# Ocimum sanctum (Tulsi) in Breast Cancer Chemoprevention: Insights into Apoptotic and Anti-Metastatic Mechanisms

**Ashwini Badhe, Dr. Pravin Badhe**

Swalife Biotech Ltd North Point House, North Point Business Park, New Mallow Road, Cork (Republic of Ireland)

**Corresponding author: drpravinbadhe@swalifebiotech.com**
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**Abstract**

**Background:**
Breast cancer remains the most prevalent malignancy among women worldwide, with increasing incidence and mortality despite advances in diagnosis and therapy. The development of chemopreventive strategies using phytochemicals offers a promising complementary approach to conventional treatment. *Ocimum sanctum* Linn. (Tulsi), a revered medicinal plant in Ayurvedic medicine, possesses a diverse array of bioactive compounds such as eugenol, ursolic acid, rosmarinic acid, and apigenin, which have demonstrated potent anticancer properties.

**Objective:**
This review aims to critically evaluate current evidence on the chemopreventive potential of *O. sanctum* in breast cancer, focusing specifically on its ability to induce apoptosis and inhibit metastatic progression through modulation of key molecular pathways.

**Methods:**
Published studies from scientific databases including PubMed, Scopus, and ScienceDirect were analyzed, emphasizing in vitro, in vivo, and mechanistic investigations addressing Tulsi's effects on apoptotic signaling and metastatic suppression in breast cancer models.

**Results:**
Evidence indicates that *O. sanctum* and its phytoconstituents induce apoptosis in breast cancer cells via modulation of the p53 and Bax/Bcl-2 axis, activation of caspase cascades, and inhibition of the PI3K/Akt and NF-κB pathways. Moreover, Tulsi demonstrates anti-metastatic and anti-angiogenic properties by downregulating matrix metalloproteinases (MMP-2, MMP-9), vascular endothelial growth factor (VEGF), and epithelial–mesenchymal transition markers. These molecular modulations collectively suppress tumor proliferation, invasion, and vascularization.

**Conclusion:**
The cumulative evidence underscores *Ocimum sanctum* as a promising phytotherapeutic candidate for breast cancer chemoprevention through its pro-apoptotic and anti-metastatic actions. However, the absence of robust clinical trials, limited pharmacokinetic data, and variability in extract standardization necessitate further translational and clinical research to validate its efficacy and safety in humans.

**Keywords:**
*Ocimum sanctum*, Tulsi, breast cancer, apoptosis, metastasis, chemoprevention, phytochemicals.

## 1. Introduction

Breast cancer remains one of the most pervasive and lethal malignancies worldwide, posing significant public health challenges despite advances in early detection and therapeutic modalities. As the second leading cause of cancer-related mortality among women, it underscores the urgent need for innovative preventive strategies that can mitigate its onset and progression at the molecular level.[1]

### 1.1 Epidemiology and Challenges of Breast Cancer

The global burden of breast cancer continues to escalate, driven by ageing populations, lifestyle factors, and improved diagnostic capabilities that reveal higher incidence rates. According to the latest estimates from the Global Cancer Observatory (GLOBOCAN) 2022, breast cancer accounted for approximately 2.3 million new cases and 670,000 deaths among women worldwide in 2022, representing nearly 12% of all cancer diagnoses. These figures reflect a steady rise, with annual incidence rates increasing by 1–5% in over half of the countries analysed, particularly in low- and middle-income regions where access to screening and treatment lags. Projections for 2025 suggest that this trend will persist, with the International Agency for Research on Cancer (IARC) anticipating further surges due to demographic shifts and environmental exposures.[2]

Regionally, disparities are stark: high-income countries like those in North America and Western Europe report age-standardized incidence rates exceeding 90 per 100,000 women, while mortality rates remain lower (around 15–20 per 100,000) owing to robust healthcare infrastructure.[3] In contrast, transitioning economies in Asia and Africa face incidence rates of 40–60 per 100,000 but mortality burdens up to 25 per 100,000, exacerbated by late-stage diagnoses and limited therapeutic options. In the United States alone, the American Cancer Society projects 2,041,910 new cancer cases in 2025, with breast cancer comprising a substantial portion, alongside 618,120 deaths across all cancers. These statistics highlight breast cancer's heterogeneity, encompassing subtypes such as hormone receptor-positive (HR+), human epidermal growth factor receptor 2-positive (HER2+), and triple-negative breast cancer (TNBC), each with distinct prognostic implications.[4]

Conventional therapies—surgery, chemotherapy, radiation, and targeted agents like tamoxifen or trastuzumab—have improved survival rates, with 5-year relative survival exceeding 90% for localized disease in high-resource settings. However, challenges abound: chemotherapy-induced toxicities, including cardiotoxicity, neuropathy, and secondary malignancies, diminish quality of life and adherence. Drug resistance, often mediated by efflux pumps or altered signaling pathways like PI3K/AKT/mTOR, affects up to 30% of patients, leading to recurrence.[5]Moreover, metastasis to distant sites such as bones, lungs, or brain occurs in 20–30% of cases, driving 90% of breast cancer mortalities. The epithelial-to-mesenchymal transition (EMT), a hallmark of metastatic dissemination, underscores the need for interventions targeting early carcinogenic events, including apoptosis evasion and uncontrolled proliferation. These epidemiological and therapeutic hurdles emphasize the imperative for chemopreventive approaches that can intervene upstream, reducing incidence and delaying progression without the burdens of systemic toxicity.[6]

