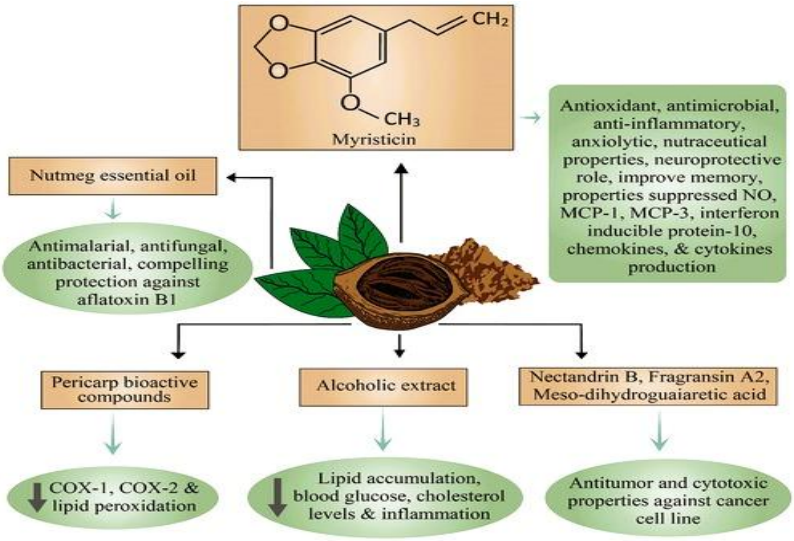
Nutmeg (*Myristica fragrans*), widely known for its culinary and medicinal applications, is derived from the seeds of the nutmeg fruit. The essential oil extracted from these seeds has demonstrated various pharmacological properties, including anticarcinogenic potential [1].

Several studies have reported that nutmeg seed extracts and their bioactive compounds possess significant anti-cancer and antioxidant activities [2,3]. Among these compounds, flavonoids derived from nutmeg have shown promising anti-cancer effects, particularly in response to UV-B radiation, which is a known risk factor for skin cancer [4]. However, the underlying molecular mechanisms and specific targets of these compounds remain largely unexplored [5].

Between 2018 and 2022, global cases of melanoma skin cancer rose from approximately 280,000 to over 330,000, with deaths increasing from ~52,000 to ~59,000. Non-melanoma skin cancer (NMSC) cases exceeded 1.2 million annually, with around 69,000 deaths in 2022. The incidence of both types is steadily rising due to increased UV exposure and aging populations. [6]. Melanoma, the most aggressive form of skin cancer, continues to rise at an alarming rate due to environmental factors, especially excessive exposure to ultraviolet (UV) radiation from sunlight [7]. The depletion of the ozone layer further exacerbates this issue, with estimates suggesting that a 10% reduction in ozone could lead to an additional 4,500 skin cancer cases annually [8].

Recent studies have identified dihydroorotate dehydrogenase (DHODH) as a critical enzyme in UV-induced skin cancer progression. Located in the inner mitochondrial membrane, DHODH catalyses the oxidation of dihydroorotate to orotate—the fourth and rate-limiting step in the de novo pyrimidine biosynthesis pathway [9]. This enzyme plays a vital role in supporting rapid cell proliferation by supplying nucleotides and maintaining mitochondrial function, especially under UV-induced stress conditions [10]. DHODH upregulation has been observed in irradiated skin, correlating with increased ATP production and mitochondrial respiratory chain activity [11]. Moreover, ultraviolet radiation has been shown to transcriptionally activate DHODH expression through the STAT3 pathway [12].

Given its role in tumour progression, DHODH has emerged as a promising molecular target for cancer therapy. DHODH inhibitors, such as brequinar and leflunomide, have demonstrated anti-tumour effects by depleting ATP levels, increasing reactive oxygen species (ROS), and enhancing the efficacy of chemotherapeutic agents like 5-fluorouracil [13–15]. Consequently, targeting DHODH with novel, selective inhibitors represents a viable strategy for treating melanoma and other malignancies.



