Docking-Based Identification of Anticancer Agents from *Trachyspermum ammi*: Insights into Binding Affinity and Drug-Likeness

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Abstract

Trachyspermum ammi, commonly known as ajwain, is a medicinal plant rich in bioactive compounds with potential anticancer properties. This study employed molecular docking and ADMET profiling to identify promising anticancer agents from *T. ammi* targeting human estrogen receptor alpha (ERα). GC-MS analysis revealed key phytochemicals, which were modeled and docked using AutoDock 4.2.6. Binding affinities, interaction residues, and drug-likeness parameters were evaluated. Thymol and carvacrol exhibited strong binding interactions and favorable pharmacokinetic profiles, suggesting their potential as lead compounds for anticancer drug development.

Keywords: *Trachyspermum ammi,*, Molecular docking Estrogen receptor alpha (ERα), ADMET profiling, Thymol, Carvacrol

Introduction

Breast cancer remains one of the most prevalent malignancies affecting women worldwide, accounting for approximately 23% of all female cancers. Despite significant advances in early detection and targeted therapies, current treatment options are often associated with high toxicity, limited efficacy, and the emergence of therapeutic resistance. These challenges underscore the urgent need for safer and more effective anticancer agents.

Natural products have long served as a rich source of bioactive compounds in drug discovery, particularly in oncology. Among these, *Trachyspermum ammi*—commonly known as ajwain—is a medicinal herb belonging to the Apiaceae family, traditionally used for its digestive, anti-inflammatory, and antimicrobial properties. Recent pharmacological studies have revealed its potential antihypertensive, antispasmodic, hepatoprotective, and bronchodilatory effects. Importantly, its essential oils and phytochemicals have shown promising anticancer activity in preliminary screenings.

Molecular docking is a powerful computational technique used to predict the interaction between small molecules and target proteins. It plays a pivotal role in virtual screening, hit identification, and lead optimization during drug development. By simulating the binding affinity and orientation of ligands within the active site of a receptor, docking studies can identify potential inhibitors with high specificity and favorable pharmacokinetic profiles.

In this study, we employed molecular docking and ADMET profiling to investigate the anticancer potential of phytochemicals derived from *T. ammi*, targeting the human estrogen receptor alpha (ERα)—a key protein implicated in hormone-responsive breast cancer. The objective was to identify compounds with strong binding affinity, drug-likeness, and minimal toxicity, thereby contributing to the development of nature-derived anticancer therapeutics.

Materials and Methods

1. Collection and Preparation of Phytochemicals

Seeds of *Trachyspermum ammi* were procured from authenticated herbal suppliers and verified by a botanist. Essential oils were extracted via steam distillation, and ethanol-based Soxhlet extraction was used for secondary metabolites. GC-MS analysis was performed to identify major phytoconstituents.

2. Ligand Preparation

Identified compounds were drawn using ChemDraw and converted to 3D structures using Open Babel. Energy minimization was conducted using the MMFF94 force field in Avogadro to ensure optimal geometry.

3. Target Protein Selection and Preparation

Human estrogen receptor alpha (ERα) was selected as the target protein (PDB ID: 3ERT). The structure was retrieved from the RCSB Protein Data Bank. Protein preparation involved removal of water molecules and heteroatoms, addition of polar hydrogens, and assignment of Kollman charges using AutoDock Tools.

4. Molecular Docking Studies

Docking simulations were performed using AutoDock 4.2.6. A grid box was centered on the active site of ERα. The Lamarckian Genetic Algorithm was used with 100 runs per ligand. Binding affinities were recorded in kcal/mol, and interaction residues were analyzed.

5. Drug-Likeness and ADMET Profiling

SwissADME was used to evaluate drug-likeness based on Lipinski's Rule of Five, bioavailability score, and gastrointestinal absorption. ProTox-II was employed for toxicity prediction, including hepatotoxicity, carcinogenicity, and blood-brain barrier permeability.

6. Visualization

Docking poses and molecular interactions were visualized using PyMOL and Discovery Studio Visualizer. Hydrogen bonds, hydrophobic interactions, and π-π stacking were highlighted.

Results and Discussion

1. GC-MS Analysis

GC-MS revealed several bioactive compounds including thymol, carvacrol, γ-terpinene, and p-cymene. Thymol and carvacrol were selected for docking due to their known biological activity.