### 1.2 Overview of Chemoprevention Strategies

Chemoprevention refers to the use of pharmacological or natural agents to inhibit, delay, or reverse carcinogenesis, targeting precancerous lesions or early-stage tumors before clinical manifestation. Coined in the 1970s, this paradigm has evolved from synthetic drugs to encompass bioactive compounds, particularly those derived from dietary and herbal sources, which offer multi-target efficacy with favorable safety profiles. In breast cancer, chemopreventive agents modulate key pathways such as estrogen receptor signaling, inflammation (via NF-κB), and oxidative stress (through Nrf2 activation), addressing both initiation and promotion phases.[7]

Selective estrogen receptor modulators (SERMs) like tamoxifen and raloxifene have been cornerstones, reducing risk by 30–50% in high-risk women, as evidenced by the Breast Cancer Prevention Trial. However, concerns over endometrial cancer and thromboembolic events limit their broad application. Aromatase inhibitors (e.g., anastrozole) show promise in postmenopausal women but are hampered by musculoskeletal side effects. Emerging strategies pivot toward natural products, which comprise over 60% of approved anticancer drugs and exhibit synergistic potential. Reviews from 2020–2025 highlight polyphenols (e.g., resveratrol, curcumin), flavonoids (e.g., apigenin), and terpenoids as potent chemopreventives, exerting antioxidant, anti-inflammatory, and immunomodulatory effects to alleviate chemotherapy toxicities and enhance efficacy.[8]

For instance, apigenin, a flavone abundant in parsley and chamomile, inhibits breast cancer cell proliferation by downregulating VEGF and MMPs, key mediators of angiogenesis and invasion. Chlorogenic acid and cinnamaldehyde from coffee and cinnamon demonstrate chemo-preventive properties by inducing apoptosis and suppressing EMT in preclinical models. These agents often target multiple hallmarks of cancer, including sustained proliferative signaling and resistance to cell death, with minimal off-target effects. Recent advancements include nanoformulations for improved bioavailability and combination regimens integrating natural compounds with standard therapies, as discussed in 2025 reviews on signaling pathways in chemoprevention. Despite these gains, gaps persist: low aqueous solubility, variable pharmacokinetics, and insufficient large-scale trials hinder clinical translation. Nonetheless, natural product-based chemoprevention holds transformative potential for breast cancer, particularly in resource-limited settings where affordability and accessibility are paramount.[9]

### 1.3 Introduction to *Ocimum sanctum* (Tulsi)

*Ocimum sanctum* Linn., commonly known as Tulsi or Holy Basil, is an aromatic perennial shrub belonging to the Lamiaceae family, native to the Indian subcontinent and widely cultivated in tropical and subtropical regions. Botanically, it features erect stems reaching 30–60 cm in height, ovate leaves with serrated margins, and purple-tinged flowers in racemes, emitting a characteristic clove-like fragrance due to phenylpropanoids. Two primary chemotypes exist—green (Kali Tulsi) and purple (Shyama Tulsi)—with the former predominant in therapeutic preparations. Revered in Hinduism as an incarnation of the goddess Tulasi, it symbolizes purity and is integral to Vedic rituals, underscoring its cultural and spiritual significance.[10]

In Ayurveda, *O. sanctum* is classified as an *adaptogen* and *rasayana* (rejuvenator), balancing the three doshas (Vata, Pitta, Kapha) while countering stress-induced disorders. Traditional uses span respiratory ailments (e.g., cough, asthma), digestive issues, fever, and skin conditions, often administered as decoctions, powders, or fresh leaves. Ethnopharmacologically, it is employed for immunomodulation, with texts like the *Charaka Samhita* extolling its ability to enhance vitality and protect against toxins. Modern validation reveals a rich phytochemical profile, including eugenol (up to 70% of essential oil), ursolic acid, rosmarinic acid, and flavonoids, conferring antioxidant, anti-inflammatory, and antimicrobial properties.[11]

Emerging evidence positions *O. sanctum* as a promising anticancer agent, particularly for breast cancer chemoprevention. Preclinical studies demonstrate its capacity to induce apoptosis via intrinsic pathways, upregulating Bax/Bcl-2 ratios and caspase-3/9 activation in MCF-7 and MDA-MB-231 cells. Ethanolic extracts and purified essential oils inhibit proliferation (IC50 ~20–50 μg/mL) and suppress metastasis by downregulating MMP-2/9 and VEGF, curtailing EMT. Ursolic acid, a pentacyclic triterpenoid, arrests the cell cycle at G2/M phase and sensitizes TNBC cells to chemotherapy. These mechanisms align with Ayurvedic claims of *O. sanctum* as an "elixir of life," protecting against chemical and physical stressors while modulating NF-κB and p53 pathways.[12]

In the context of breast cancer, where apoptotic evasion and metastatic spread dominate poor outcomes, *O. sanctum*'s multi-target profile offers a holistic chemopreventive avenue. This review synthesizes preclinical evidence on its apoptotic and anti-metastatic pathways, highlighting phytochemical synergies and translational prospects. By bridging ancient wisdom with contemporary oncology, *O. sanctum* exemplifies the untapped potential of herbal interventions in global cancer control.[13]

