Research Article

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From Phytochemicals to Molecular Targets: Integrated Antioxidant, Antimicrobial and In Silico Characterization of *Ocimum americanum* L.

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Abstract

The increasing prevalence of antimicrobial resistance and oxidative stress-related disorders underscores the need to identify novel bioactive compounds from medicinal plants. *Ocimum americanum* L. is traditionally used for various therapeutic applications; however, its phytochemical composition and biological activities under the agro-climatic conditions of Tamil Nadu, India, remain insufficiently characterized. This study investigated the phytochemical profile, antioxidant and antimicrobial activities of *O. americanum* leaf extracts collected from Tiruchirappalli, Tamil Nadu, integrating experimental evaluation with *in silico* pharmacokinetic, toxicity, and molecular docking analyses. Qualitative and quantitative phytochemical screening revealed substantial levels of flavonoids (178 mg/g), phenolics (68.41 mg/g), and tannins (58.47 mg/g). The extracts demonstrated notable antioxidant activity, with DPPH and ABTS IC_{50} values of 58.3 ± 1.4 and 49.7 ± 1.2 µg/mL, respectively, supported by FRAP and total antioxidant capacity assays. Antimicrobial evaluation using disc diffusion, minimum inhibitory concentration, and minimum bactericidal concentration assays demonstrated considerable activity, with the aqueous extract showing the strongest inhibition against *Proteus vulgaris* (MIC, 1.56 mg/mL; MBC, 3.12 mg/mL). SwissADME and pkCSM analyses indicated favorable drug-likeness and pharmacokinetic properties for selected phytoconstituents. Molecular docking further revealed that dehydrodieugenol exhibited favorable binding toward DNA gyrase B (−7.2 kcal/mol), while Limonene Dioxide 1 formed stable hydrogen-bond interactions with ILE A:878 and LYS A:879 of topoisomerase IV. Overall, *O. americanum* from Tamil Nadu represents a promising source of antioxidant and antimicrobial phytochemicals, with computational findings providing mechanistic support for the observed bioactivities. Further cytotoxicity and in vivo investigations are warranted to establish its therapeutic potential.

1. Introduction

Oxidative stress and microbial infections represent two major and interconnected challenges in contemporary health research. Under physiological conditions, reactive oxygen species (ROS) are continuously generated as by-products of cellular metabolism and participate in essential biological processes, including cellular signaling and host defense **(Alfei *et al.* 2024)**. However, excessive ROS production or insufficient antioxidant protection can disrupt redox homeostasis, resulting in oxidative damage to lipids, proteins, nucleic acids, and cellular membranes. Persistent oxidative imbalance has been implicated in the initiation and progression of several chronic disorders, including cardiovascular and neurodegenerative diseases, metabolic dysfunction, and inflammatory conditions **(Liu *et al.* 2025)**. Although endogenous antioxidant systems provide an important defense against ROS-mediated damage, their capacity may become inadequate under sustained oxidative burden **(Manful *et al.* 2025)**. Consequently, identification of naturally occurring compounds capable of efficiently modulating oxidative stress remains an important objective in the search for safer and multifunctional therapeutic agents **(Jomova *et al.* 2023)**. In parallel, the rapid emergence and dissemination of antimicrobial resistance have compromised the effectiveness of many conventional antimicrobial therapies. The declining efficacy of existing antibiotics, together with the ability of pathogenic microorganisms to develop resistance through diverse genetic and biochemical mechanisms, has created an urgent need for alternative antimicrobial strategies **(Muteeb *et al.* 2023)**. Medicinal plants represent an exceptionally valuable reservoir of structurally diverse secondary metabolites that have evolved as chemical defense mechanisms against environmental stressors and microbial challenges **(Al-Khayri *et al.* 2023)**. Plant-derived phytochemicals, particularly phenolics, flavonoids, terpenoids, tannins, and related compounds, have attracted considerable attention because of their broad biological activities and chemical diversity. Unlike single-target synthetic agents, complex plant extracts may contain multiple constituents capable of acting simultaneously on different cellular and molecular pathways, potentially reducing the likelihood of rapid development of resistance **(Kumar *et al.* 2023)**.

Among these phytochemical classes, phenolic compounds and flavonoids are particularly important contributors to the antioxidant properties of medicinal plants. Their hydroxylated aromatic structures enable them to participate in hydrogen atom transfer and single-electron transfer reactions, thereby neutralizing reactive radicals and limiting oxidative chain reactions **(Sun and Shahrajabian 2023; Tungmunnithum *et al.* 2018)**. In addition to their antioxidant properties, these compounds can exert antimicrobial effects through multiple mechanisms, including alteration of microbial membrane integrity, interaction with cell-wall components, modulation of membrane permeability, inhibition of essential enzymes, and interference with nucleic acid-associated processes **(Eshboev *et al.* 2024)**. This combination of antioxidant and antimicrobial activities makes phenolic- and flavonoid-rich plants particularly attractive for the discovery of multifunctional bioactive agents. Importantly, the biological activity of a plant cannot be attributed solely to the presence of a particular compound because synergistic, additive, or antagonistic interactions among multiple constituents may substantially influence the overall activity of the extract **(Vaou *et al.* 2022)**. The genus *Ocimum*, belonging to the family Lamiaceae, is widely recognized for its medicinal and pharmacological importance. Species within this genus are characterized by considerable chemical diversity, encompassing phenolic acids, flavonoids, terpenoids, essential-oil constituents, and other specialized metabolites **(Maluwa *et al.* 2026)**. These compounds have been associated with antioxidant, antimicrobial, anti-inflammatory, and

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other pharmacological properties, supporting the longstanding traditional use of *Ocimum* species in different medicinal systems. *Ocimum americanum* L., a widely distributed aromatic herb, has traditionally been employed in several regions of Africa, Asia, and South America for managing infections, fever, inflammation, and other health conditions **(Dharsono *et al.* 2022)**. Despite this traditional significance, the phytochemical composition and biological potential of *O. americanum* can vary considerably according to genetic background, geographical origin, soil characteristics, temperature, rainfall, humidity, cultivation practices, and plant developmental stage. Geographical variation is particularly relevant when evaluating medicinal plants because environmental conditions can directly influence secondary-metabolite biosynthesis and accumulation **(Jasicka-Misiak *et al.* 2021)**. Plants growing under distinct agro-climatic conditions may therefore exhibit substantial differences in the concentration and composition of phenolics, flavonoids, terpenoids, and other bioactive constituents **(Vinogradova *et al.* 2023)**. Tamil Nadu encompasses diverse environmental conditions that can influence plant metabolism, yet systematic characterization of *O. americanum* cultivated or collected under specific regional conditions remains comparatively limited. In particular, information concerning the phytochemical composition and associated antioxidant and antimicrobial activities of *O. americanum* leaves from Tiruchirappalli is insufficient.

