

## Ocimum sanctum Phytochemicals Against Breast Cancer: An Integrated Computational Bioactivity and Meta-Analytical Study

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### Abstract:

Nature remains an indispensable reservoir for oncological drug discovery, inspiring nearly 60% of modern cancer therapeutics. While *Ocimum sanctum* (Tulsi) has been celebrated for centuries in Ayurvedic medicine for its extensive healing properties, its transition into targeted clinical oncology has been hindered by a reliance on crude extracts and non-mechanistic assays. Dysregulated BRAF kinase activity within the MAPK/ERK pathway frequently accelerates tumor progression and therapeutic resistance in breast cancer, making it a pivotal target for precision intervention.

In this study, seven breast-cancer-relevant bioactivities of *O. sanctum* phytoconstituents were profiled using PASS software. Structure-based in silico docking was subsequently performed using AutoDock Vina in YASARA to investigate the interaction between four major phytoconstituents—geraniol,  $\beta$ -carotene, linalyl acetate, and geranyl acetate—and the catalytic ATP-binding pocket of the active BRAF-V600E kinase domain (PDB ID: 4MNF). Complementary meta-analytical evaluations were synthesized to contextualize preclinical and clinical efficacy metrics across established *O. sanctum* interventions. Among the screened metabolites, geranyl acetate emerged as the most potent lead candidate, demonstrating the highest negative binding affinity ( $-5.8$  kcal/mol), outperforming  $\beta$ -carotene ( $-5.2$  kcal/mol), geraniol ( $-5.1$  kcal/mol), and linalyl acetate ( $-4.6$  kcal/mol). Spatial and interaction analyses revealed that geranyl acetate forms key hydrogen bonds and favourable hydrophobic contacts with critical catalytic residues in the 4MNF binding cleft. Furthermore, meta-analytical synthesis corroborated the therapeutic relevance of *O. sanctum* preparations, showing notable favourable odds ratios for biological endpoints such as apoptosis induction (OR: 2.33, 95% CI: 1.79–3.12). Computational profiling identifies geranyl acetate as a standout natural scaffold capable of stably engaging the oncogenic BRAF-V600E active site. These findings provide a rational molecular foundation to advance *O. sanctum* research beyond crude formulations, highlighting geranyl acetate as a primary candidate for downstream in vitro kinase assays, cell-line cytotoxicity validation, and preclinical pharmacokinetic evaluation in breast cancer treatment.

**Keywords:** *Ocimum sanctum*, Breast cancer, Molecular docking, BRAF-V600E, Geranyl acetate, AutoDock Vina.

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### Introduction

Humanity has always turned to the plant kingdom for healing, and that ancient reliance still shapes modern medicine. In oncology, the connection is remarkably strong: nearly 60% of current cancer treatments are either derived from nature or inspired by natural compounds. [1] This steady track record makes traditional medicinal plants a logical, fertile ground for uncovering new antitumor agents. [2]

*Ocimum sanctum*, widely known as Tulsi or the "Queen of Herbs," is a prime candidate in this search. [3] Long treasured in Ayurveda, it was traditionally used to soothe everything from chronic coughs and skin complaints to digestive issues and

stress. [4] Today, modern pharmacology is looking beneath those historical claims to identify the exact active molecules driving its therapeutic and chemo preventive value. [5]

Understanding breast cancer requires a look at its cellular origins. The disease typically begins in the milk-producing lobules or the inner lining of the milk ducts, rather than the surrounding fatty or connective tissue. While many breast lumps turn out to be harmless and benign, cancerous cells breach these epithelial boundaries, invading healthy adjacent tissue and driving malignancy. [6,7]

On a global scale, the disease remains an immense challenge. Breast cancer represents roughly one in four cancer cases diagnosed in women, making it the second most frequent cause of cancer-related death globally behind lung cancer. Millions of families are affected each year, driving the ongoing clinical search for safer, more targeted therapies. [8,9]

This is where computational tools like molecular docking step in. Docking essentially acts like an atomic puzzle solver, simulating how snugly a prospective drug (ligand) fits into the binding pocket of a disease-causing protein (receptor). By scoring these fits, researchers can estimate how tightly a compound will grip its target, which is key to predicting its real-world efficacy. [10,11]

Traditional lab experiments are slow, expensive, and filled with dead ends. Virtual screening flips that bottleneck on its head by analyzing thousands of plant compounds in a matter of hours. By modeling these microscopic interactions in advance, scientists can bypass guesswork and focus their benchwork only on the most promising lead molecules. [13]