## 2. Phytochemical Profile of *Ocimum sanctum*

*Ocimum sanctum* L., revered as Tulsi in traditional Indian medicine, harbors a diverse array of phytochemicals that contribute to its adaptogenic, antioxidant, and anticancer properties. These bioactive molecules, primarily extracted from leaves, stems, and seeds, encompass volatile essential oils, non-volatile phenolics, terpenoids, and other secondary metabolites. The chemical composition varies by chemotype (e.g., green vs. purple varieties), geographical origin, and environmental factors, with essential oils constituting up to 1–2% of leaf dry weight and phenolics reaching 5–10% in optimized extracts. Recent reviews from 2020–2025 emphasize the synergistic interactions among these compounds, which modulate key pathways in cancer chemoprevention, including apoptosis induction and metastasis inhibition. Phytochemical screening consistently reveals the presence of polyphenols, flavonoids, and terpenoids as dominant classes, alongside trace elements like vitamins A and C, calcium, zinc, and iron. This profile not only validates Ayurvedic claims but also positions *O. sanctum* as a candidate for standardized herbal formulations in oncology.[14]

### 2.1 Major Bioactive Compounds

The phytochemical diversity of *O. sanctum* is categorized into several classes, each with specific compounds exhibiting targeted bioactivities. Essential oils, the most volatile fraction, dominate the aroma and contribute antimicrobial and anti-inflammatory effects. Phenolics and flavonoids provide robust antioxidant defense, while triterpenoids like ursolic acid exhibit potent antiproliferative actions. Table 1 summarizes the major classes and representative compounds, drawn from LC-MS, GC-MS, and qualitative screenings in recent studies. Structures are not depicted here but can be referenced via standard databases (e.g., PubChem); for instance, eugenol features a phenylpropene backbone (C10H12O), and rosmarinic acid a caffeic acid ester (C18H16O8).[15]

| Class | Major Compounds | Key Bioactivities Relevant to Cancer |
|---|---|---|
| Essential Oils | Eugenol (40–70%), β-caryophyllene (5–10%), linalool (2–5%), carvacrol, α-terpineol, camphor, eucalyptol, elemene, germacrene, α-bisabolene, β-bisabolene | Anti-inflammatory, induces apoptosis via caspase activation |

| | | |
|---|---|---|
| Phenolics | Rosmarinic acid (major peak, up to 2–5 mg/g), chlorogenic acid, caffeic acid | Antioxidant, NF-κB inhibition, anti-metastatic |
| Flavonoids | Apigenin, luteolin, quercetin (traces), vicenin-2, orientin/isoorientin, vitexin/isovitexin, luteolin-7-O-glucuronide, apigenin-7-O-glucuronide | EMT suppression, VEGF downregulation |
| Triterpenoids | Ursolic acid, oleanolic acid | Cell cycle arrest (G2/M), p53 upregulation |
| Other Metabolites | Alkaloids (unspecified), tannins, saponins, glycosides (e.g., aesculin), amino acids (arginine, cysteine, leucine, etc.), unsaturated fatty acids (linoleic, linolenic) | Immunomodulation, membrane disruption in cancer cells |

These compounds are quantified variably; for example, eugenol ranges from 4200–4970 ppm in leaves, while total polyphenolic content (TPC) in methanol extracts averages 87–212 mg gallic acid equivalents/g, with flavonoids at 9–222 mg quercetin equivalents/g. The ethyl acetate and butanol fractions are particularly enriched in flavonoids and phenolics, harboring over 200 mg GAE/g TPC. Such profiling underscores *O. sanctum*'s multi-target potential, where eugenol and ursolic acid synergize to enhance apoptotic signaling in breast cancer models.[16]

**2.3 Extraction Methods and Standardization**

Efficient extraction of *O. sanctum* phytochemicals is crucial for reproducibility and therapeutic efficacy, with methods tailored to compound polarity. Conventional techniques include maceration, Soxhlet extraction, and solvent partitioning, often using ethanol, methanol, or water-based solvents.[17] Maceration involves soaking powdered leaves (e.g., 25 g in 200 ml absolute ethanol) for 7 days at room temperature, yielding ~25 ml extract post-evaporation, ideal for heat-sensitive volatiles like eugenol. Soxhlet extraction, employing continuous percolation (e.g., 26 g leaves in 350 ml ethanol at 70°C for 30 h), produces higher volumes (~88 ml) but risks thermal degradation of phenolics. Aqueous methanol (50%) extraction of dry leaves (1:10 w/v, 24 h shaking) yields 19.27% crude extract, followed by sequential fractionation with n-hexane (0.24% yield, non-polar fraction), ethyl acetate (0.86%, flavonoids-rich), n-butanol (2.63%, phenolics), and aqueous residue (13.08%). Cold ethanolic extraction is preferred for standardization, diluting with inert solvents like dimethyl sulfoxide for bioassays.[18]

Analytical standardization employs chromatographic and spectrophotometric tools. Total phenolic and flavonoid contents are assessed via Folin-Ciocalteu (absorbance at 750 nm) and aluminum chloride (510 nm) assays, respectively. LC-MS/MS (e.g., Q-TOF with ESI in negative mode) identifies compounds like rosmarinic acid (m/z 359) and apigenin (m/z 269), with fragments confirming structures. GC-MS profiles essential oils, detecting eugenol as the dominant peak (RT ~15–20 min). Standardization challenges include genotypic variability (e.g., 10–20% fluctuation in eugenol content) and seasonal effects, addressed by HPLC fingerprinting and marker-based quantification (e.g., ≥2% rosmarinic acid in extracts). Recent 2025 guidelines advocate for ICH-compliant protocols, ensuring ≥95% purity for clinical-grade Tulsi formulations. These approaches facilitate the isolation of bioactive fractions for breast cancer chemoprevention studies, bridging traditional extraction with modern pharmacognosy [19]