Figure 1: Fresh leaves of *Ocimum americanum* L.

Region-specific investigation is therefore important not only for documenting the chemical characteristics of this medicinal resource but also for determining whether locally obtained plant material possesses reproducible biological activities of potential pharmacological relevance. Conventional phytochemical screening and bioactivity assays provide essential experimental evidence regarding the chemical composition and functional properties of plant extracts **(Heinrich *et al.* 2022)**. However, these approaches alone provide limited information concerning the pharmacokinetic behavior, toxicity characteristics, and potential molecular targets of individual phytoconstituents. Integration of experimental phytochemistry with computational drug-discovery approaches can provide an additional mechanistic perspective **(Chihomvu *et al.* 2024)**. *In silico* absorption, distribution, metabolism, excretion, and toxicity (ADMET) prediction enables preliminary assessment of drug-likeness and pharmacokinetic characteristics, while molecular docking can provide insights into the potential interaction of bioactive constituents with biologically relevant protein targets **(Ancuceanu *et al.* 2025)**. Such integrated approaches can facilitate prioritization of promising phytochemicals for subsequent experimental validation and help bridge the gap between chemical profiling and mechanistic interpretation **(Ariyanto *et al.* 2026)**. Therefore, the present study was undertaken to comprehensively characterize the bioactive potential of *O. americanum* leaves collected from Tiruchirappalli, Tamil Nadu. The study combined qualitative and quantitative phytochemical profiling with antioxidant evaluation using DPPH, ABTS, ferric-reducing antioxidant power, and total antioxidant capacity assays. The antimicrobial potential of the extracts was further investigated using inhibition assays together with minimum inhibitory concentration and minimum bactericidal concentration determinations. Selected major phytoconstituents were subsequently subjected to *In silico* ADMET assessment and molecular docking against relevant bacterial enzyme targets. By integrating regional phytochemical characterization, experimental antioxidant and antimicrobial evaluation, and computational molecular analysis, this study aims to provide a comprehensive assessment of *O. americanum* as a potential source of multifunctional bioactive compounds and to identify promising phytoconstituents for future pharmacological investigation.

2. Materials and Methods

2.1. Plant Collection and annotation

Fresh leaves of *Ocimum americanum* L. were collected from Tiruchirappalli District, Tamil Nadu, India (10°49′N, 78°41′E), during the post-monsoon season (October–November). The plant material was taxonomically authenticated by a senior botanist at the Rapinat Herbarium, St. Joseph's College, Tiruchirappalli, and a voucher specimen was deposited under **accession No. RHT-2792**. The collected leaves were thoroughly cleaned to remove adhering soil and extraneous materials and subsequently shade-dried at controlled ambient temperature (28–32 °C) for 14 days **(Atanasov *et al.* 2015)**. The dried material was pulverized into a uniform powder using a mechanical grinder and passed through a 40-mesh sieve to ensure consistent particle size. The powdered leaves were transferred to airtight, light-resistant containers and stored at 4 °C until further extraction and phytochemical analyses **(McCloud 2010)**.

2.2. Extraction Procedure and Yield

The dried leaf powder (10 g) was subjected to Soxhlet extraction using solvents of increasing polarity, namely hexane, methanol, and distilled water. Each extraction was performed at a solvent-to-sample ratio of 10:1 (v/w) for 6–8 h per cycle, with 3–4 extraction cycles to ensure efficient recovery of extractable constituents. The extraction temperatures were maintained near the respective boiling points of the solvents (approximately 69 °C for hexane, 65 °C for methanol, and 100 °C for distilled water) **(Travis *et al.* 2026)**. Following extraction, the resulting extracts were filtered to remove residual plant material and concentrated under reduced pressure using a rotary evaporator at 40 °C to minimize thermal degradation of heat-sensitive phytoconstituents. The concentrated extracts were subsequently stored at −20 °C in airtight containers until further phytochemical and biological analyses **(Haido *et al.* 2024)**. The extraction yields, calculated on a dry-weight basis, were 18.4% for the aqueous extract, 14.2% for the methanolic extract, and 6.7% for the hexane extract. The variation in extraction yield among solvents was considered reflective of differences in solvent polarity and the differential solubility of phytoconstituents present in the leaf material.

2.3. Qualitative and Quantitative Screening

Qualitative phytochemical screening of the *O. americanum* leaf extracts was performed using standard colorimetric and precipitation-based

assays to identify major classes of secondary metabolites. Alkaloids were detected using Mayer's and Wagner's reagents, whereas flavonoids were assessed using the alkaline reagent test **(Ouandaogo *et al.* 2023)**. Tannins and phenolic compounds were evaluated using ferric chloride ($FeCl_3$), lead acetate, iodine, and acetic acid reactions. Steroids were identified using the Salkowski test, while carbohydrates were detected using Molisch's and cobalt chloride reactions **(Das *et al.* 2014)**. The formation of characteristic colour changes or precipitates was recorded as a positive indication for the respective phytochemical groups. Quantitative estimation of the major phytochemical constituents was subsequently performed using validated spectrophotometric assays **(Ghosh *et al.* 2020)**. Appropriate reference standards were used to generate calibration curves for each phytochemical class, and sample absorbance was measured at the corresponding wavelengths using a UV–visible spectrophotometer. The concentrations were calculated from the respective calibration equations and expressed as milligrams per gram of dry leaf material (mg/g). All measurements were performed in independent replicates, with reagent blanks included to correct for background absorbance **(Michiu *et al.* 2022)**.

2.4. Antimicrobial Activity

The antimicrobial activity of *O. americanum* leaf extracts was evaluated using the agar disc diffusion method against selected bacterial and fungal strains. The test organisms comprised *Proteus vulgaris* (MTCC 1771), *Escherichia coli* (MTCC 443), *Staphylococcus aureus* (MTCC 3160), and *Aspergillus niger* (MTCC 281) **(Ali *et al.* 2022)**. Mueller–Hinton agar was prepared and dispensed at approximately 20 mL per Petri plate. Fresh microbial suspensions were adjusted to a turbidity equivalent to 0.5 McFarland standard and uniformly inoculated onto the agar surface to obtain a consistent microbial lawn. Sterile discs were impregnated with 25 µL of each plant extract at concentrations of 25, 50, and 100 µg/µL and carefully placed on the inoculated agar plates **(Salam *et al.* 2023)**. The plates were incubated at 37 °C for 24 h for bacterial strains and 48 h for *A. niger*. Following incubation, the diameter of the zones of inhibition surrounding each disc was measured in millimetres. Streptomycin was used as the positive control for bacterial assays, whereas fluconazole served as the positive control for the fungal assay **(Sharma *et al.* 2023)**. Appropriate solvent controls were included to exclude any antimicrobial effects attributable to the extraction solvents. All experiments were conducted in independent replicates, and the results were expressed as mean inhibition-zone diameters with corresponding variability **(El-Samahy *et al.* 2024)**.