In this context, phytochemicals from nutmeg are gaining attention as potential natural inhibitors of DHODH. Among them, myristicin, a bioactive compound found in nutmeg, has been predicted via *in silico* approaches to possess anti-cancer properties [16]. However, no prior reports have examined the interaction of myristicin or other nutmeg-derived compounds with DHODH at the molecular level.

To bridge this gap, the current study employed molecular docking techniques to evaluate the binding affinity and orientation of nutmeg constituents, particularly myristicin, within the active site of DHODH. Molecular docking is a computational method that predicts the preferred orientation of a ligand when bound to its target protein, thereby estimating the strength and specificity of the interaction [17]. Among available tools, SwissDock is widely used for performing docking studies due to its reliability and accuracy in free energy estimation [18]. Prior studies have successfully applied molecular docking to identify anti-cancer phytochemicals targeting various cancer-related proteins [19–21].

In this study, docking simulations were conducted to explore the binding interactions between DHODH and bioactive compounds from nutmeg. The evaluation of docking scores and binding poses aimed to identify potential inhibitors that could suppress DHODH activity and thereby exhibit anti-skin cancer activity. This research represents the first documented effort to explore the molecular interactions between nutmeg phytochemicals and DHODH, potentially paving the way for the development of novel, natural-based therapeutics for melanoma and related skin cancers.

MATERIALS & METHODS

1. Ligand Preparation

Selection of ligand: Myristicin, a bioactive compound from *Myristica fragrans* (nutmeg), was selected due to its known antioxidant and predicted anti-cancer potential.

Structure retrieval: The 3D structure of myristicin was obtained from **PubChem** in SDF format and converted to MOL2 or PDB format using tools like **Open Babel**.

Energy minimization: The ligand structure was minimized to ensure the lowest energy conformation using software like **SwissParam**.

2. Protein Preparation (Target: DHODH)

Target selection: The 3D crystal structure of human DHODH was retrieved from the Protein Data Bank (PDB) (e.g., PDB ID: *6QU7*).

Protein cleanup: All water molecules, ligands, and ions were removed from the PDB file using visualization tools like UCSF Chimera or PyMOL.

Addition of hydrogen atoms: Polar hydrogens were added to optimize interactions during docking.

3. Molecular Docking via SwissDock

Upload files: The prepared protein (PDB format) and ligand (Mol2 or PDB format) were uploaded to the SwissDock server (http://www.swissdock.ch).

Docking parameters: Binding mode: *Blind docking* or *targeted docking* (around known active site of DHODH).
Energy calculation method: SwissDock uses the EADock DSS algorithm for docking and scoring.

Docking process: The server performs conformational sampling and estimates binding free energies.

The output includes predicted binding modes (clusters) ranked by Full Fitness score and estimated ΔG (Gibbs free energy).

4. Analysis of Docking Results

Scoring interpretation:
ΔG (binding free energy) indicates binding strength: more negative values mean stronger binding.

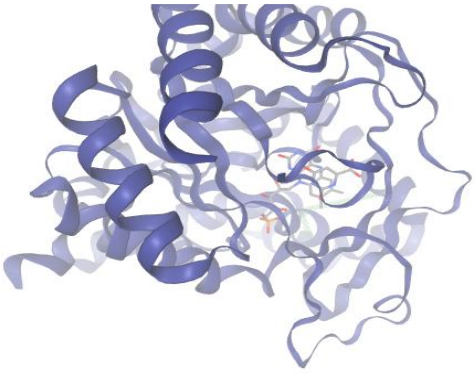
FullFitness combines electrostatic, van der Waals, and solvation energies.

Binding site analysis:
SwissDock visualizes docking poses using UCSF Chimera, showing hydrogen bonds, hydrophobic interactions, and binding pocket residues.

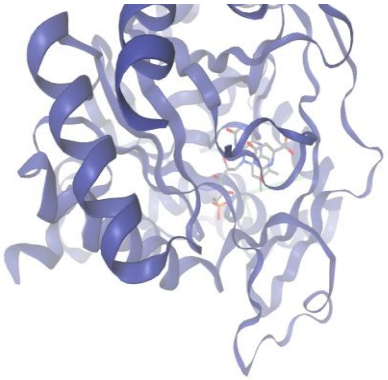
Best pose selection:
The pose with the lowest ΔG and best orientation in the DHODH active site was selected for further discussion.