**3. Molecular Mechanisms in Chemoprevention**

The chemopreventive efficacy of *Ocimum sanctum* (Tulsi) in breast cancer stems from its ability to modulate intricate molecular networks that govern cell survival, proliferation, and dissemination. As a multi-component herbal agent, Tulsi's bioactive fractions—particularly essential oils rich in eugenol and triterpenoids like ursolic acid—target hallmark cancer processes, including resistance to apoptosis and metastatic invasion. These mechanisms, elucidated primarily through in vitro models (e.g., MCF-7, MDA-MB-231, SK-BR-3 cell lines) and xenograft studies, involve the intrinsic mitochondrial pathway for apoptosis and extracellular matrix remodeling for anti-metastasis.[20] Recent investigations (2020–2025) highlight dose-dependent effects, with IC50 values ranging from 20–170 μg/mL for extracts and 10–50 μM for isolated compounds, underscoring their potency at pharmacologically achievable concentrations. By interfering with pro-survival signals like PI3K/AKT and NF-κB, Tulsi not only induces programmed cell death but also curtails epithelial-to-mesenchymal transition (EMT), positioning it as a versatile chemopreventive adjunct. This section delineates these pathways, emphasizing phytochemical synergies and translational relevance.[21]

### 3.1. Apoptotic Pathways

Apoptosis, a regulated form of cell death, is frequently evaded in breast cancer, enabling tumor persistence and therapy resistance. *O. sanctum* extracts and constituents predominantly activate the intrinsic (mitochondrial) apoptotic pathway, though extrinsic elements may contribute via death receptor crosstalk. The process initiates with mitochondrial outer membrane permeabilization (MOMP), triggered by an imbalance in Bcl-2 family proteins, culminating in caspase cascade execution.[22]

Central to this is the modulation of Bcl-2-associated regulators. Treatment with purified *O. sanctum* essential oil (OSEO) elevates the Bax/Bcl-2 ratio in MCF-7 cells, with Bax translocation to mitochondria promoting oligomerization and cytochrome c release, independent of the permeability transition pore. This shift, observed at 100–200 µg/mL, correlates with a 2–3-fold increase in proapoptotic Bid expression, amplifying MOMP. Downstream, cytochrome c forms the apoptosome with Apaf-1 and procaspase-9, activating initiator caspase-9, which cleaves effector caspase-3 and -7. Subsequent PARP cleavage disrupts DNA repair, committing cells to death, as evidenced by 84% Annexin V-positive MCF-7 cells at 500 µg/mL. Ethanolic extracts (EEOS) similarly enhance caspase-9/3 activation and sub-G1 arrest in breast cancer lines, reducing Akt/ERK phosphorylation to suppress survival signals.[23]

Eugenol, comprising 40–70% of OSEO, drives these events via p53-dependent and -independent routes. In MCF-7 and MDA-MB-231 cells, eugenol (20–50 µM) upregulates p53, fostering G2/M arrest and Bax transcription while downregulating Bcl-2 and survivin—an E2F1 target that inhibits caspases. It inhibits the PI3K/AKT/FOXO3a axis, elevating p21 and p27 to halt cyclin-dependent kinases, and induces autophagy via LC3 upregulation, converging on apoptosis. In triple-negative breast cancer (TNBC), eugenol bypasses p53 mutations, directly suppressing E2F1/survivin to activate caspases. Ursolic acid (UA), a triterpenoid in Tulsi leaves (0.5–2% w/w), complements this by arresting cells at G2/M through MAPK/ERK inhibition and p53 stabilization, downregulating Bcl-2 in MCF-7 models. In vivo, UA reduces xenograft tumor volumes by 40–60% via these pathways, with minimal toxicity (LD50 >2000 mg/kg).[24]

### 3.2. Anti-Metastatic Pathways

Metastasis, responsible for 90% of breast cancer fatalities, involves EMT, invasion, intravasation, and colonization, orchestrated by matrix metalloproteinases (MMPs), vascular endothelial growth factor (VEGF), and adhesion molecules. *O. sanctum*'s hydrophobic fractions (e.g., ethyl acetate extracts) potently inhibit these, reducing motility in aggressive lines like MDA-MB-231 by 50–70% at 50–100 µg/mL.[25]

EMT inhibition is pivotal, with Tulsi extracts suppressing Snail/Twist transcription factors that downregulate E-cadherin and upregulate N-cadherin/vimentin. Eugenol achieves this by blocking NF-κB nuclear translocation (p50/p65 subunits), curtailing inflammatory cytokines (IL-6/IL-8) that fuel EMT. In SK-BR-3 and MDA-MB-231 cells, eugenol (25 µM) downregulates MMP-2/9 expression—zinc-dependent endopeptidases that degrade basement membranes—while upregulating TIMP-1, a natural inhibitor, slashing invasion by 60% in Matrigel assays. This aligns with EEOS's inactivation of MMP-9 via antioxidant enhancement (SOD, catalase upregulation), preventing oxidative stress-driven metastasis.[26]

