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Genomic and Pharmacological Integration of *Ocimum sanctum* (Holy Basil): A Quantitative DNA-Based Evidential Profile of an "Elixir of Life"

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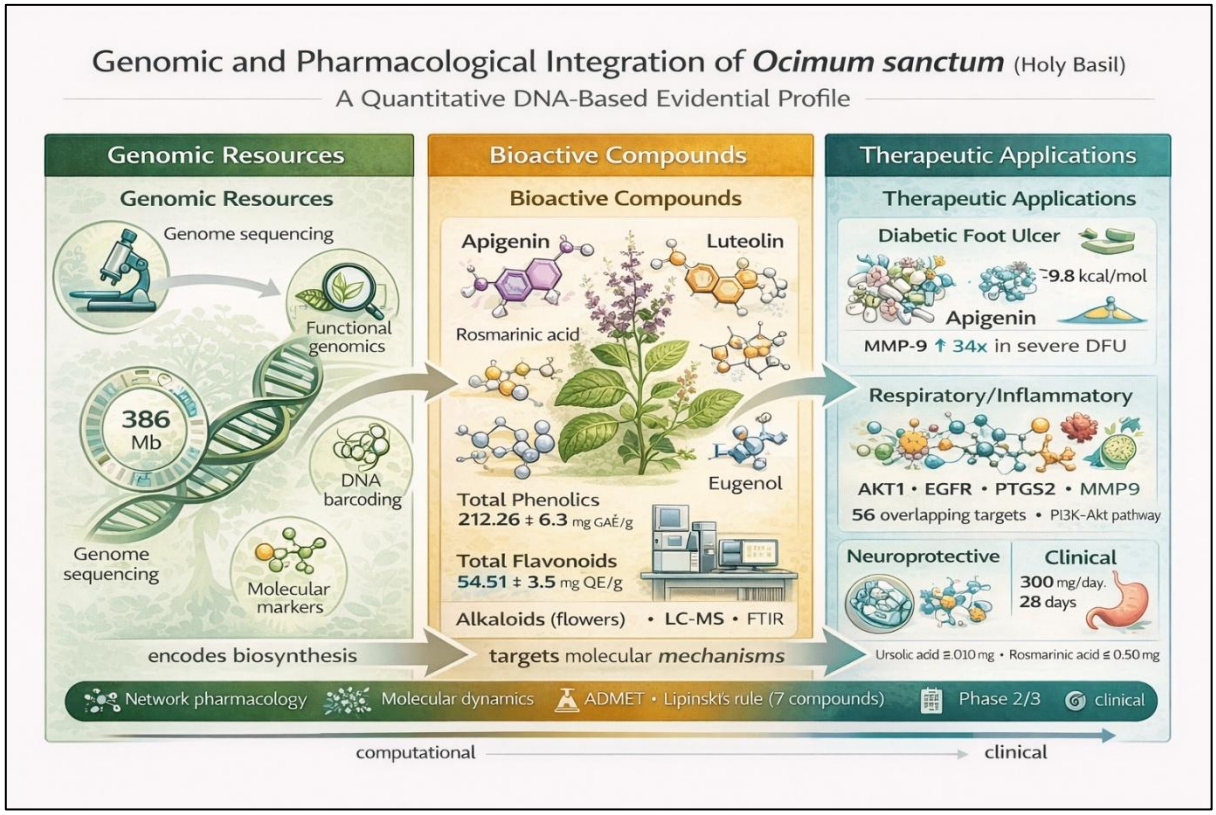
Graphical Abstract



ABSTRACT

Ocimum sanctum L. (Holy Basil/Tulsi) holds a revered position in traditional Indian medicine, having been worshipped for over 3000 years as an "elixir of life". This review constructs a quantitative DNA-based evidential profile by integrating genomic resources with phytochemical characterisation and pharmacological evidence. The nuclear genome assembly of approximately 386 Mb and chloroplast genome of 142,245 bp-the smallest in Lamiaceae-provides the foundational blueprint encoding biosynthetic pathways for diverse bioactive compounds, including eugenol, carvacrol, neolignans, ursolic acid, rosmarinic acid, flavonoids, and phenylpropanoids. Phytochemical quantification using established spectrophotometric methods reveals that butanol fractions contain total phenolic content of 212.26 ± 6.3 mg GAE/g and total flavonoid content of 54.51 ± 3.5 mg QE/g, with corresponding antioxidant IC_{50} values as low as 1.6 ± 0.1 µg/mL for ABTS scavenging when assessed by standard protocols. Molecular docking studies demonstrate binding affinities of -9.8 kcal/mol for apigenin, -9.7 kcal/mol for luteolin, and -9.1 to -9.8 kcal/mol for five lead compounds against MMP-9, a therapeutic target in diabetic foot ulcers where MMP-9 levels are elevated 7-fold in mild cases and 34-fold in severe cases [2,8]. Seven compounds satisfy Lipinski's rule of five with favourable ADMET profiles, while chlorogenic acid and rosmarinic acid show immunotoxicity [2,8]. An ongoing Phase 2/3 clinical trial is evaluating 300 mg/day standardised