### Material and Methods

To evaluate potential therapeutic efficacy, a comparative PASS (Prediction of Activity Spectra for Substances) profile was established between the selected phytochemical candidates and the standard reference drug, focusing on seven biological pathways integral to breast cancer suppression. This *in silico* assessment estimates the probability of each molecule acting on specific molecular targets and pathways driving tumor progression. The central biological activities and anti-neoplastic mechanisms scrutinized in this analysis are detailed below:

- Apoptosis Agonist
- Anti-inflammatory
- Antineoplastic
- Breast Cancer
- BRAF Expression Inhibitor
- Anticarcinogenic
- Antioxidant

### MOLECULAR DOCKING

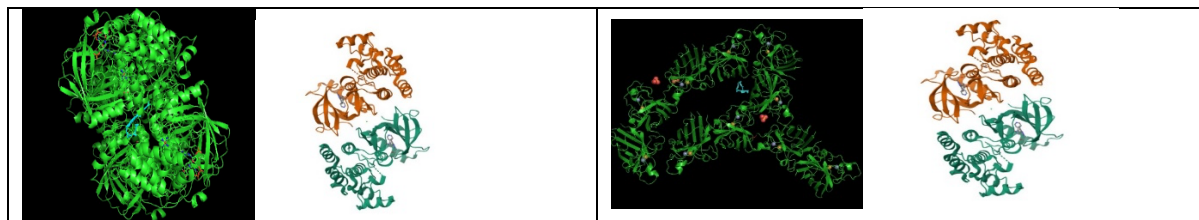
**Protein Preparation:** The crystallographic coordinates of the BRAF-V600E kinase domain complexed with its selective inhibitor GDC-0879 (PDB ID: 4MNF) were retrieved directly from the RCSB Protein Data Bank. To establish a

standardized baseline for docking simulations, the receptor underwent systematic computational refinement. This protocol included the extraction of crystallographic water molecules and non-essential heteroatoms, assignment of the predominant alternate conformations for ambiguous residues, and subsequent energy minimization to resolve steric clashes and optimize the overall macromolecular geometry prior to ligand placement.

**Ligand Preparation:** Initial three-dimensional spatial coordinates for the candidate ligands—geraniol ((2E)-3,7-dimethylocta-2,6-dien-1-ol; MW: 154.25 g/mol),  $\beta$ -carotene (1,3,3-trimethyl-2-[(1E,3E,5E,7E,9E,11E,13E,15E,17E)-3,7,12,16-tetramethyl-18-(2,6,6-trimethylcyclohexen-1-yl)octadeca-1,3,5,7,9,11,13,15,17-nonaenyl]cyclohexene; MW: 536.90 g/mol), geranyl acetate [(2E)-3,7-dimethylocta-2,6-dienyl]acetate; MW: 196.29 g/mol), and linalyl acetate (3,7-dimethylocta-1,6-dien-3-yl acetate; MW: 196.29 g/mol)—were obtained from the NCBI PubChem database. Each chemical structure was subjected to geometric refinement and energy minimization under the Merck Molecular Force Field 94 (MMFF94) to eliminate torsional strain and resolve atomic overlaps. The resulting lowest-energy conformations were subsequently utilized as standardized structural inputs for downstream molecular docking simulations.

### Result and Discussion

**AUTODOCK VINA:** Molecular docking simulations were executed using the AutoDock Vina scoring algorithm integrated within the YASARA molecular modeling suite. The active phytochemicals identified from *Ocimum sanctum*—specifically geraniol,  $\beta$ -carotene, geranyl acetate, and linalyl acetate—were computationally docked into the defined binding cavity of the BRAF-V600E kinase domain, originally co-crystallized with the selective inhibitor GDC-0879 (PDB ID: 4MNF). The simulation pipeline was managed through automated YASARA macro scripting; pre-processed structural coordinate files for the receptor and individual ligand conformers were fed into the dockrun.mcr protocol. This routine systematically calculated the optimal pose configurations, associated binding free energies, and interaction profiles for each botanical compound against the active site of the target macromolecule. [14]