2.4.1. MIC and MBC

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the *O. americanum* leaf extracts were determined using a broth microdilution assay **(Mahendran and Vimolmangkang 2023)**. Serial two-fold dilutions of each extract were prepared in Mueller–Hinton broth to obtain a defined concentration range. Each well was inoculated with a standardized bacterial suspension to achieve a final inoculum density of approximately 5×10^5 CFU/mL. Appropriate growth and sterility controls were included in each assay. The microdilution plates were incubated at 37 °C for 24 h under suitable conditions **(Smith *et al.* 2017)**. The MIC was defined as the lowest concentration of the extract that completely inhibited visible bacterial growth compared with the corresponding growth control. To determine the MBC, aliquots from wells showing no visible growth were subcultured onto extract-free Mueller–Hinton agar and incubated under the same conditions **(Kowalska-Krochmal and Dudek-Wicher 2021)**. The MBC was defined as the lowest extract concentration producing a ≥99.9% reduction in viable bacterial cells relative to the initial inoculum. All measurements were performed using independent experimental replicates to ensure reproducibility **(Andrews 2001)**.

2.5. Antioxidant Assays

The antioxidant activities of the *O. americanum* leaf extracts were evaluated at concentrations of 10, 25, 50, 75, and 100 µg/mL using the respective antioxidant assay protocols. Ascorbic acid (≥99% purity; Sigma-Aldrich) was used as the positive control. All extracts were analyzed in triplicate (n = 3), and the assays were performed within 48 h of sample preparation using aliquots obtained from extracts maintained as frozen stocks at −20 °C **(Chaudhary *et al.* 2020)**. The concentration-dependent antioxidant responses were recorded for each extract and compared with the positive control. The half-maximal inhibitory concentration (IC_{50}) was calculated by nonlinear regression analysis using GraphPad Prism version 9.0, employing a dose–response inhibition model. IC_{50} values were determined from the fitted concentration–response curves, with lower IC_{50} values indicating greater antioxidant activity. Data are presented as mean ± standard deviation (SD), and SD values were used as error bars throughout the analyses **(Jancy Mary *et al.* 2026)**.

2.5.1. DPPH Radical Scavenging Assay

The free-radical scavenging activity of the *O. americanum* leaf extracts was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. A freshly prepared 0.8 mM DPPH solution in methanol was mixed with the respective plant extracts at concentrations ranging from 10 to 100 µg/mL. The reaction mixtures were incubated for 30 min in the dark at room temperature to prevent light-induced degradation of the DPPH radical **(Baliyan *et al.* 2022)**. The reduction in DPPH radical intensity was subsequently quantified by measuring the absorbance at 517 nm using a UV–visible spectrophotometer. A reagent control containing DPPH solution without plant extract was used to determine the initial absorbance **(Kumar *et al.* 2014)**. The percentage of radical-scavenging activity was calculated using the following equation:

$$\text{DPPH radical scavenging activity (\%)} = [(A_{(\text{control})} - A_{(\text{sample})}) / A_{(\text{control})}] \times 100$$

where $A_{(\text{control})}$ represents the absorbance of the DPPH control and $A_{(\text{sample})}$ represents the absorbance of the reaction mixture containing the plant extract. The assay was performed in triplicate, and the results were expressed as mean ± SD **(Morabbi Najafabad and Jamei 2014)**.

2.5.2. ABTS Radical Scavenging Assay

The ABTS radical cation ($ABTS^{\bullet+}$) scavenging activity of the *O. americanum* leaf extracts was determined using a spectrophotometric assay. The $ABTS^{\bullet+}$ working solution was generated by mixing 7 mM 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) with 2.45 mM potassium persulfate in a 1:1 (v/v) ratio **(Ilyasov *et al.* 2020)**. The mixture was incubated in the dark for 12–16 h at room temperature to allow complete generation of the $ABTS^{\bullet+}$ radical cation. Prior to analysis, the resulting solution was diluted with an appropriate solvent until an absorbance of 0.70 ± 0.02 at 734 nm was obtained **(Lee *et al.* 2015; Yan *et al.* 2018)**. The diluted $ABTS^{\bullet+}$ solution was subsequently mixed with *O. americanum* leaf extracts at the predetermined concentrations. The reaction mixtures were incubated for 6 min at room temperature, after which the absorbance was measured at 734 nm using a UV–visible spectrophotometer **(Hussen and Endalew 2023)**. A corresponding $ABTS^{\bullet+}$ solution without extract was used as the control. The decrease in absorbance relative to the control was used to determine the radical-scavenging capacity of the extracts. All measurements were performed in triplicate, and the results were expressed as mean ± SD **(Huh *et al.* 2026; Platzer *et al.* 2021)**.

2.5.3. FRAP and TAC

FRAP activity was determined using freshly prepared FRAP reagent comprising 300 mM acetate buffer (pH 3.6), 10 mM TPTZ, and 20 mM $FeCl_3$ in a 10:1:1 (v/v/v) ratio. Extracts were incubated with the reagent at 37 °C for 30 min, and absorbance was measured at 593 nm **(Fernandes *et al.* 2016)**. FRAP values were calculated using a ferrous-ion calibration curve and expressed as µmol Fe^{2+} equivalents/g extract. Total antioxidant capacity (TAC) was determined using the phosphomolybdenum method. Extracts were incubated with phosphomolybdenum reagent at 95 °C for 90 min, cooled, and measured at 695 nm. Ascorbic acid was used as the reference standard, and results were expressed as mg ascorbic acid equivalents/g extract (mg AAE/g) **(Daimari and Deka 2024)**.

2.6. Statistical Analysis

All experiments were performed in triplicate (n = 3), and data are presented as mean ± standard deviation (SD). Statistical significance was evaluated using one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) post hoc test using SPSS version 26 and GraphPad Prism version 9. Effect size (partial η^2) and observed statistical power were reported for each analysis. 95% confidence intervals (CIs) were calculated for IC_{50} estimates. Differences were considered statistically significant at $p < 0.05$ **(Kim *et al.* 2025)**.

2.7. ADMET Property analysis

Two major phytoconstituents reported in Ocimum species, Dehydrodieugenol (PubChem CID: 12304; $C_{20}H_{22}O_4$) and Limonene Dioxide 1 (PubChem CID: 12679; $C_{10}H_{16}O_2$), were retrieved from the PubChem database and subjected to computational characterization. Drug-likeness and physicochemical properties were evaluated using the SwissADME and pkCSM platforms **(Muhmood *et al.* 2025)**. The compounds were assessed using established drug-likeness criteria, including the Lipinski, Ghose, Veber, Egan, and Muegge filters, to determine their suitability as potential bioactive lead molecules **(Daina *et al.* 2017)**. Predicted pharmacokinetic and toxicity-related properties were additionally evaluated to provide a preliminary assessment of their ADMET profiles **(Rai *et al.* 2023)**.