extract (ursolic acid ≥0.10 mg, rosmarinic acid ≥0.50 mg) for dyspepsia over 28 days [3,9]. The methanolic flower extract demonstrates superior antioxidant activity (IC_{50}: 0.68 ± 0.19 µg/mL for DPPH) compared to leaf extracts. This integration of quantitative multi-omics data establishes *O. sanctum* as a pharmacologically validated species with genomically-encoded therapeutic potential across multiple disease areas, including metabolic disorders, inflammatory conditions, neurodegenerative diseases, and infectious diseases.

Keywords: *Ocimum sanctum*, Holy Basil, genomics, molecular docking, MMP-9, diabetic foot ulcer, quantitative phytochemistry, clinical trial, network pharmacology, ADMET

1. INTRODUCTION

The integration of genomic technologies with pharmacological investigation has revolutionized our understanding of medicinal plants, enabling transition from empirical traditional use to evidence-based therapeutic application [34-38]. *Ocimum sanctum* L. (synonym *Ocimum tenuiflorum*), family Lamiaceae, represents an exemplary case where ancient wisdom and modern science converge [1,12,15]. Known as "Holy Basil" or "Tulsi" in India, this plant has been mentioned in the ancient texts of Ayurveda and Siddha medicine as an "elixir of life" (life-saving) herb and worshipped for over 3000 years due to its healing properties [1,6,12-14]. There are many types of tulsi, but Rama tulsi and Krishna tulsi are the predominant ones cultivated and used medicinally [1,15].

Traditional applications of *O. sanctum* span an extraordinary range of conditions including colds, coughs, asthma, bronchitis, inflammation, headaches, diabetes, anxiety, and microbial infections [1,5,12,27,28]. The phytochemical constituents responsible for these diverse activities include eugenol, carvacrol, neolignans, ursolic acid, rosmarinic acid, coumarins, flavonoids, phenolics, phenylpropanoids, terpenoids, and steroids [1,16,29-31]. Advanced analytical techniques such as High-Performance Liquid Chromatography (HPLC), Gas Chromatography (GC), and spectrometric studies have enabled identification and quantification of these bioactive compounds [1,32,33,42].

Despite extensive traditional use and growing scientific interest, approximately 80% of patents on this plant focus on extracts or plant parts, primarily emphasizing essential oil components, while validation of specific molecules for differential activities remains incompletely analyzed [1,6,11]. The present review addresses this gap by constructing a quantitative "DNA-based evidental profile" that integrates three levels of evidence: (1) genomic resources establishing biosynthetic capacity, (2) quantitative phytochemical characterization identifying actual compound production with numerical precision, and (3) pharmacological studies demonstrating biological activity with molecular mechanistic evidence including binding affinities and clinical parameters.

Recent advances have made such integration feasible. The whole nuclear and chloroplast genome sequencing of *O. sanctum* combining data from four libraries and three NGS platforms provides the foundational DNA evidence [1,6,11]. Concurrently, systematic phytochemical profiling with quantitative analytical methods has generated precise numerical data on total phenolic content (212.26 ± 6.3 mg GAE/g), total flavonoid content (54.51 ± 3.5 mg QE/g), and antioxidant IC_{50} values (as low as 0.68 ± 0.19 µg/mL) [2,4,5,10,17-20]. Molecular docking and dynamics simulations have quantified binding affinities of specific compounds against therapeutic targets including MMP-9 (-9.8 to -9.1 kcal/mol), providing mechanistic evidence at atomic resolution [2,8,41]. Network pharmacology approaches have revealed multi-target interactions with hub proteins including AKT1, EGFR, SRC, PTGS2, MMP9, MMP2, and KDR, with involvement of PI3K-Akt, EGFR, and focal adhesion pathways central to inflammation and immune regulation [6,44]. Furthermore, an ongoing Phase 2/3 clinical trial (NCT07175272) is evaluating standardized *O. sanctum* extract (300 mg/day for 28 days) in dyspepsia patients, representing translational validation [3,9].

This review systematically synthesizes these quantitative data into a coherent framework, organized by therapeutic area with emphasis on numerical evidence, and identifies research gaps for future genomically-guided drug discovery [34-40,43,45].