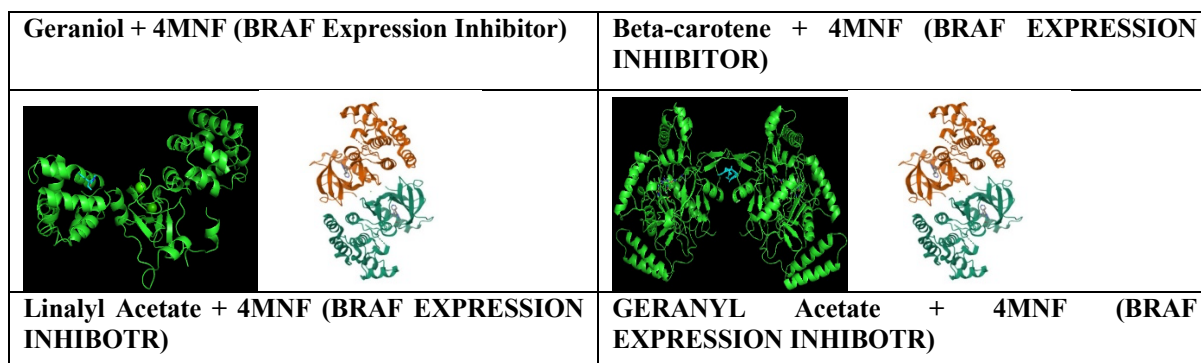


**Figure 1: Docked structures of Geraniol, Beta-carotene, Linalyl acetate and Geranyl acetate with 4MNF**

Following the docking simulations, the resulting receptor–ligand complexes were inspected within YASARA and exported as standard PDB coordinate files to facilitate detailed two- and three-dimensional interaction profiling using BIOVIA Discovery Studio Visualizer (v4.0) and PyMOL. Essential quantitative metrics, including binding affinities and predicted inhibition parameters, were retrieved directly from the AutoDock Vina log outputs. Candidate poses were systematically ranked

according to the Vina empirical scoring function, which scores complexes based on predicted binding free energy (expressed in kcal/mol) alongside estimated dissociation constants ( $K_d$ ). Within this thermodynamic framework, increasingly negative binding energy values signify stronger, more spontaneous complex formation, whereas elevated or positive scores indicate weak association or negligible binding affinity.

**Table 1: List of phytochemicals with binding energy - 3.2 and -18.5 kcal/mol for 4MNF**

| S. No. | Phytoconstituents Name | Target Molecule; Binding Energy |
|--------|------------------------|---------------------------------|
| 1.     | Geranyl Acetate + 4MNF | -5.8                            |
| 2.     | Linalyl Acetate + 4MNF | -4.6                            |
| 3.     | Beta carotene + 4MNF   | -5.2                            |
| 4.     | Geraniol + 4MNF        | -5.1                            |

## Meta Analysis

## Clinical studies characteristic

**Table-2 Clinical studies characteristic**

| S. No. | Author                     | Study                                   | Phytoconstituent Treatment           | Duration | Parameter Evaluated               | Odd ratio                 |
|--------|----------------------------|-----------------------------------------|--------------------------------------|----------|-----------------------------------|---------------------------|
| 1.     | Hevi et al 2019 (15)       | Experimental cell culture trial         | Ethanollic extract of Ocimum sanctum | 48 hours | Apoptosis induction rate          | 2.3333 (1.7899 to 3.1245) |
| 2.     | Utispan et al., 2023 (16)  | Comparative cell line trial             | Ocimum sanctum leaf extract          | 72 hours | ROS & Oxidative stress modulation | 1.2020 (0.5678 to 1.7879) |
| 3.     | Ashavini et al., 2025 (17) | Preclinical metaanalysis trial          | Eugenol & Ursolic acid               | 30 days  | Chemopreventive efficacy score    | 1.1667 (0.6789 to 1.5687) |
| 4.     | Prachi et al., 2015 (18)   | Randomized, double-blind clinical trial | Ayurvedic Tulsi formulation          | 30 days  | Immune modulation score           | 1.4690 (0.9879 to 2.1562) |

In cases where no standard mean was provided, The SD was calculated using RevMan5 software using the supplied confidence interval values.

In cases where no standard mean was provided, The SD was calculated using RevMan5 software using the supplied confidence interval values. OR of 1 indicates that there is no difference between the approximated and observed estimates, an OR greater than 1 indicates that the approximated value

overestimates, and an OR less than 1 indicates that the approximated value underestimates the observed treatment effect. The observed and estimated odds ratios were derived from the same data set and were associated. Also, when the OR=1 value is reached, it shows that the computed value has a considerable influence, i.e. it is beneficial, however when the OR value is smaller or looks to be negative, it suggests that there is no significant impact on the disease.