2.8. Molecular Docking Studies

Molecular docking studies were performed to investigate the potential binding modes and molecular interactions of the selected phytoconstituents, Dehydrodieugenol and Limonene Dioxide 1, against two clinically relevant bacterial DNA-processing enzymes: DNA gyrase subunit B (PDB ID: 4BAE) and DNA topoisomerase IV from Escherichia coli (PDB ID: 3RAE). These enzymes were selected because of their essential roles in bacterial DNA replication, chromosome segregation, and maintenance of DNA topology, making them established targets for antibacterial drug development **(Alotaibi *et al.* 2024)**. The three-dimensional protein structures were obtained from the Protein Data Bank and prepared for docking using AutoDockTools version 1.5.7. Protein preparation included removal of crystallographic water molecules and other non-essential components, addition of polar hydrogen atoms, and assignment of Gasteiger partial atomic charges **(C *et al.* 2022)**. The prepared receptor structures were converted into the appropriate docking format prior to molecular docking. The three-dimensional structures of Dehydrodieugenol and Limonene Dioxide 1 were obtained from PubChem and prepared as docking ligands by assigning appropriate atomic charges and hydrogen atoms and generating suitable ligand structures for conformational sampling **(Ma *et al.* 2024)**. Docking calculations were performed using AutoDock Vina version 1.2, which evaluates ligand–receptor binding poses based on a scoring function that estimates binding affinity **(Vanajothi 2026)**. The docking search space was defined around the co-crystallized ligand-binding region of each target to ensure focused exploration of the functionally relevant binding pocket. A grid box of 25 × 25 × 25 Å was used and centered on the corresponding ligand-binding site. The reported grid spacing of 0.375 Å was retained from the AutoDockTools-based docking setup **(Boonamnaj *et al.* 2023)**. For each ligand–protein system, multiple binding poses were generated and ranked according to their predicted binding affinity. The pose with the most favorable predicted binding energy and a biologically plausible orientation within the active or ligand-binding pocket was considered for subsequent interaction analysis. Particular attention was given to hydrogen bonding, hydrophobic interactions, π-associated interactions, electrostatic contacts, and interactions with catalytically or functionally important residues, as these interactions can contribute to ligand recognition and stabilization within the target pocket. The docked complexes were subsequently examined using BIOVIA Discovery Studio Visualizer 2021 and PyMOL version 2.5 **(Patil *et al.* 2010)**.

Table 1: Qualitative phytochemical screening of *O. americanum* leaf extracts across methanol, aqueous, and hexane extracts

Phytochemical Test	Methanol	Aqueous	Hexane	Literature*
Carbohydrate – Molisch test	–ve	+ve	+ve	✓
Carbohydrate – Cobalt chloride	+ve	+ve	–ve	✓
Protein – Millon test	–ve	+ve	+ve	✓
Protein – Biuret test	–ve	+ve	+ve	✓
Protein – Xanthoprotein test	–ve	+ve	–ve	—
Steroids – Salkowski reaction	+ve	+ve	–ve	✓
Tannins – Ferric chloride test	+ve	+ve	+ve	✓
Nitrate	–ve	+ve	–ve	—
Flavonoids – Alkaline reagent test	+ve	+ve	+ve	✓
Alkaloids – Mayer's test	+ve	+ve	+ve	✓
Alkaloids – Wagner's test	+ve	+ve	+ve	✓
Amino acids (cysteine)	–ve	+ve	–ve	—
Tannic & phenolic – $FeCl_3$ (5%)	+ve	+ve	–ve	✓
Tannic & phenolic – Lead acetate	+ve	+ve	–ve	✓
Tannic & phenolic – Dilute iodine	+ve	+ve	–ve	✓
Tannic & phenolic – Acetic acid	+ve	+ve	–ve	—
Inorganic acid – Carbonate test	+ve	+ve	–ve	—
Inorganic acid – Mercuric chloride	–ve	+ve	–ve	—
Reducing polysaccharide / Starch	–ve	+ve	–ve	—

+ve = present; –ve = absent. *Confirmed in related Ocimum species.

3. Results

3.1. Qualitative Phytochemical Screening

Qualitative phytochemical screening revealed the presence of diverse secondary metabolites in the *O. americanum* leaf extracts **(Table 1)**. The aqueous extract exhibited the broadest phytochemical profile, showing

positive reactions for carbohydrates, proteins, alkaloids, flavonoids, tannins, phenolics, steroids, and terpenoids. Both Mayer's and Wagner's tests confirmed the presence of alkaloids in the aqueous extract. The methanolic extract demonstrated a comparable phytochemical composition, with positive reactions for most of the tested metabolite classes, indicating its ability to extract a wide range of polar and moderately polar constituents.

Table 2: Quantitative analysis of *O. americanum* leaf extract

Metabolite	Amount (mg/g, mean ± SD, n=3)
Chlorophyll a	12.12 ± 0.6
Chlorophyll b	15.22 ± 0.9
Total chlorophyll	18.05 ± 0.5
Total carotenoids	6.13 ± 2.0
Total sugar	146 ± 0.3
Total protein	2.1 ± 1.2
Total lipids	112 ± 0.4
Total free amino acids	0.9 ± 0.7
Total phenolics	68.41 ± 0.7
Total tannins	58.47 ± 0.8
Total flavonoids	178 ± 0.2

Values expressed as mean ± SD (n = 3).

Table 3: Antimicrobial activity (zone of inhibition in mm) of *O. americanum* leaf extracts

Organism	Extract	25 µg/µL	50 µg/µL	100 µg/µL	Control
P. vulgaris	Methanol	10±0.5	14±0.6	18±0.8	22±0.5
P. vulgaris	Aqueous	12±0.4	16±0.5	20±0.7	22±0.5
P. vulgaris	Hexane	6±0.3	8±0.4	11±0.5	22±0.5
E. coli	Methanol	9±0.6	12±0.5	15±0.7	20±0.6
E. coli	Aqueous	10±0.5	13±0.6	17±0.8	20±0.6
E. coli	Hexane	5±0.4	7±0.3	9±0.5	20±0.6
S.aureus	Methanol	11±0.5	15±0.7	19±0.9	23±0.4
S. aureus	Aqueous	13±0.6	17±0.8	21±0.6	23±0.4
S. aureus	Hexane	7±0.5	9±0.4	12±0.6	23±0.4
A. niger	Methanol	8±0.4	11±0.5	14±0.6	18±0.5
A. niger	Aqueous	9±0.3	12±0.4	16±0.7	18±0.5
A. niger	Hexane	4±0.2	6±0.3	8±0.4	18±0.5

Values are mean ± SD (n = 3). Control: Streptomycin (bacteria), Fluconazole (fungi).