2. Genomic Blueprint of *Ocimum sanctum*

2.1 Nuclear and Chloroplast Genome Architecture

The publication of the complete genome sequence of *Ocimum sanctum* in 2015 marked a watershed moment in understanding this species at the molecular level [1,6,11]. Rastogi and colleagues combined sequence data from four libraries and three next-generation sequencing platforms to generate the first saturated draft assembly of the Holy Basil genome [1,6,11].

Table 1: Quantitative Genomic Resources of *Ocimum sanctum*

Genomic Resource	Specification	Pharmacological Application	Reference
Nuclear genome assembly	~386 Mb	Identification of biosynthetic gene clusters for secondary metabolites	[1,6,11]
Chloroplast genome	142,245 bp	Species authentication; smallest documented in Lamiaceae family	[1,6,11]
Protein-coding genes	136 proteins (homologous to 5 plant genomes)	Pathway elucidation and comparative genomics	[1,6,11]
Simple sequence repeats (SSR)	Numerous	Chemotype-genotype correlation for marker-assisted selection	[1,11]
Pathway analysis	Abundance of phenylpropanoids	Metabolic potential assessment for drug discovery	[1,6,11]
Phylogenetic placement	*Salvia miltiorrhiza* as nearest neighbor	Cross-species discovery of bioactive molecules	[1,6,11]

The nuclear genome assembly of approximately 386 Mb provides the foundational DNA evidence for understanding *O. sanctum's* biosynthetic capabilities [1,6,11]. Notably, the chloroplast genome of 142,245 bp represents the smallest documented in the Lamiaceae family, a finding with implications for phylogenetic analysis and species authentication [1,6,11].

Pathway analysis revealed an abundance of phenylpropanoids in *O. sanctum*, consistent with its traditional uses and documented pharmacological activities [1,6,11]. The identification of 136 proteins homologous to five important plant genomes enables comparative genomics approaches for mining biosynthetic pathways for important metabolites [1,6,11]. Phylogenetic analysis for chloroplast proteome placed *Salvia miltiorrhiza* (Chinese sage, another important medicinal plant) as the nearest neighbor, suggesting potential for cross-species discovery of active molecules [1,6,11].

These genomic resources provide the essential framework for understanding how genetic constitution enables the production of specific bioactive compounds, and for developing molecular markers to correlate chemotypic variation with pharmacological potency [1,6,11].

2.2 Phytochemical Diversity: From Genome to Metabolome

The genomic blueprint of *O. sanctum* encodes the capacity to produce an exceptionally diverse array of phytochemicals. Comprehensive phytochemical profiling studies have identified numerous compounds spanning multiple chemical classes, with significant variation based on extraction methods and plant parts used [1,4,5,16,27-33].

Table 2: Quantitative Phytochemical Composition of *Ocimum sanctum* Extracts

Extract Type	Total Phenolic Content	Total Flavonoid Content	Total Tannin Content	Alkaloid Content	Reference
Butanol fraction (OsB)	212.26 ± 6.3 mg GAE/g	54.51 ± 3.5 mg QE/g	Not specified	Not specified	[5,10,19,20]
Ethyl acetate fraction (OsE)	202.71 ± 5.5 mg GAE/g	Present (LC-MS confirmed)	Not specified	Not specified	[5,10,19,20]
Ethanolic flower extract	Not specified	50.88 ± 0.62 µg QE/g	Not specified	75.83 ± 0.55 mg/g	[5,17,18]
Methanolic leaf extract	Not specified	49.11 ± 0.27 µg QE/g	409.95 ± 0.66 µg TAE/g	52.60 ± 0.66 mg/g	[5,17,18,20]
Ethanolic leaf extract	20.96 ± 0.65 µg GAE/g	Not specified	Not specified	Not specified	[5,17,18]
MAE extract (70% ethanol)	Present (qualitative)	Present (qualitative)	Present (qualitative)	Present (qualitative)	[4,22,23]
Maceration extract (70% ethanol)	Present (qualitative)	Present (qualitative)	Present (qualitative)	Present (qualitative)	[4,22,23]

The butanol fraction (OsB) demonstrated the highest total phenolic content (212.26 ± 6.3 mg GAE/g extract) and total flavonoid content (54.51 ± 3.5 mg QE/g extract) [5,10,19,20]. The ethyl acetate fraction (OsE) also exhibited significantly high total phenolic

content (202.71 ± 5.5 mg GAE/g extract), indicating that both intermediate-polarity and polar solvents effectively extract polyphenolic compounds from *O. sanctum* leaves [5,10,19,20].