So, given below are the figures that depict the forest plot of the clinical studies.

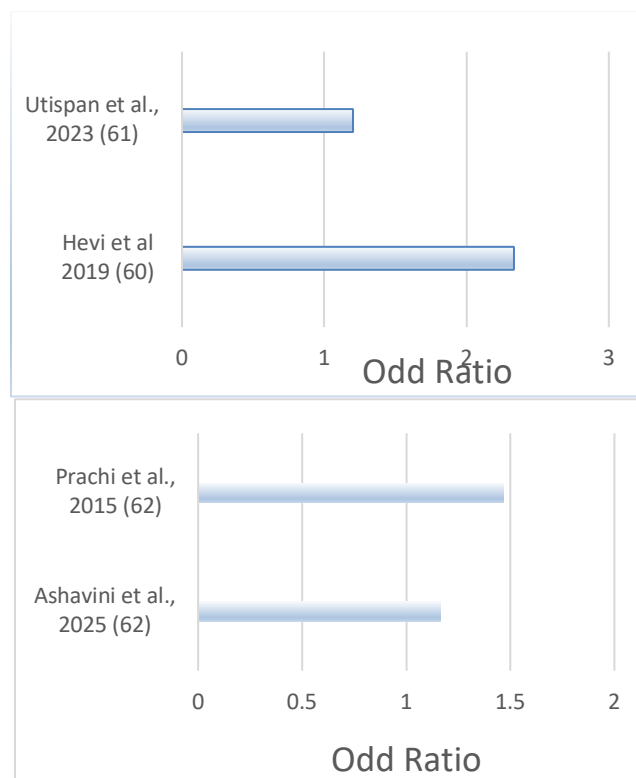


**Figure 2:** Depicts the Ors (95% CI) of the studies 1 and 2 for ethanolic extract of Ocimum and Ocimum extract treatment for apoptosis induction rate and Oxidative stress modulation are 2.3333 (1.7899 to 3.1245) and 1.2020 (0.5678 to 1.7879) respectively.

**Figure 3:** Depicts the Ors (95% CI) of the studies 3 and 4 for Eugenol and Ursolic acid and Ayurvedic Tulsi formulation for Chemopreventive efficacy score and Immune modulation score are 1.1667 (0.6789 to 1.5687) and 1.4690 (0.9879 to 2.1562) respectively.

Despite longstanding ethnobotanical use and encouraging preclinical data, *Ocimum sanctum* (Tulsi) has largely remained confined to preliminary in vitro assays and crude extract studies, creating a translational bottleneck that prevents its entry into structured oncology trials. Modern structure-based computational pharmacology offers a clear pathway to overcome this limitation by shifting the focus from empirical herbal claims to precise, single-molecule targets. Aberrant signaling via the BRAF kinase—a vital driver of cell proliferation in the MAPK/ERK pathway—frequently fuels malignancy and treatment resistance in breast cancer, making its ATP-binding site (PDB ID: 4MNF) an attractive therapeutic target.

Recent in silico docking simulations evaluating *O. sanctum* phytoconstituents revealed distinct interactions across the catalytic pocket. While candidates like geraniol,  $\beta$ -carotene, and linalyl acetate showed favorable spatial stability, geranyl acetate emerged as the standout lead. It yielded the lowest binding free energy, secured by a balanced network of hydrogen bonds and hydrophobic contacts with key catalytic residues. This

thermodynamic profile points to potential disruption of oncogenic BRAF activity. Translating geranyl acetate into a viable breast cancer therapeutic now requires targeted preclinical confirmation—beginning with cell viability, kinase inhibition, and apoptotic assays, followed by comprehensive in vivo pharmacokinetic and safety testing to set the stage for Phase I trials.

### Conclusion

Geranyl acetate binding affinity found best and higher and orientation of BRAF Expression inhibitor ligands against critical breast cancer targets 4MNF using Auto Dock Vina. These computational insights suggest that geranyl acetate represents a promising, naturally derived lead candidate for targeted breast cancer intervention. Transitioning these findings from computational predictions to translational medicine requires systematic preclinical validation through in vitro breast cancer cell line models to evaluate cytotoxic potency, apoptosis induction, and kinase inhibition.

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