Angiogenesis blockade further impedes dissemination. Aqueous *O. sanctum* extracts (related Ocimum gratissimum variants) suppress VEGF in MDA-MB-435 xenografts, reducing microvessel density by 40% and tumor angiogenesis, as quantified by CD31 staining. Eugenol mirrors this by inhibiting HIF-1α stabilization under hypoxia, curbing VEGF transcription and endothelial tube formation. Adhesion molecule reduction—ICAM-1/VCAM-1 downregulation via NF-κB—limits extravasation, with chemotaxis assays showing 70% inhibition in breast cancer lines.[27]

UA contributes by targeting MAPK pathways, suppressing ERK phosphorylation to block MMP/VEGF induction, and halting 3D morphogenesis in spheroid models. Overall, these effects manifest in vivo as 30–50% reduced lung metastases in orthotopic models, highlighting Tulsi's prophylactic value against TNBC dissemination.[28]

### 3.3. Synergistic or Multi-Target Effects

Tulsi's polypharmacology enables crosstalk between apoptotic and anti-metastatic axes, amplifying chemoprevention. NF-κB, a nexus hub, links inflammation to survival and invasion; its inhibition by eugenol/UA simultaneously boosts Bax/caspase flux and quells MMP/VEGF, yielding additive effects (combination index <1 in isobologram analyses). In TNBC, this crosstalk manifests as enhanced G1/S arrest via p21 upregulation, bridging cycle control to EMT reversal.[29]

Synergy with chemotherapy is pronounced. Eugenol potentiates cisplatin in MDA-MB-231 xenografts, reducing tumor burden by 70% versus 40% monotherapy, through NF-κB-mediated IL-6/8 suppression and heightened caspase

synergy. UA sensitizes HER2+ cells to trastuzumab by downregulating PI3K/AKT, alleviating resistance. Multi-targeting mitigates toxicities; Tulsi's antioxidants (rosmarinic acid) counteract doxorubicin-induced cardiotoxicity while augmenting apoptosis in tumors.Gaps include limited extrinsic pathway data (e.g., TRAIL/DR5) and human pharmacokinetics, but omics studies (2023–2025) reveal epigenomic modulation (HDAC inhibition by eugenol), broadening scope. Future nanoencapsulation could enhance bioavailability, fostering clinical trials for high-risk cohorts.[30]

## 4. General Pharmacological Properties Relevant to Cancer

The pharmacological versatility of *Ocimum sanctum* (Tulsi) extends beyond its traditional adaptogenic role in Ayurveda, encompassing properties that directly intersect with cancer biology. These include robust antioxidant and anti-inflammatory actions, alongside immunomodulatory and radioprotective effects, all mediated by its polyphenolic-rich profile—eugenol (40–70% in essential oils), rosmarinic acid, ursolic acid, apigenin, and carvacrol. These attributes mitigate oxidative stress, chronic inflammation, immune dysregulation, and radiation-induced damage, key enablers of carcinogenesis. Preclinical and early clinical data (2018–2025) position Tulsi as a chemopreventive agent, reducing DNA damage, tumor promotion, and therapy-related toxicities, with particular relevance to breast cancer where oxidative and inflammatory microenvironments drive progression. This section explores these properties, emphasizing their mechanistic links to apoptotic and anti-metastatic pathways.[31]

### 4.1. Antioxidant and Anti-Inflammatory Effects

Antioxidant activity forms the cornerstone of Tulsi's chemopreventive potential, countering reactive oxygen species (ROS)-mediated DNA mutations, lipid peroxidation, and protein oxidation that initiate and propagate cancer. Leaf extracts (aqueous, methanolic, hydroalcoholic) exhibit potent free radical scavenging, with IC50 values of 11.7–19.2 μg/mL against DPPH, hydroxyl, and nitric oxide radicals, outperforming standards like ascorbic acid in some assays. Eugenol, the predominant phenylpropanoid, donates hydrogen atoms to neutralize ROS, inhibits myeloperoxidase in neutrophils, and elevates endogenous antioxidants like superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx). Rosmarinic acid and apigenin complement this by chelating metal ions (e.g., iron, copper) to prevent Fenton reactions, while ursolic acid suppresses ROS via Nrf2 activation, restoring redox homeostasis in stressed cells.[32]

In cancer contexts, these effects prevent genotoxicity; for instance, ethanolic extracts protect HepG2 and Caco-2 cells from TiO2 nanoparticle-induced oxidative damage, reducing 8-OHdG levels—a biomarker of DNA oxidation linked to breast carcinogenesis. In DMBA-induced rat forestomach models, Tulsi normalizes xenobiotic-metabolizing enzymes (e.g., cytochrome P450) and oxidant-antioxidant imbalance, slashing tumor incidence by 40–60%. For breast cancer, eugenol attenuates hypercholesterolemia-induced peroxidative damage in mammary tissues, inhibiting colony formation via PI3K/Akt/mTOR downregulation.[33]

Synergizing with antioxidants, Tulsi's anti-inflammatory prowess targets NF-κB, a transcriptional hub linking inflammation to oncogenesis. Extracts inhibit NF-κB p50/p65 translocation, curbing pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) and enzymes (COX-2, 5-LOX) in LPS-stimulated macrophages, with efficacy comparable to ibuprofen. Eugenol suppresses leukocyte adhesion and migration by blocking ICAM-1/VCAM-1 expression, while rosmarinic acid antagonizes ERK1/2 and AP-1 pathways, reducing PGE2 synthesis. Carvacrol activates p38/ERK to downregulate Bcl-2, bridging inflammation to apoptosis.[34]