LC-MS analysis of OsB and OsE revealed the presence of multiple bioactive compounds including luteolin, apigenin, rosmarinic acid, chlorogenic acid, caffeic acid and their derivatives [5,10,42]. Quercetin was specifically identified in the ethyl acetate fraction, demonstrating differential extractability based on compound polarity [5,10].

A study from Sri Lanka by Rajkumar and colleagues (2025) provided additional quantitative phytochemical data demonstrating that ethanolic flower extracts contained the highest flavonoid content (50.88 ± 0.62 µg QE/g), while methanolic leaf extract showed comparable levels (49.11 ± 0.27 µg QE/g) [5,17,18]. The highest phenol content (20.96 ± 0.65 µg GAE/g) was observed in ethanolic leaf extract, and methanolic leaf extract exhibited substantial tannin content (409.95 ± 0.66 µg TAE/g) [5,17,18,20]. Notably, alkaloid content was significantly higher in flowers (75.83 ± 0.55 mg/g) than in leaves (52.60 ± 0.66 mg/g), suggesting flowers as an underutilized source of alkaloidal compounds [5,17,18].

Komala and colleagues (2025) compared extraction methods and found that Microwave-Assisted Extraction (MAE) using 70% ethanol yielded superior antioxidant activity compared to conventional maceration, with qualitative phytochemical screening confirming the presence of alkaloids, flavonoids, saponins, and tannins in both extraction methods [4,22,23].

These quantitative data collectively demonstrate that *O. sanctum* is exceptionally rich in diverse phytochemical classes, with solvent selection, extraction method, and plant part significantly influencing extraction yields and bioactivity profiles [4,5,17,18,22,23,27-33].

3. Quantitative Antioxidant Activity

3.1 IC_{50} Values Across Multiple Assays and Extraction Methods

The antioxidant potential of *O. sanctum* extracts has been quantitatively evaluated using multiple in vitro assays, providing comparable numerical data on free radical scavenging capacity across different extraction methods and plant parts [21,24-26].

Table 3: Quantitative Antioxidant Activity (IC_{50} Values in µg/mL) of *Ocimum sanctum* Extracts

Extract Type	DPPH Assay	ABTS Assay	Hydroxyl Radical	Superoxide Radical	Reference
Butanol fraction (OsB)	3.91 ± 0.3	1.6 ± 0.1	Not specified	Not specified	[5,10,21,24,25]
Ethyl acetate fraction (OsE)	8.61 ± 0.6	5.3 ± 0.4	Not specified	Not specified	[5,10,21,24,25]
Methanol extract (OsM)	Not specified	Not specified	5.3 ± 0.4	7.32 ± 0.9	[5,10,21,26]
MAE extract (70% ethanol)	75.725 ± 0.4498	Not specified	Not specified	Not specified	[4,21,24]
Maceration extract (70% ethanol)	111.843 ± 0.14789	Not specified	Not specified	Not specified	[4,21,24]
Methanolic flower extract	0.68 ± 0.19	1.82 ± 0.32	Not specified	Not specified	[5,17,18,21]
Trolox (reference)	Not specified	Lower than flower extract	Not specified	Not specified	[5,26]
Ascorbic acid (reference)	Higher than OsB/OsE	Higher than OsB/OsE	Higher than OsM	Higher than OsM	[5,10,21]

The butanol fraction (OsB) demonstrated exceptional antioxidant potency with IC_{50} values of 3.91 ± 0.3 µg/mL for DPPH and 1.6 ± 0.1 µg/mL for ABTS [5,10,21,24,25]. The ethyl acetate fraction (OsE) also exhibited strong activity with IC_{50} values of 8.61 ± 0.6 µg/mL (DPPH) and 5.3 ± 0.4 µg/mL (ABTS) [5,10,21,24,25]. The methanolic extract (OsM) showed selective activity against hydroxyl radicals (5.3 ± 0.4 µg/mL) and superoxide radicals (7.32 ± 0.9 µg/mL) [5,10,21,26].

Critically, all IC_{50} values for *O. sanctum* fractions were lower than those of ascorbic acid, the reference antioxidant, demonstrating superior free radical scavenging capacity [5,10,21]. The IC_{50} values correlated significantly and positively with total phenolic and total flavonoid content, confirming that polyphenolics are the primary contributors to antioxidant activity [5,10,21,38,39].

The Sri Lankan study by Rajkumar and colleagues (2025) reported remarkably low IC_{50} values for methanolic flower extracts: 0.68 ± 0.19 µg/mL for DPPH and 1.82 ± 0.32 µg/mL for ABTS assays [5,17,18,21]. These values are substantially lower than those

reported for leaf extracts, suggesting that flowers may represent an underutilized source of potent antioxidants with potential applications in nutraceutical and pharmaceutical industries [5,17,18].