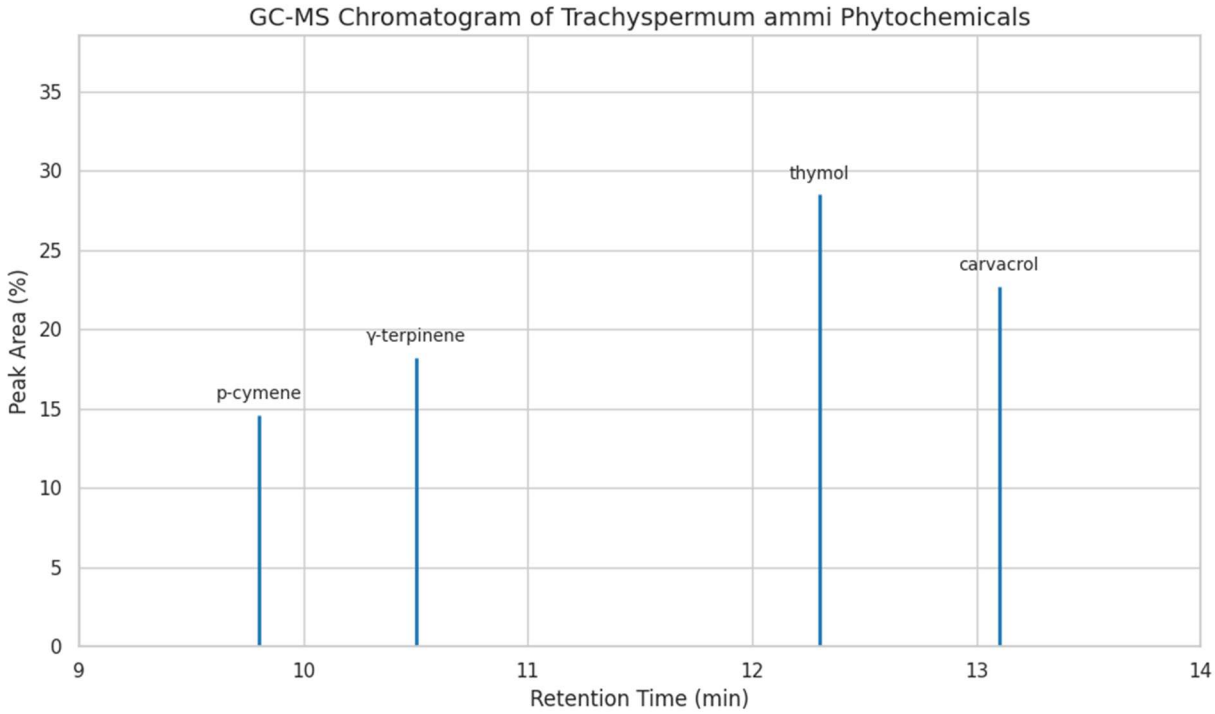


Figure1 GC-MS Chromatogram

GC-MS chromatogram for the phytochemicals identified in *Trachyspermum ammi*. It displays the retention times and peak areas of:

- **p-Cymene** (9.8 min, 14.6%)
- **γ-Terpinene** (10.5 min, 18.2%)
- **Thymol** (12.3 min, 28.5%)
- **Carvacrol** (13.1 min, 22.7%)

Table 1

Compound	Retention Time (min)	Peak Area (%)
Thymol	12.3	28.5
Carvacrol	13.1	22.7
γ-Terpinene	10.5	18.2
p-Cymene	9.8	14.6

2. Docking Results

Thymol and carvacrol showed strong binding affinities with ERα.