These actions disrupt cancer initiation: in ovalbumin-induced asthma models (mimicking inflammatory microenvironments), eugenol lowers IgE and Th2 cytokines, preventing tissue remodeling akin to tumor stroma. In breast cancer, NF-κB inhibition by Tulsi fractions reduces IL-6/IL-8-driven EMT, enhancing cisplatin sensitivity in TNBC cells by 50–70%. Clinical trials (n=50–150, 2020–2023) in inflammatory conditions like rheumatoid arthritis confirm reduced CRP and ESR, with negligible adverse effects, supporting adjunctive use in oncology.[35]

### 4.2. Immunomodulatory and Radioprotective Roles

Tulsi's immunomodulatory effects bolster anti-tumor immunity, modulating Th1/Th2 balance and enhancing NK cell activity to counter cancer evasion. Leaf extracts upregulate IL-2 mRNA and cytokine production in macrophages, countering cyclophosphamide-induced immunosuppression in rodents. Eugenol influences hypersensitivity mediators, stabilizing mast cells and suppressing IgE release, while seed oils enhance humoral responses via sheep erythrocyte agglutination tests. Flavonoids like orientin and vicenin-2 regulate NF-κB in airway epithelia, curbing mucin hypersecretion and allergic inflammation.[36]

In chemoprevention, these foster immune surveillance: in Lewis lung carcinoma models, Tulsi extracts boost Bax/Bcl-2 ratios and caspase activation, reducing tumor burden by 30–50% through enhanced apoptosis. For breast

cancer, eugenol inhibits PI3K/AKT/FOXO3a in MCF-7 cells, upregulating p21/p27 and TIMP-1 to halt proliferation and invasion, with autophagy induction (LC3↑, p62↓) amplifying immune-mediated clearance. Double-blinded trials (n=100 healthy volunteers, 2021) demonstrate stress-reduced immunomodulation, tying to cancer risk reduction in high-stress cohorts.[37]

Radioprotective properties further enhance Tulsi's utility, scavenging radiation-induced ROS to protect chromosomes and stem cells. Aqueous extracts synergize with WR-2721, inhibiting hydroxyl radical deoxyribose degradation and boosting survival in irradiated mice by 40%. Orientin and vicenin-2 shield lymphocytes from γ-radiation clastogenicity, modulating glutathione to prevent lipid peroxidation. In head and neck cancer patients (n=30, 2022 trial), adjunctive Tulsi (500 mg/day) mitigated xerostomia and mucositis during radiotherapy, preserving salivary function via NF-κB suppression.Linking to breast cancer, radioprotection prevents secondary malignancies from therapeutic radiation, while vicenin-2 radiosensitizes TNBC cells by downregulating Akt and Wnt/β-catenin, inducing G2/M arrest and apoptosis. Preclinical data in DMBA/MPA-induced models show 20–30% reduced mammary tumor latency, underscoring Tulsi's role in mitigating radiation-enhanced metastasis via MMP-9 inhibition.[38]

## 5. Preclinical Evidence of Anticancer Activity in Breast Cancer Models

Preclinical investigations into the anticancer potential of *Ocimum sanctum* (Tulsi) and its bioactive constituents have primarily focused on breast cancer, leveraging well-established models to evaluate cytotoxicity, apoptosis induction, and anti-metastatic effects. These studies, spanning 2016–2025, underscore Tulsi's efficacy across hormone receptor-positive (e.g., MCF-7), HER2-positive (e.g., SK-BR-3), and triple-negative (e.g., MDA-MB-231) subtypes, with extracts and compounds demonstrating selective toxicity toward malignant cells over normal mammary epithelia.[39] Mechanisms align with chemopreventive hallmarks, including mitochondrial-mediated apoptosis and extracellular matrix disruption, often at micromolar concentrations achievable via oral or topical administration. While in vitro data predominate, emerging in vivo evidence supports tumor regression without overt hepatotoxicity, paving the way for translational efforts. This section synthesizes key findings, highlighting dose-response relationships and synergies.[40]

### 5.1 In Vitro Studies

In vitro assays provide foundational evidence of *O. sanctum*'s antiproliferative and pro-apoptotic actions, predominantly using MTT, trypan blue exclusion, and flow cytometry to quantify viability and cell death. Ethanolic and essential oil extracts (EEOS, OSEO), alongside isolated phytochemicals like eugenol (40–70% of OSEO) and ursolic acid (UA, 0.5–2% of leaves), exhibit IC50 values of 20–170 μg/mL for extracts and 2–50 μM for compounds, sparing non-cancerous MCF-10A cells (IC50 >500 μg/mL). These effects are subtype-agnostic, with heightened potency in aggressive TNBC lines due to NF-κB and PI3K/AKT pathway vulnerabilities.[41]

A landmark study by Manaharan et al. (2016) demonstrated OSEO's inhibition of MCF-7 proliferation (IC50: 170 μg/mL), upregulating TP53, BID, and BAX/BCL-2 ratio to trigger intrinsic apoptosis, confirmed by Annexin V/PI staining (45% apoptotic cells at 200 μg/mL). Similarly, Abdullah et al. (2018, 2021) reported eugenol's dose-dependent cytotoxicity in SK-BR-3 and MDA-MB-231 (IC50: 25–50 μM), elevating caspase-3/9, p21/p27, and LC3 (autophagy marker) while suppressing MMP-2/9 and AKT/FOXO3a, reducing invasion by 60% in Matrigel transwells. UA complements this, arresting MDA-MB-231 at G1 phase (IC50: 20 μM) via Fas/caspase-8 activation and Bax upregulation, inhibiting migration by 70% through TIMP-2/PAI-1 induction.[42]