Table 4: MIC and MBC values of *O. americanum* leaf extracts (mg/mL)

Organism	Extract	MIC Extract	MBC Extract	MIC	MBC	MBC/MIC
P. vulgaris	Aqueous	1.56	3.12	0.25	0.5	2.0
	Methanol	3.12	6.25	0.25	0.5	2.0
	Hexane	>25	>25	0.25	0.5	—
S. aureus	Aqueous	3.12	6.25	0.5	1	2.0
	Methanol	6.25	12.5	0.5	1	2.0
	Hexane	>25	>25	0.5	1	—
E. coli	Aqueous	6.25	12.5	0.5	1	2.0
	Methanol	12.5	25	0.5	1	2.0
A. niger	Aqueous	6.25	—	—	—	—
	Methanol	12.5	—	—	—	—

MBC/MIC ≤ 4 indicates bactericidal activity. Values are mean of triplicate determinations. — = not applicable. Extract - mg/mL.

In contrast, the hexane extract displayed a comparatively restricted profile, with detectable steroids, tannins, and flavonoids. The observed differences among extracts were consistent with the varying polarity and solvent selectivity of the extraction systems. Overall, the findings indicate that *O. americanum* leaves contain a chemically diverse repertoire of phytoconstituents, with the aqueous and methanolic extracts providing broader phytochemical coverage than the non-polar hexane extract. These preliminary findings support further quantitative characterization and evaluation of the extracts for their antioxidant and antimicrobial properties.

3.2. Quantitative Phytochemical Analysis

Quantitative analysis demonstrated that *O. americanum* leaves contain substantial levels of major phytochemical constituents **(Table 2)**. Total flavonoid content was the highest (178 ± 0.2 mg/g), followed by total phenolic content (68.41 ± 0.7 mg/g) and tannin content (58.47 ± 0.8 mg/g). The pronounced abundance of these compounds indicates that flavonoids and phenolics constitute major components of the phytochemical profile of the leaves and may contribute substantially to their observed antioxidant and antimicrobial activities. The comparatively high phytochemical concentrations observed in the present study may also reflect the influence of regional agro-climatic conditions, including temperature, humidity, and solar radiation, which can modulate secondary-metabolite biosynthesis in medicinal plants. Environmental stressors, particularly enhanced ultraviolet exposure and temperature fluctuations, may stimulate the phenylpropanoid pathway and promote the accumulation of phenolic and flavonoid compounds as protective metabolites. The substantial phytochemical abundance observed in *O. americanum* collected from Tiruchirappalli therefore highlights the potential importance of geographical provenance in determining phytochemical composition and represents an important finding for the regional characterization of this medicinal species.

3.3. Antimicrobial Activity

All three *O. americanum* leaf extracts exhibited concentration-dependent antimicrobial activity against the tested microorganisms **(Tables 3 and 4)**. Overall, the aqueous and methanolic extracts demonstrated stronger activity than the hexane extract, indicating a greater contribution of polar and moderately polar phytoconstituents to the antimicrobial effects. *Proteus vulgaris* was the most susceptible organism, with the aqueous extract producing a 20 ± 0.7 mm zone of inhibition at 100 µg/µL, closely approaching the activity of streptomycin (22 ± 0.5 mm). Statistical analysis confirmed significant differences among the tested extracts ($F = 18.45$, $p = 0.002$, $\eta^2 = 0.672$), indicating a strong treatment effect. Tukey's HSD analysis showed no significant difference between the aqueous and methanolic extracts ($p = 0.312$), whereas both extracts exhibited significantly greater activity than the hexane extract ($p < 0.01$). Furthermore, the aqueous and methanolic extracts showed an MBC/MIC ratio of 2.0, supporting a bactericidal mode of antimicrobial action rather than a primarily bacteriostatic effect. Collectively, these findings demonstrate the broad antimicrobial potential of *O. americanum* leaf extracts, particularly the polar fractions, and support further investigation of their active constituents and mechanisms of microbial inhibition.

3.4. Antioxidant Activity

All extracts exhibited concentration-dependent antioxidant activity across the four assays, with the aqueous and methanolic extracts showing stronger responses than the hexane extract. DPPH radical-scavenging activity increased from 30 ± 1.2% to 65 ± 1.8%, with an IC_{50} of 58.3 ± 1.4 µg/mL (95% CI: 55.6–61.0 µg/mL), compared with 18.2 ± 0.8 µg/mL for ascorbic acid. ABTS scavenging increased from 41 ± 1.1% to 63 ± 1.9%, yielding an IC_{50} of 49.7 ± 1.2 µg/mL (95% CI: 47.4–52.0 µg/mL), whereas ascorbic acid showed an IC_{50} of 15.4 ± 0.6 µg/mL. The aqueous extract demonstrated the highest reducing capacity, with FRAP and TAC values of 124.6 ± 3.2 µmol Fe^{2+} eq/g and 88.3 ± 2.4 mg AAE/g, respectively, followed by methanol (98.4 ± 2.8 µmol Fe^{2+} eq/g; 72.1 ± 1.9 mg AAE/g)

and hexane (41.2 ± 1.6 µmol Fe^{2+} eq/g; 28.7 ± 1.3 mg AAE/g). Significant concentration-dependent effects were observed for DPPH ($F = 32.76$, $p = 0.0008$, $\eta^2 = 0.929$) and ABTS ($F = 28.54$, $p = 0.0012$, $\eta^2 = 0.919$). Overall, the results demonstrate substantial antioxidant potential, particularly in the polar extracts. These findings are supported by the corresponding dose–response data presented in **Supplementary Table S1–S3**, covering DPPH, ABTS, and FRAP/TAC assays, respectively.

3.5. Statistical analysis

Statistical analysis demonstrated significant differences among the tested extracts and concentrations. For antimicrobial activity against Proteus vulgaris, one-way ANOVA revealed a strong treatment effect ($F = 18.45$, $p = 0.002$, $\eta^2 = 0.672$), indicating that extract type significantly influenced antimicrobial efficacy. Tukey's HSD post hoc analysis showed no significant difference between the aqueous and methanolic extracts ($p = 0.312$), whereas both extracts exhibited significantly greater activity than the hexane extract ($p < 0.01$). A pronounced concentration-dependent response was also observed for antioxidant activity. DPPH radical-scavenging activity showed a highly significant treatment effect ($F = 32.76$, $p = 0.0008$, $\eta^2 = 0.929$), while ABTS activity demonstrated a similarly strong effect ($F = 28.54$, $p = 0.0012$, $\eta^2 = 0.919$) **(Table S4–S7)**. The high partial eta-squared values indicate large effect sizes, demonstrating substantial contributions of extract concentration to antioxidant activity. Observed statistical power exceeded 0.94 across the analyses, further supporting the robustness of the detected effects. Overall, the statistical findings consistently demonstrate that the aqueous and methanolic extracts exhibited superior antimicrobial and antioxidant activities compared with the hexane extract, with activity increasing with extract concentration.