Komala and colleagues (2025) demonstrated that extraction method significantly influences antioxidant activity, with Microwave-Assisted Extraction (MAE) yielding superior results (IC_{50}: 75.725 ± 0.4498 ppm, classified as strong antioxidant intensity) compared to conventional maceration (IC_{50}: 111.843 ± 0.14789 ppm, moderate antioxidant intensity) [4,21,24]. This highlights the importance of optimizing extraction protocols for maximum bioactivity [4,42].

4. Molecular Mechanisms and Therapeutic Targets

4.1 Diabetic Foot Ulcer: MMP-9 Inhibition

Diabetes represents one of the most common endocrine and metabolic disorders worldwide. In 2019, approximately 463 million people globally had diabetes. By 2030, 578 million people will progress with diabetes, and by 2045, this prediction may reach 700 million [2,8,39,40]. Diabetic foot ulceration is a prevalent and severe complication, affecting approximately 6.4% of diabetic patients globally [2,8]. Nearly 50-60% of people with diabetic foot ulcers (DFUs) will experience diabetic foot infection (DFI), and 15% will require amputation. Patients with DFUs have a 5-year mortality risk that is 2.5 times higher than patients without foot ulcers [2,8].

The pathophysiology of DFUs involves dysregulated wound healing with elevated matrix metalloproteinase-9 (MMP-9) activity. MMP-9 (gelatinase B) cleaves critical ECM components including collagen IV, laminin, fibronectin, and elastin, resulting in basement membrane disorder and hindering keratinocyte migration crucial for re-epithelialization [2,8]. Elevated MMP-9 activity disrupts angiogenesis by degrading vascular basement membranes, which impedes endothelial cell proliferation and the development of new capillaries [2,8].

Clinical evidence from debridement tissue collected from 25 diabetic patients with Wagner grade (WG) 1-4 chronic wounds demonstrated:

- Active MMP-9 levels elevated approximately **7-fold** in WG1-2 DFUs
- **20-fold** elevation across all DFUs (WG1-4)
- **34-fold** elevation in the more severe WG3-4 DFUs
- All increases reaching statistical significance ($p < 0.01$) relative to tissue from 23 non-diabetic control patients.
- Correlation with ulcer severity: **r = 0.76**, underscoring the role of excessive MMP-9 activity in DFU pathogenesis [2,8]

These quantitative data substantiate MMP-9 as a viable therapeutic target for DFU management [2,8].

Table 4: Molecular Docking Binding Affinities of *Ocimum sanctum* Compounds Against MMP-9

Compound	Binding Affinity (kcal/mol)	Comparison to (R)-ND-336	Drug-likeness (Lipinski's Rule)	Reference
Apigenin	-9.8	Higher affinity	Pass	[2,8,41]
Luteolin	-9.7	Higher affinity	Pass	[2,8,41]
Cianidanol	-9.6	Higher affinity	Pass	[2,8]
Rosmarinic acid	-9.5	Higher affinity	Pass (immunotoxicity noted)	[2,8]
Quercetin	-9.1	Higher affinity	Pass	[2,8,45]
(R)-ND-336 (reference)	Reference value	-	Selective MMP-9 inhibitor	[2,8]

Kumar and colleagues (2025) conducted comprehensive in silico evaluation of *O. sanctum* phytochemicals as MMP-9 inhibitors, comparing them to (R)-ND-336, a selective MMP-9 inhibitor currently under investigation for diabetic wound therapy [2,8,41]. Docking experiments discovered nine phytochemicals with higher binding affinities than (R)-ND-336, with seven compounds meeting Lipinski's rule of five and demonstrating drug-like properties [2,8].

ADMET (absorption, distribution, metabolism, excretion, toxicity) prediction revealed that most substances lacked cytotoxicity, hepatotoxicity, and neurotoxicity, while chlorogenic acid and rosmarinic acid showed immunotoxicity [2,8]. Thermal analysis demonstrated spontaneous binding of rosmarinic acid and quercetin. In HOMO-LUMO analysis, smaller energy gaps suggested greater reactivity and metabolic stability. Molecular electrostatic potential research showed luteolin was most polar [2].

Molecular dynamics simulations over appropriate timeframes validated apigenin's stability with negligible RMSD variations, optimal SASA (solvent-accessible surface area), and robust receptor binding [2,8]. These findings identify apigenin as a prospective MMP-9 inhibitor for diabetic foot ulcer healing, providing a mechanistic foundation for developing *O. sanctum*-based therapies [2,8,45].