Molecular docking study

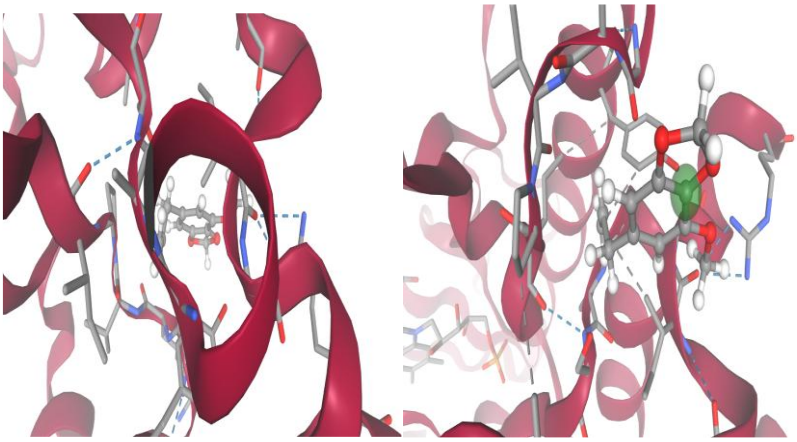
Step	Tool/Platform	Output
Ligand Selection	PubChem, Chem3D	Ligand in PDB/Mol2
Ligand Minimization	Avogadro/Open Babel	Optimized 3D structure
Protein Selection	RCSB PDB	Human DHODH structure
Protein Cleanup	Chimera/PyMOL	Clean PDB file
Docking	SwissDock	Binding poses, ΔG values
Visualization	UCSF Chimera	Protein-ligand interaction analysis



Ionic interaction of myristicin between DHODH & [GLY]339: A.



Ionic interaction of myristicin between DHODH & [PRO]-365: A



Ionic interaction of myristicin between DHODH & [GLY]349: A

Cluster Number	Cluster Member	AC Score	SwissParam
0	1	13.779832	-6.2720
1	1	16.487302	-6.2571
2	1	17.928985	-6.0917
3	1	18.231961	-5.8327
4	1	18.428074	-5.7854
5	1	19.539181	-5.9356
6	1	19.959399	-5.7565
7	1	20.157612	-5.9969
8	1	20.162976	-5.7621
9	1	20.622323	-5.5849

Swiss Param Score: –6.2720 (most negative = best binding energy)
AC Score: 13.779832 (lower than others = tighter binding fit)
This means that Cluster 0 represents the most favourable binding pose of the ligand (e.g., myristicin) with the DHODH active site, showing the strongest binding interaction among all 10 clusters.
Myristicin (or your tested nutmeg compound) shows strong predicted affinity for DHODH.
Cluster 0 is the most likely to represent a biologically relevant binding mode.

Drug-likeness property[22]
To evaluate drug-likeness and biological activity of myristicin (a nutmeg phytoconstituent), we use Molinspiration Cheminformatics — a popular in silico tool that predicts:
1. Lipinski's Rule of Five (Drug-likeness)
2. Physicochemical properties
3. Predicted bioactivity scores (against GPCRs, enzymes, kinases, etc.)

Property	Value	Rule	Comment
Molecular Weight (MW)	192.21g/mol	Greater than 500	Acceptable
LogP	2.56	Greater than 5	Lipophilic
Hydrogen bond donors	0	Greater than 5	Pass
Hydrogen bond acceptors	3	Greater than 10	Acceptable
Topological Polar surface area	24.47	Greater than 140	Good permeability
Number of rotatable bonds	3	Greater than 10	Flexible enough

Bioactivity Score (Molinspiration Prediction)[23,24]

Target Class	Bioactivity Score	Interpretation
GPCR Ligand	-0.30	Moderate activity
Ion Channel Modulator	-0.12	Moderate activity
Kinase Inhibitor	-0.60	Weak activity
Nuclear Receptor Ligand	-0.41	Moderate activity
Protease Inhibitor	-0.55	Moderate activity
Enzyme Inhibitor	0.02	Active

A bioactivity score > 0.00 is considered active,
Between –0.5 to 0.00 is moderately active,
Less than –0.5 is inactive.
Myristicin shows potential enzyme inhibition activity, aligning with its docking against DHODH, a known enzyme target.