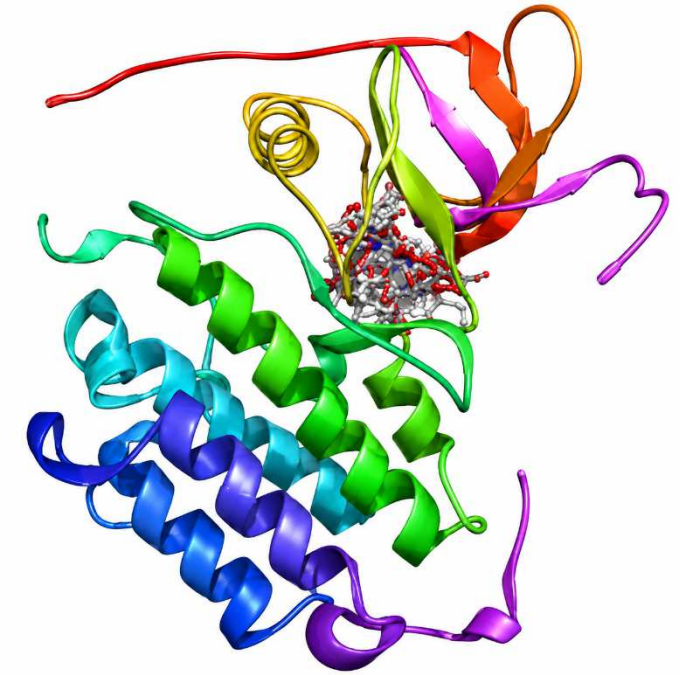

Figure 2: 3D ribbon representation of Dehydrodieugenol (grey sticks with red oxygen atoms) docked within the binding pocket of DNA gyrase B (PDB: 4BAE). Protein secondary structure colored from N-terminus (purple) to C-terminus (red).

3.6. ADMET Analysis

Limonene Dioxide 1 (MW 168.23 g/mol; $C_{10}H_{16}O_2$) exhibited favorable physicochemical and pharmacokinetic characteristics, with a consensus Log P of 2.00, TPSA of 25.06 Å², and an ESOL-predicted Log S of −1.54, indicating good aqueous solubility. The compound was predicted to have high gastrointestinal (GI) absorption and blood–brain barrier (BBB) permeability, with no predicted inhibition of major cytochrome P450 enzymes and a bioavailability score of 0.55. The SwissADME radar plot

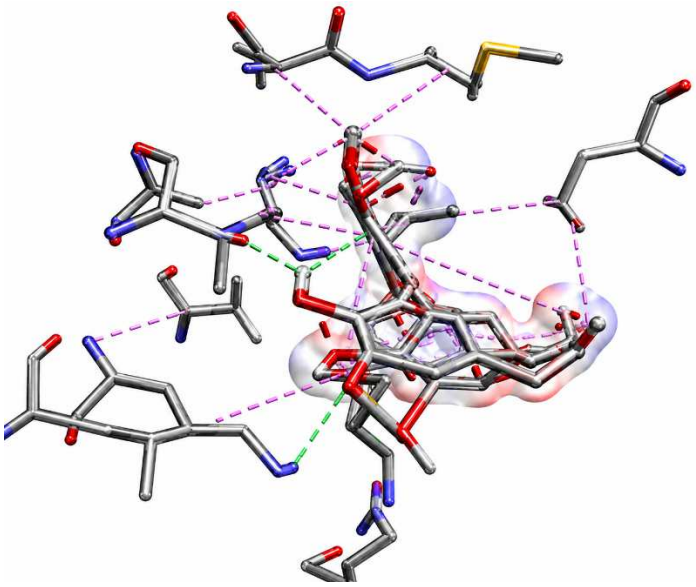

Figure 3a. 3D view of Dehydrodieugenol interactions within the DNA gyrase B active site.

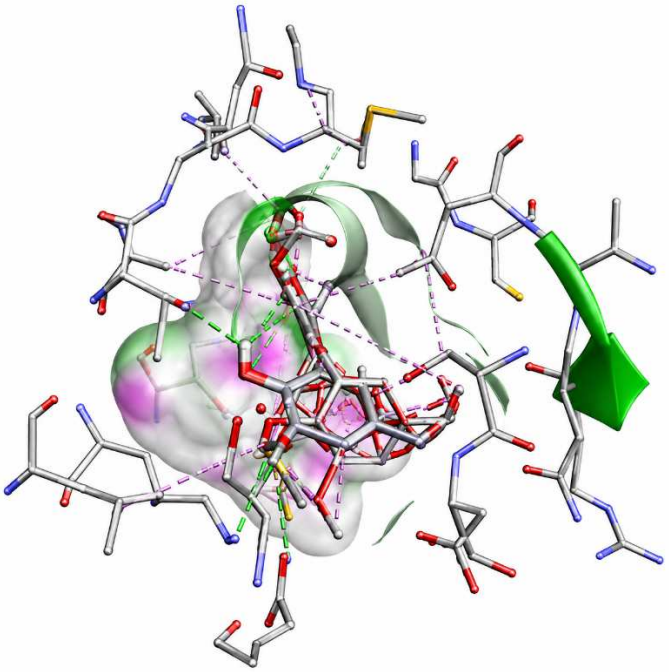

Figure 3b. Active site surface map of Dehydrodieugenol binding site in DNA gyrase B.

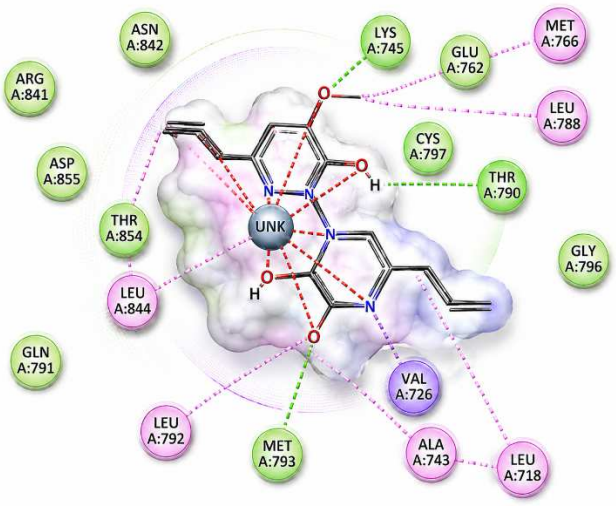

Figure 3c. 2D protein-ligand interaction map of Dehydrodieugenol in DNA gyrase B active site.