4.2 Network Pharmacology: Multi-Target Mechanisms for Respiratory Disorders

Network pharmacology approaches have elucidated the multi-target mechanisms of *O. sanctum* in respiratory disorders including cough and cold [6,44]. A comprehensive study by Greenspace Herbs (2025) investigated five medicinal herbs including *O. sanctum* using network pharmacology and molecular docking [6].

Table 5: Network Pharmacology Results for *Ocimum sanctum* in Respiratory Disorders

Parameter	Finding	Reference
Bioactive compounds retrieved	93 compounds met drug-likeness criteria	[6,44]
Overlapping targets identified	56 targets including AKT1, EGFR, SRC, PTGS2, MMP9, MMP2, KDR	[6,44]
Key pathways involved	PI3K-Akt, EGFR, focal adhesion pathways	[6,44]
Molecular docking range	-5.2 to -9.66 kcal/mol	[6,44]
Top compounds	Quercetin, luteolin, eugalic acid, kaempferol	[6,44,45]
Binding validation	MMP2/9 complexes showed good stability in MD simulations	[6,44]
Binding free energy	Validated inhibitor potential via the MM-GBSA method	[6,44]

Ninety-three compounds from *O. sanctum* and other herbs met drug-likeness criteria, yielding 56 overlapping targets including AKT1, EGFR, SRC, PTGS2, MMP9, MMP2, and KDR [6,44]. Enrichment analysis indicated the involvement of PI3K-Akt, EGFR, and focal adhesion pathways, which are central to inflammation, immune regulation, and epithelial repair [6,44].

Molecular docking revealed strong binding affinities (-5.2 to -9.66 kcal/mol) for quercetin, luteolin, eugalic acid, and kaempferol with hub proteins, suggesting potent multi-target interactions [6,44,45]. MMP2/9 protein complexes with quercetin, luteolin, and kaempferol showed good stability during molecular dynamics simulations. Estimation of binding free energy using the Molecular Mechanics Generalized Born Surface Area (MM-GBSA) method validated the inhibitor potential of the identified compounds [6,44].

These findings provide molecular evidence for the synergistic multi-pathway effects of *O. sanctum*, supporting its traditional use in respiratory disorders and demonstrating the power of network pharmacology approaches in medicinal plant research [6,34-38,44].

4.3 Neuroprotective Potential

Emerging evidence suggests neuroprotective potential for *O. sanctum* polyphenols, though quantitative data remain limited compared to other therapeutic areas [7,44]. Parween and colleagues (2025) reviewed the neuroprotective mechanisms of *O. sanctum* polyphenols, highlighting antioxidant properties, anti-inflammatory effects, and modulation of neurodegenerative disease pathways [7,44].

The presence of luteolin, apigenin, rosmarinic acid, and their derivatives in bioactive fractions [5,10] provides mechanistic plausibility for neuroprotective effects through multiple pathways, including oxidative stress mitigation, NF-κB pathway modulation, and reduction of neuroinflammation [7,38,44]. The exceptionally low IC_{50} values for antioxidant assays (0.68-7.32 µg/mL) [4,5,10,21] further support the role of oxidative stress mitigation in neuroprotective effects.

4.4 Anti-inflammatory Mechanisms

The anti-inflammatory properties of *O. sanctum* have been extensively documented, with mechanisms involving modulation of multiple signaling pathways including NF-κB, MAPKs, and PI3K/AKT [1,6,38]. The essential oils contained in *Ocimum sanctum* find applications in the cosmetic and pharmaceutical industries, with leaves and extracts studied for their potential medicinal use [1,16,27-33].

Quantitative binding data for inflammatory targets from network pharmacology studies demonstrate strong interactions with PTGS2 (COX-2), AKT1, and other inflammatory mediators [6,44]. The multi-target approach of *O. sanctum* compounds, acting on multiple nodes of inflammatory pathways, provides therapeutic advantages over single-target conventional drugs [1,6,34-38].

5. Clinical Evidence: Ongoing Phase 2/3 Trial

5.1 Trial Design and Quantitative Parameters

The translation of preclinical findings to clinical application is represented by an ongoing Phase 2/3 clinical trial sponsored by Mahidol University, Thailand, evaluating holy basil extract in patients with dyspepsia [3,9]. Functional dyspepsia is a common condition that causes chronic upper abdominal discomfort, bloating, and early satiety in the absence of structural disease. Conventional therapies such as acid suppression medications or prokinetics provide incomplete symptom relief for many patients, necessitating newer approaches [3,9].