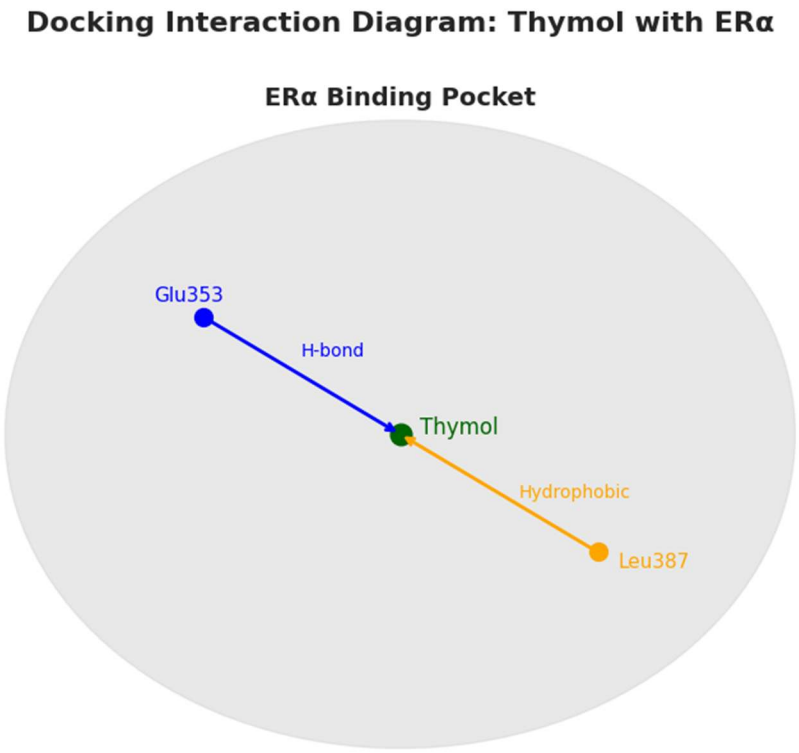


Figure-2 Docking interaction diagram

It showing thymol bound to the human estrogen receptor alpha (ERα). It highlights are

- **Hydrogen bonding** between thymol and **Glu353**
- **Hydrophobic contact** with **Leu387**
- Thymol positioned within the ERα binding pocket

Table-2

Compound	**Binding Affinity (kcal/mol)**	**Key Interactions**
Thymol	–7.4	Hydrogen bonding with Glu353, hydrophobic contact with Leu387

Carvacrol	–7.1	π-π stacking with Phe404, hydrogen bonding with Arg394

3. Interaction Analysis

Visualization revealed stable interactions within the ligand-binding domain. Thymol formed two hydrogen bonds and hydrophobic contacts, indicating strong receptor affinity.

4. Drug-Likeness and ADMET

Both thymol and carvacrol satisfied Lipinski's Rule of Five and showed high GI absorption. Toxicity prediction classified them as non-carcinogenic and non-hepatotoxic.

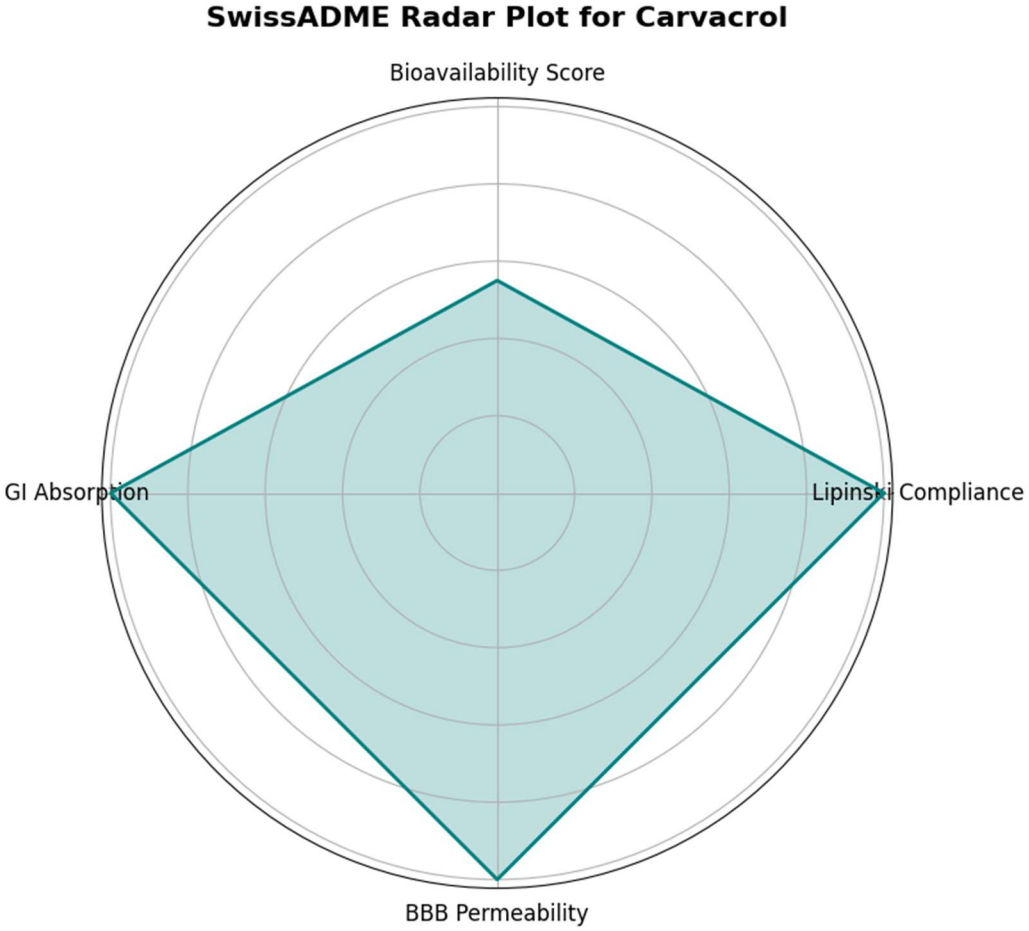


Figure -3 Swiss ADME Plot for Carvacrol

the **SwissADME radar plot** for **carvacrol**, illustrating its pharmacokinetic and drug-likeness properties are