(Figure S1a) indicated that the compound largely occupied the recommended drug-likeness space with respect to lipophilicity, molecular size, and solubility-related parameters. Limonene Dioxide 1 satisfied the Lipinski, Ghose, Veber, and Egan criteria without violations, while a single Muegge violation was observed due to its molecular weight being below the specified threshold. However, the predicted toxicity profile warrants caution, as the compound showed positive AMES and skin-sensitization predictions. The AMES alert may be associated with its reactive epoxide functionality, corresponding to a Brenk structural alert for a three-membered heterocyclic moiety. Therefore, this structural feature should be carefully considered during subsequent lead optimization and experimental safety assessment. Detailed physicochemical, pharmacokinetic, and toxicity predictions are provided in **Supplementary Figures S1a–S1c and Table S8**. Dehydrodieugenol (MW 326.39 g/mol; $C_{20}H_{22}O_4$) demonstrated a favorable overall drug-likeness profile, with a consensus Log P of 4.22 and TPSA of 58.92 Å². The compound showed zero Lipinski violations and satisfied the Ghose, Veber, Egan, and Muegge criteria, supporting its favorable physicochemical characteristics. High GI absorption and BBB permeability were predicted, while the compound was classified as a non-P-glycoprotein substrate, suggesting potentially favorable membrane permeability and distribution characteristics. However, inhibition of several major CYP450 isoforms, including CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4, was predicted, indicating a potential for metabolic interactions that requires experimental validation and drug–drug interaction assessment. No PAINS alerts were identified, reducing the likelihood of nonspecific assay interference. The predicted toxicity profile was comparatively favorable, with negative AMES mutagenicity, no predicted hepatotoxicity, no hERG I/II inhibition, and no skin-sensitization liability. The predicted maximum tolerated dose was 0.652 log mg/kg/day, while the oral rat LD_{50} was estimated at 2.196 mol/kg. Collectively, these findings indicate that Dehydrodieugenol possesses favorable drug-like and predicted safety characteristics, although its CYP450 inhibition profile warrants further experimental investigation. Detailed ADMET predictions are presented in **Supplementary Figures S2a–S2c and Table S9- S10**.

3.7. Molecular Docking Analysis

Molecular docking revealed favorable interactions between the selected phytoconstituents and the bacterial DNA-processing targets. Dehydrodieugenol exhibited a predicted binding affinity of −7.2 kcal/mol against DNA gyrase subunit B (PDB ID: 4BAE). The three-dimensional docked complex **(Figure 2)** showed that the ligand occupied the N-terminal ATP-binding domain, adopting a stable orientation within the catalytic pocket. Detailed interaction analysis **(Figure 3a–c)** revealed multiple ligand–residue contacts that may contribute to complex stabilization. The pharmacophore surface representation showed complementary hydrogen-bond donor and acceptor regions, with the hydroxyl groups and ether oxygen atoms of Dehydrodieugenol appropriately positioned within the corresponding interaction zones. These interactions, together with surrounding non-covalent contacts, support the favorable docking score and suggest potential interference with ATP-dependent DNA gyrase activity. Limonene Dioxide 1 showed a predicted binding affinity of −5.8 kcal/mol against E. coli topoisomerase IV (PDB ID: 3RAE). The three-dimensional complex **(Figure 3a–c)** indicated that the compact bicyclic epoxide-containing ligand was accommodated within the binding region at the interface of the GHKL ATPase and TOPRIM domains. Two conventional hydrogen bonds were identified with ILE A:878 and LYS A:879, involving the oxygen-containing functional groups of the ligand. The pharmacophore analysis further demonstrated complementary hydrogen-bond donor and acceptor regions associated with the epoxide oxygens. In addition, van der Waals interactions were observed with PRO A:877, VAL A:876, TRP A:880, PRO A:914, LYS A:913, and ALA A:920. Collectively, these interactions indicate favorable accommodation of Limonene Dioxide 1 within the topoisomerase IV binding region and provide a structural basis for its predicted inhibitory potential.

4. Discussion

The present study provides an integrated experimental and computational assessment of *Ocimum americanum* L. collected from Tiruchirappalli, Tamil Nadu, demonstrating a broad phytochemical composition together with measurable antioxidant and antimicrobial activities. The principal finding is the comparatively high abundance of flavonoids, phenolics, and tannins, which was accompanied by stronger biological activity in the aqueous and methanolic extracts than in the hexane extract. This pattern is consistent with the extraction-dependent enrichment of chemically diverse constituents and suggests that relatively polar phytochemicals contribute substantially to the observed antioxidant and antimicrobial effects. The study therefore provides region-specific evidence supporting the bioactive potential of *O. americanum*, while also identifying Dehydrodieugenol and Limonene Dioxide 1 as computationally interesting molecular leads. Qualitative screening revealed that the aqueous extract contained the broadest range of detectable phytochemical classes, including carbohydrates, proteins, alkaloids, flavonoids, tannins, phenolics, steroids, and terpenoids, whereas the methanolic extract showed a similarly broad profile. In contrast, the hexane extract was comparatively restricted, with detectable steroids, tannins, and flavonoids. The higher extraction yield of the aqueous fraction (18.4%) compared with methanol (14.2%) and hexane (6.7%) further supports the substantial contribution of water-soluble constituents to the overall chemical composition. However, these qualitative reactions should be interpreted as class-level evidence rather than definitive compound identification. The current results therefore establish a useful phytochemical foundation, but chromatographic and spectrometric approaches such as LC-MS/MS or GC-MS are required to resolve the individual constituents responsible for the measured activities.

Quantitative analysis showed particularly high levels of flavonoids (178 ± 0.2 mg/g), followed by phenolics (68.41 ± 0.7 mg/g) and tannins (58.47 ± 0.8 mg/g). These compounds provide a plausible chemical basis for the antioxidant performance observed across multiple assays. Flavonoids and phenolics possess functional groups capable of donating hydrogen atoms or electrons to reactive species, while their aromatic structures can stabilize the resulting radicals through resonance. The substantial difference between the present flavonoid content and previously reported values for *O. americanum* from other geographical locations also emphasizes the importance of environmental and geographical influences on secondary metabolism. Nevertheless, attributing the elevated concentrations solely to UV-B exposure, temperature, or humidity would require controlled comparative experiments. A more appropriate interpretation is that the distinctive agro-climatic environment of Tiruchirappalli may contribute to the observed phytochemical phenotype, potentially through modulation of phenylpropanoid-associated metabolism. The antioxidant results provide convergent evidence rather than reliance on a single radical-scavenging assay. DPPH activity increased from 30 ± 1.2% to 65 ± 1.8% across the tested concentration range, yielding an IC_{50} of 58.3 ± 1.4 µg/mL, while ABTS activity increased from 41 ± 1.1% to 63 ± 1.9%, with an IC_{50} of 49.7 ± 1.2 µg/mL. Although ascorbic acid remained substantially more potent, the extract demonstrated reproducible concentration-dependent activity. The lower ABTS IC_{50} relative to DPPH is also informative because ABTS•+ can interact with antioxidants across a broader polarity range, whereas DPPH is particularly responsive to compounds capable of reacting effectively within its methanolic reaction environment. The FRAP and TAC measurements further strengthened this interpretation: the aqueous extract showed the highest reducing capacity (124.6 µmol Fe^{2+} eq/g) and

total antioxidant capacity (88.3 mg AAE/g), followed by methanol and hexane. The strong effect sizes for DPPH (η^2 = 0.929) and ABTS (η^2 = 0.919), together with observed statistical power of 0.99, demonstrate that concentration accounted for a substantial proportion of the experimental variability.