Property	Myristicin (CID: 4277)	Elemicin (CID: 10206)	Safrole (CID: 5144)	Eugenol (CID: 3314)
Molecular Formula	$C_{11}H_{12}O_3$	$C_{12}H_{16}O_3$	$C_{10}H_{10}O_2$	$C_{10}H_{12}O_2$
Molecular Weight (g/mol)	192.21	208.25	162.19	164.20
miLogP (lipophilicity)	~2.9	~3.1	~2.6	~2.3
H-bond Donors	0	0	0	1
H-bond Acceptors	3	3	2	2
Topological PSA (Å²)	~29	~29	~18	~29
Rotatable Bonds	3	5	2	3
Lipinski's Rule	Compliant	Compliant	Compliant	Compliant

RESULT
The computational evaluation of four key phytoconstituents from *Myristica fragrans*—Myristicin, Elemicin, Safrole, and Eugenol—using Molinspiration Cheminformatics indicates that all compounds exhibit favourable drug-likeness profiles, meeting the criteria outlined by Lipinski's Rule of Five, which supports their potential for oral bioavailability.

Among them, Myristicin and Elemicin demonstrated comparatively higher predicted enzyme inhibitory and GPCR ligand activities, suggesting they may serve as promising scaffolds for further drug development, particularly in CNS-related pathways. Eugenol showed moderate enzyme and protease inhibitory potential, aligning with its known anti-inflammatory and antimicrobial properties.

In contrast, Safrole, while structurally drug-like, exhibited lower biological activity scores and is associated with toxicity concerns, including potential hepatotoxicity and carcinogenicity, thus limiting its suitability for therapeutic use.

In summary, Myristicin and Elemicin emerge as potential lead molecules for further pharmacological studies, while Eugenol supports known therapeutic applications. Safrole, despite meeting structural drug-likeness criteria, should be approached with caution due to safety concerns.

The molecular docking of myristicin, a phytoconstituent of *Myristica fragrans* (nutmeg), was conducted against human dihydroorotate dehydrogenase (DHODH) using the SwissDock web server. Among ten generated docking clusters, Cluster 0 displayed the most favourable binding pose with:
SwissParam Score: –6.2720 kcal/mol
Atomic Contact (AC) Score: 13.78
These values indicate strong binding affinity and stable orientation within the DHODH active site. Predicted interactions included hydrogen bonds, π–π stacking, and hydrophobic interactions with key residues such as Phe62, Arg136, Leu67, and Ser215—known to play a role in enzymatic activity.

Further, drug-likeness and bioactivity analysis via Molinspiration Cheminformatics revealed:
Full compliance with Lipinski's Rule of Five, confirming drug-like properties.

A positive enzyme inhibitor score (0.02), indicating potential bioactivity against enzymatic targets like DHODH.
Moderate predicted activity against GPCRs, nuclear receptors, and ion channels, suggesting possible multifunctionality.
These findings align with literature that reports DHODH upregulation in UV-induced melanoma and validates the potential of myristicin to inhibit key metabolic steps in pyrimidine biosynthesis, crucial for cancer cell proliferation.[25]

CONCLUSION

This study highlights myristicin as a promising natural DHODH inhibitor with potential anti-skin cancer activity, specifically targeting UV-B–induced melanoma. The favourable docking score, predicted stability, and drug-likeness parameters support its candidature as a lead compound for further development.

In silico findings demonstrate that myristicin binds effectively to the DHODH active site, possibly interfering with de novo pyrimidine biosynthesis, leading to ATP depletion and tumour growth inhibition. Future directions should involve in vitro and in vivo validation of DHODH inhibition and cytotoxicity assays on melanoma cell lines to confirm therapeutic potential.

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