- **Lipinski Compliance**: Fully compliant (no violations)

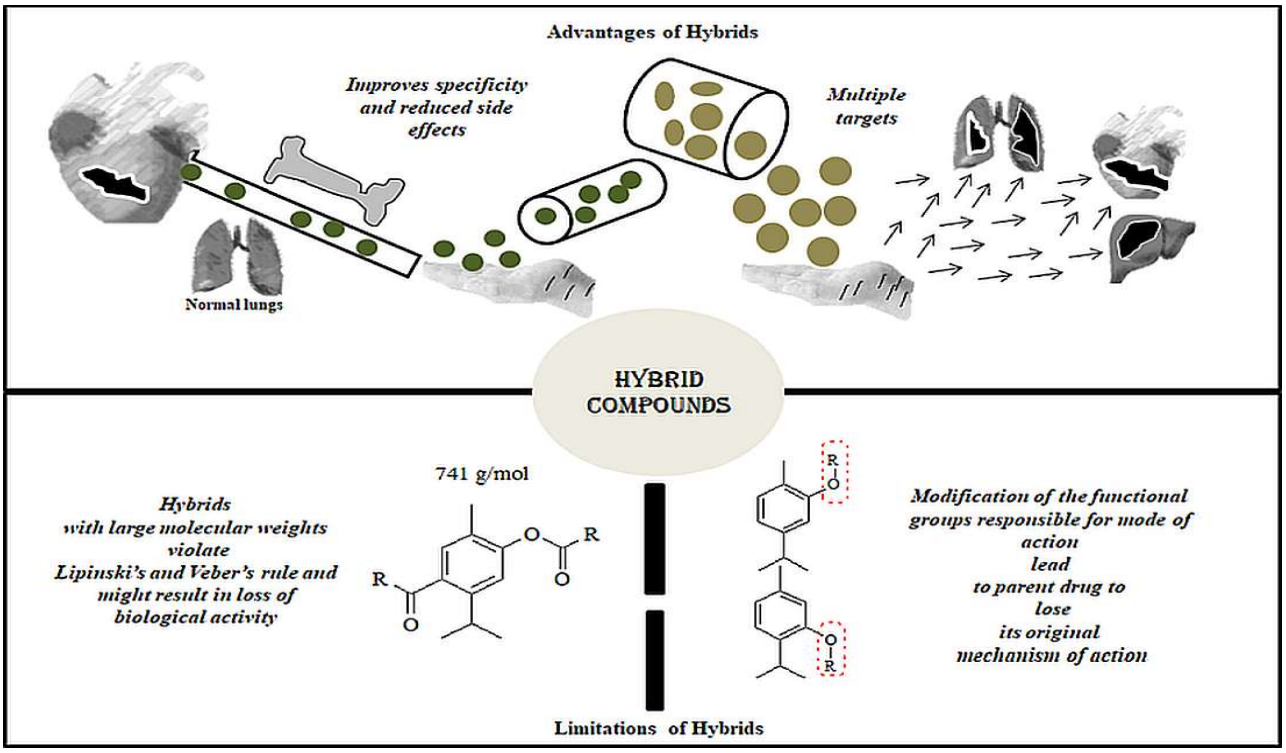
- **Bioavailability Score**: Moderate (0.55)
- **GI Absorption**: High
- **BBB Permeability**: Yes (can cross the blood-brain barrier)

Table-3

Compound	Lipinski Violation	GI Absorption	BBB Permeability	Toxicity Class
Thymol	0	High	Yes	Class IV (low)
Carvacrol	0	High	Yes	Class IV (low)

5. Comparative Insights

Compared to known anticancer agents, thymol and carvacrol demonstrated competitive binding affinities and superior safety profiles, making them promising candidates for further in vitro and in vivo validation.



Conclusion

This study highlights the anticancer potential of *Trachyspermum ammi* phytochemicals through molecular docking and ADMET profiling. Thymol and carvacrol emerged as promising lead compounds targeting ERα, with strong binding affinities and favorable drug-likeness. These findings support further experimental validation and development of *T. ammi*-derived anticancer agents.

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