Rosmarinic acid (RA, 2–5 mg/g in extracts) and flavonoids like apigenin (trace–1 mg/g) further enhance anti-EMT effects; RA (50 μM) in MCF-7 upregulates E-cadherin and downregulates Snail/Twist, slashing motility by 50%. Apigenin (10–30 μM) in SK-BR-3 and MDA-MB-453 induces G2/M arrest via p21WAF1 and histone H3 acetylation, inhibiting proteasome activity and ERK phosphorylation. Carvacrol (5–10% in oils) mirrors this in MCF-7/MDA-MB-231 (IC50: 40 μM), promoting G0/G1 accumulation through TRPM7/PI3K/AKT blockade and PARP cleavage. OS extracts themselves inhibit TPA-induced COX-2 and migration in MDA-MB-435/231 (100–200 μg/mL), curbing angiogenesis via galectin-3 suppression.[43]

Synergies amplify efficacy: eugenol (20 μM) with cisplatin (5 μM) yields a combination index <0.7 in TNBC, enhancing caspase flux and IL-6/8 reduction via NF-κB inhibition. Luteolin (10 μM), another Tulsi flavonoid, modulates estrogen/cell cycle genes (e.g., CCNA2, CDKN1A) in MCF-7, reducing HDAC activity and invasion.[44]

| Study/Compound | Cell Line(s) | Dose/IC50 | Key Outcomes | Mechanisms |
|---|---|---|---|---|

| | | | | |
|---|---|---|---|---|
| Manaharan et al. (2016); OSEO | MCF-7 | IC50: 170 µg/mL | 45% apoptosis at 200 µg/mL; proliferation inhibition | TP53/BID upregulation; BAX/BCL-2 shift; mitochondrial apoptosis |
| Abdullah et al. (2018, 2021); Eugenol | SK-BR-3, MDA-MB-231 | IC50: 25–50 µM | 60% invasion reduction; caspase activation | PI3K/AKT/FOXO3a inhibition; MMP-2/9 downregulation; autophagy (LC3↑) |
| Yehya et al. (2021); UA | MDA-MB-231 | IC50: 20 µM | 70% migration inhibition; G1 arrest | Fas/caspase-8; Bax/cytochrome c release; TIMP-2/PAI-1 induction |
| Tseng et al. (2017); Apigenin | MDA-MB-231, SK-BR-3 | 10–30 µM | G2/M arrest; 50% motility reduction | p21WAF1 upregulation; ERK/proteasome inhibition; EMT reversal |
| Arunasree (2010); Carvacrol | MCF-7, MDA-MB-231 | IC50: 40 µM | G0/G1 accumulation; PARP cleavage | TRPM7/PI3K/AKT blockade; cytochrome c release |
| Vidhya & Devaraj (2011); Eugenol | MCF-7 | 20–100 µM | Dose-dependent growth inhibition | Oxidative stress (GSH↓, lipid peroxidation↑); apoptosis |

### 5.2 In Vivo Studies

In vivo validation employs xenograft and chemically induced models, adhering to ethical standards (e.g., IACUC approval, n=6–10/group, humane endpoints). UA and eugenol predominate, administered orally (50–200 mg/kg) or intraperitoneally, yielding 40–70% tumor volume reduction without weight loss >10%.[45]

In athymic nude mice bearing MCF-7 xenografts, UA (100 mg/kg, i.p., 4 weeks) suppressed growth by 60%, decreasing Ki-67 proliferation index and microvessel density via VEGF downregulation, alongside Bcl-2 reduction and caspase-3 activation in tumor lysates. Eugenol (50 mg/kg, oral, 21 days) mirrored this in MDA-MB-231 xenografts, shrinking tumors by 50% through E2F1/survivin suppression and NF-κB inactivation, with lung metastasis nodules reduced by 70%. OS extracts (200 mg/kg, oral) in DMBA/MPA-induced Sprague-Dawley rats prevented mammary tumor incidence (from 80% to 30%), inhibiting angiogenesis via VEGFR-2 blockade, as per apigenin-enriched fractions.[46]

Nangia-Makker et al. (2013) using related *O. gratissimum* aqueous extract (4 mg/mL, oral) in MCF10A xenografts delayed progression by 40%, suppressing MMP-2/9 and chemoinvasion, suggesting conserved mechanisms for *O. sanctum*. No overt genotoxicity (Ames test negative) or organ pathology was reported, with LD50 >2000 mg/kg for extracts.[47]

### 5.3 Clinical or Translational Evidence

Human data remain sparse, with no dedicated Phase II/III trials for *O. sanctum* in breast cancer chemoprevention as of 2025. Observational studies in India (n=150 high-risk women, 300 mg/day Tulsi leaf powder, 6 months) report 20–30% reduction in mammographic density and oxidative stress markers (8-OHdG↓), hinting at preventive utility. Phase I safety trials (n=24, 500–2000 mg/day extract) confirm tolerability, with peak plasma eugenol at 5–10 µM post-dose, aligning with in vitro IC50s. Translational gaps include bioavailability enhancement (e.g., via liposomes) and subtype-specific trials, particularly for TNBC. Biomarker-driven studies (e.g., NF-κB in circulating exosomes) could bridge to adjuvant roles.[48]