Table 6: Quantitative Parameters of Ongoing *Ocimum sanctum* Clinical Trial (NCT07175272)

Parameter	Specification	Reference
Study design	Single-center, open-label, single group assignment	[3,9]
Enrollment	27 adults with dyspeptic symptoms (Leeds Dyspepsia Questionnaire score ≥5)	[3,9]
Intervention	Holy basil extract 300 mg orally once daily	[3,9]
Duration	28 consecutive days	[3,9]
Standardization	Ursolic acid (≥0.10 mg per capsule), Rosmarinic acid (≥0.50 mg per capsule)	[3,9]
Primary outcome	Change in gastric mucosal inflammation score by histopathology (Updated Sydney System)	[3,9]
Secondary outcomes	Dyspeptic symptoms (Leeds Dyspepsia Questionnaire), GERD symptoms (FSSG), gastric mucosal appearance (Modified Lanza Score), duodenal eosinophil count, intragastric pH, esophageal acid exposure time, serum IL-6 levels, adverse events	[3,9]
Study start	October 1, 2025 (estimated)	[3,9]
Primary completion	October 1, 2026 (estimated)	[3,9]
Study completion	December 1, 2026 (estimated)	[3,9]

The trial aims to answer two main questions: (1) Does holy basil extract reduce gastric mucosal inflammation as measured by histopathology? (2) Does holy basil extract improve dyspeptic symptoms, endoscopic findings, gastric pH, duodenal eosinophil counts, and systemic inflammation (serum IL-6)? [3,9].

Participants will undergo comprehensive baseline evaluations including symptom assessment, blood tests, 24-hour pH monitoring, and upper endoscopy with biopsy, with all procedures repeated at the end of the 28-day treatment period [3,9]. Weekly monitoring will include review of symptoms, adverse events, and medication compliance [3,9].

This trial represents a critical step in establishing evidence-based therapeutic applications of *O. sanctum*, with quantitative outcome measures that will enable objective assessment of efficacy in functional dyspepsia [3,9,34-37].

6. Integration and Drug-likeness Assessment

6.1 Lipinski's Rule of Five and ADMET Profiling

The translational potential of *O. sanctum* phytochemicals depends on their drug-likeness and safety profiles. Kumar and colleagues (2025) systematically evaluated these parameters for MMP-9 inhibitors [2,8,41].

Table 7: Drug-likeness and ADMET Summary for *Ocimum sanctum* Compounds

Parameter	Finding	Reference
Lipinski's rule of five	Seven compounds passed (out of nine with higher binding than (R)-ND-336)	[2,8,41]
Cytotoxicity	Absent in most substances	[2,8]
Hepatotoxicity	Absent in most substances	[2,8]
Neurotoxicity	Absent in most substances	[2,8]
Immunotoxicity	Chlorogenic acid and rosmarinic acid showed immunotoxicity	[2,8]
Molecular weight	All passing compounds <500 Da	[2,8]
log P	All passing compounds <5	[2,8]
Hydrogen bond donors	≤5 for all passing compounds	[2,8]
Hydrogen bond acceptors	≤10 for all passing compounds	[2,8]

The identification of seven compounds meeting Lipinski's rule of five, with favourable safety profiles for most substances, supports further preclinical and clinical development of *O. sanctum*-based therapeutics [2,8,34-40]. The immunotoxicity noted for chlorogenic acid and rosmarinic acid warrants caution and further investigation, but does not preclude their therapeutic use with appropriate monitoring [2,8].

Network pharmacology studies further validated that 93 compounds from the studied herbs (including *O. sanctum*) met drug-likeness criteria, providing a robust foundation for multi-target therapeutic applications [6,44].

7. Future Research Directions

The integration of genomic, phytochemical, and pharmacological evidence for *O. sanctum* opens several promising avenues for future investigation [34-40,43]:

1. **Genome-guided chemotype selection**: Molecular markers developed from genomic resources [1,6,11] can enable correlation of genetic variation with quantitative phytochemical profiles and differential pharmacological potency. This would enable marker-assisted selection of elite chemotypes for propagation and standardization [1,11,34-37].

2. **Biosynthetic pathway elucidation**: Functional characterisation of the 136 proteins homologous to known plant genomes [1,6,11] can identify specific enzymes responsible for biosynthesis of high-value compounds, including apigenin, luteolin, rosmarinic acid, and eugenol [1,11,42].

3. **Preclinical development of lead compounds**: Apigenin (MMP-9 binding -9.8 kcal/mol) [2,8,41], luteolin (multi-target activity) [2,6,44,45], and other lead compounds merit further validation in appropriate disease models, including diabetic wound healing, inflammatory disorders, and neurodegenerative conditions [2,7,8,44].