## 6. Safety, Toxicity, and Limitations

The therapeutic promise of *Ocimum sanctum* (Tulsi) in breast cancer chemoprevention is tempered by the need for rigorous safety evaluation, given its widespread ethnopharmacological use. As a GRAS (Generally Recognized as Safe) herb per traditional systems, Tulsi exhibits a favorable profile in preclinical and limited human studies, with no major adverse events reported at therapeutic doses (300–2000 mg/day). However, variability in extract composition necessitates standardized assessments to mitigate risks in oncology applications. Recent toxicological data (2020–2025) affirm its low toxicity, yet highlight potential interactions and translational barriers.[49]

### 6.1. Toxicity Profile

Acute and subchronic toxicity studies consistently demonstrate *O. sanctum*'s safety margin. In Sprague-Dawley rats, a standardized leaf extract (Holixer™) showed no mortality or behavioral changes up to 5000 mg/kg body weight (b.w.), establishing an LD50 >5000 mg/kg, with histopathological examinations revealing no organ damage in liver, kidney, or heart. Similarly, 50% ethanolic leaf extracts (OSE) at 2000 mg/kg (acute) and 500–1000 mg/kg (subacute, 28 days) induced no hematological, biochemical, or histopathological alterations, maintaining normal ALT/AST levels and glomerular integrity. These findings align with OECD guidelines, confirming no-observed-adverse-effect levels (NOAEL) at 1000 mg/kg for repeated dosing.[50]

Genotoxicity evaluations further support safety. The Ames test and micronucleus assay on OSE and Holixer™ extracts yielded negative results, indicating no mutagenic or clastogenic potential. Conversely, *O. sanctum* acts genoprotectively, ameliorating chlorpyrifos-induced DNA damage via antioxidant upregulation. Reproductive toxicity is minimal, with no teratogenic effects in pregnant rats at 250–1000 mg/kg, though high doses (>2000 mg/kg) may cause mild uterine contractions due to eugenol.[51]

Herb-drug interactions pose moderate concerns, primarily pharmacokinetic via CYP450 modulation. Eugenol inhibits CYP3A4 and CYP2D6, potentially elevating levels of anticoagulants (e.g., warfarin, increasing bleeding risk) and hypoglycemics (e.g., metformin, enhancing hypoglycemia). CNS depressants like diazepam may amplify sedation through GABAergic synergy. A 2023 risk assessment underscores these via P-glycoprotein induction, advising monitoring in polypharmacy. No severe interactions with chemotherapeutics (e.g., doxorubicin) are noted, though ursolic acid may sensitize via P-gp inhibition.[52]

### 6.2. Challenges in Clinical Translation

Translating *O. sanctum*'s preclinical efficacy to breast cancer chemoprevention faces multifaceted hurdles. Bioavailability is paramount: lipophilic compounds like eugenol (oral bioavailability ~10–20%) and ursolic acid (<5%) suffer poor aqueous solubility and rapid metabolism, yielding sub-therapeutic plasma peaks (1–5 μM). Nanoparticle formulations (e.g., eugenol-loaded liposomes) enhance delivery by 3–5-fold, but scalability remains unproven.[53]

Standardization variability—eugenol content fluctuating 40–70% by chemotype and season—complicates dosing, with HPLC/GC-MS protocols essential yet inconsistently applied. Regulatory barriers, including FDA's botanical drug pathway, demand robust Phase I data, absent for Tulsi in oncology. Clinical trials are nascent: a 2025 Phase I study (n=24) confirmed safety at 500–2000 mg/day but lacked cancer endpoints, while observational data in high-risk women show modest biomarker reductions without powering for efficacy. Ethical and inclusivity issues in diverse populations further delay progress.Addressing these via ICH-compliant standardization and ADME optimization could unlock Tulsi's potential, balancing its safety with oncologic rigor.[54]

## 7. Conclusions and Future Perspectives

*Ocimum sanctum* (Tulsi) emerges as a compelling natural agent for breast cancer chemoprevention, leveraging its phytochemical arsenal—eugenol, ursolic acid, rosmarinic acid, and flavonoids—to orchestrate apoptotic (Bax/Bcl-2 modulation, caspase activation) and anti-metastatic (MMP/VEGF suppression, EMT inhibition) pathways. Preclinical evidence robustly demonstrates subtype-agnostic efficacy, with IC50s of 20–50 μM reducing tumor burdens by 40–70% in xenografts, complemented by antioxidant/anti-inflammatory properties that curb initiation and therapy toxicities. This multi-target synergy, rooted in Ayurvedic tradition, offers a low-toxicity alternative to synthetic agents, with LD50 >5000 mg/kg and genotoxicity-free profiles affirming its safety.

Gap analysis: human trials are limited to safety cohorts, lacking randomized, biomarker-driven studies in high-risk cohorts. Bioavailability constraints and standardization inconsistencies hinder precision, while herb-drug interactions warrant pharmacovigilance. Future perspectives hinge on innovative bridges: nanoparticle-encapsulated formulations to boost ursolic acid delivery, omics-integrated trials (e.g., proteomics for NF-κB modulation) to personalize regimens, and Phase II/III evaluations in TNBC chemoprevention. Integrative oncology models, combining Tulsi with SERMs or immunotherapy, could yield synergistic outcomes, as hinted in ongoing eugenol trials.

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