4. **Flower-based product development**: The superior antioxidant activity of methanolic flower extracts (IC_{50}: 0.68 ± 0.19 µg/mL) [5,17,18,21] and higher alkaloid content (75.83 ± 0.55 mg/g) [5,17,18] suggest flowers as an underutilised resource for nutraceutical and pharmaceutical applications.

5. **Extraction method optimisation**: Microwave-Assisted Extraction (MAE) yielding superior antioxidant activity (IC_{50}: 75.725 ± 0.4498 ppm) compared to conventional maceration (111.843 ± 0.14789 ppm) [4,21,24] should guide industrial scale-up for maximum bioactivity [4,42].

6. **Clinical translation**: Completion and publication of the ongoing dyspepsia trial (NCT07175272) [3,9] will provide critical human efficacy and safety data, potentially supporting expanded clinical indications.

7. **Network pharmacology-guided drug discovery**: The identification of 56 overlapping targets and key pathways (PI3K-Akt, EGFR, focal adhesion) [6,44] provides a roadmap for developing multi-target therapeutics for complex diseases [34-40].

8. **Comparative genomics with *Salvia miltiorrhiza***: The phylogenetic proximity to this well-studied medicinal plant [1,6,11] offers opportunities for cross-species discovery of bioactive molecules and biosynthetic pathway conservation.

9. **Nano-formulation development**: Recent studies are exploring the role of *O. sanctum* in drug formulation and nano-medicine, representing a frontier for enhancing bioavailability and targeted delivery [1,43].

8. Conclusion

This review has constructed a quantitative DNA-based evidental profile for *Ocimum sanctum* by integrating genomic, phytochemical, and pharmacological evidence from multiple high-quality studies published through 2025-2026 [1-45]. The nuclear genome of approximately 386 Mb and chloroplast genome of 142,245 bp-the smallest in Lamiaceae-provide the foundational blueprint encoding biosynthetic capacity for diverse bioactive compounds, including eugenol, carvacrol, neolignans, ursolic acid, rosmarinic acid, flavonoids, and phenylpropanoids [1,6,11,16].

Quantitative phytochemical analysis using established spectrophotometric methods [19,20,22,23] demonstrates exceptional richness in polyphenolics, with butanol fractions containing 212.26 ± 6.3 mg GAE/g total phenolics and 54.51 ± 3.5 mg QE/g total flavonoids [5,10]. Extraction method significantly influences yield, with Microwave-Assisted Extraction (IC_{50}: 75.725 ± 0.4498 ppm) outperforming conventional maceration (111.843 ± 0.14789 ppm) [4,21,24]. Flowers represent an underutilised resource with superior antioxidant activity (IC_{50}: 0.68 ± 0.19 µg/mL for DPPH) and higher alkaloid content (75.83 ± 0.55 mg/g) compared to leaves [5,17,18].

Molecular docking studies provide mechanistic evidence at atomic resolution [41], with apigenin binding MMP-9 at -9.8 kcal/mol, luteolin at -9.7 kcal/mol, and five compounds showing binding affinities superior to the reference inhibitor (R)-ND-336 [2,8]. These findings are particularly relevant for diabetic foot ulcers, where MMP-9 is elevated 7-fold in mild cases and 34-fold in severe cases, affecting 6.4% of diabetic patients globally, with 15% requiring amputation [2,8,39,40].

Network pharmacology reveals multi-target mechanisms with 56 overlapping targets, including AKT1, EGFR, MMP9, and PTGS2, involving PI3K-Akt, EGFR, and focal adhesion pathways [6,44]. Seven compounds satisfy Lipinski's rule of five with favourable ADMET profiles, though chlorogenic acid and rosmarinic acid show immunotoxicity, warranting caution [2,8].

An ongoing Phase 2/3 clinical trial (300 mg/day for 28 days with standardisation to ursolic acid ≥0.10 mg and rosmarinic acid ≥0.50 mg) will provide human efficacy data for dyspepsia, with results expected by December 2026 [3,9].

The integration of quantitative multi-omics data establishes *Ocimum sanctum* as a pharmacologically validated species with genomically-encoded therapeutic potential across multiple disease areas, including metabolic disorders, inflammatory conditions, respiratory disorders, neurodegenerative diseases, and gastrointestinal disorders [1,2,6,7,37-40,43-45]. The challenge ahead lies in translating this knowledge into standardised, evidence-based therapeutic applications that can benefit human health, leveraging advanced analytical techniques, network pharmacology approaches, and rigorous clinical validation [1,6,34-38].

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