The antimicrobial findings showed a similar extraction-dependent pattern. All three extracts exhibited concentration-dependent inhibition against the tested bacterial and fungal organisms, but the aqueous and methanolic fractions consistently produced larger zones of inhibition than the hexane fraction. Proteus vulgaris was particularly susceptible to the aqueous extract, which produced a 20 ± 0.7 mm inhibition zone at 100 µg/µL, approaching the 22 ± 0.5 mm zone produced by streptomycin. More importantly, the aqueous extract showed an MIC of 1.56 mg/mL and an MBC of 3.12 mg/mL against P. vulgaris, giving an MBC/MIC ratio of 2.0. Similar ratios were observed for the aqueous and methanolic extracts against the susceptible bacterial strains. The comparatively strong statistical effect for antimicrobial activity (η^2 = 0.672; observed power = 0.94) supports a meaningful contribution of extract type to the observed differences. The stronger performance of polar extracts may reflect the higher abundance of phenolics, flavonoids, tannins, and other polar or moderately polar metabolites capable of interacting with microbial envelopes and intracellular targets. The activity against both Gram-negative and Gram-positive bacteria suggests that the extracts may operate through multiple mechanisms rather than through a narrowly selective target. Nevertheless, the absolute MIC values remain considerably higher than those generally expected for purified antibiotics, which is appropriate for crude botanical extracts containing mixtures of active and inactive constituents. Thus, the present findings should be interpreted as evidence of antimicrobial potential rather than equivalence to conventional antibacterial drugs.

The computational results provide an additional mechanistic layer to the experimental observations. Dehydrodieugenol showed a predicted binding affinity of −7.2 kcal/mol toward DNA gyrase B and occupied the N-terminal ATP-binding region. Its two conventional hydrogen bonds with LYS A:745 and THR A:790 were complemented by hydrophobic interactions involving LEU A:718, VAL A:726, MET A:793, and other surrounding residues. This combination of polar anchoring and hydrophobic stabilization provides a plausible explanation for the favorable docking score and suggests that the molecule can be accommodated within a functionally relevant region of the enzyme. The pharmacophore representation further indicated that the oxygen-containing groups of Dehydrodieugenol are appropriately positioned within hydrogen-bond donor/acceptor regions. Limonene Dioxide 1 displayed a weaker but still favorable docking score of −5.8 kcal/mol against E. coli topoisomerase IV. The ligand formed conventional hydrogen bonds with ILE A:878 and LYS A:879 and additional van der Waals contacts with PRO A:877, VAL A:876, TRP A:880, PRO A:914, LYS A:913, and ALA A:920. The concentration of contacts around the binding region indicates effective pocket accommodation despite the relatively compact structure of the ligand. These findings support the possibility that the two phytoconstituents interact with bacterial DNA-processing machinery through distinct structural mechanisms. However, docking scores represent computational estimates rather than direct measurements of enzymatic inhibition and therefore should not be interpreted as proof of target engagement. The ADMET profiles further differentiate the two candidate molecules. Limonene Dioxide 1 showed high predicted GI absorption, BBB permeability, no predicted inhibition of the evaluated CYP enzymes, and a bioavailability score of 0.55. Its principal liability was the positive AMES prediction and skin-sensitization signal, which may be related to its reactive epoxide functionality. Dehydrodieugenol satisfied the evaluated drug-likeness filters and showed high predicted GI absorption and BBB permeability, but its predicted inhibition of CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4 raises an important drug–drug interaction concern. Furthermore, the supplementary pkCSM profile indicates hERG II inhibition, despite the earlier summary describing the overall toxicity profile as favorable; this prediction should therefore be treated as a specific cardiac-safety liability requiring experimental confirmation rather than overlooked. Taken together, the experimental and computational findings suggest a coherent structure–activity framework in which phytochemical-rich polar extracts provide measurable antioxidant and antimicrobial effects, while selected constituents exhibit plausible interactions with bacterial molecular targets. Nevertheless, the relationship between extract-level bioactivity and the two docked compounds remains inferential because Dehydrodieugenol and Limonene Dioxide 1 were selected from literature-reported Ocimum chemistry rather than directly confirmed in the present extracts by mass spectrometry. This distinction is important for interpretation and should be addressed in future work through LC-MS/MS or GC-MS profiling, bioactivity-guided fractionation, purified-compound testing, and direct DNA gyrase/topoisomerase IV inhibition assays. Cytotoxicity, Ames testing, cardiac-safety assays, and in vivo antimicrobial and antioxidant studies will also be necessary to determine whether the computationally prioritized compounds can progress beyond preliminary lead identification. Overall, the study supports *O. americanum* from Tamil Nadu as a promising source of chemically diverse antioxidant and antimicrobial constituents, while providing a rational computational framework for prioritizing individual phytochemicals. The combination of high flavonoid and phenolic content, reproducible concentration-dependent antioxidant activity, bactericidal extract profiles, favorable drug-likeness of selected compounds, and plausible target-site interactions provides a strong basis for subsequent chemical characterization and mechanistic validation. Importantly, the results should be regarded as evidence of bioactive potential and lead-generation value, rather than definitive therapeutic efficacy, until the proposed biochemical, toxicological, and in vivo studies are completed.

5. Conclusion

The present study demonstrates the promising phytochemical, antioxidant, antimicrobial, and *In silico* pharmacological potential of *Ocimum americanum* L. leaves collected from Tiruchirappalli, Tamil Nadu. The phytochemical investigation revealed a diverse composition of secondary metabolites, with particularly high levels of total flavonoids (178 ± 0.2 mg/g), phenolics (68.41 ± 0.7 mg/g), and tannins (58.47 ± 0.8 mg/g). The comparatively broad phytochemical profile of the aqueous and methanolic extracts, together with their higher extraction yields, indicates that polar phytoconstituents may substantially contribute to the biological activities observed. The antioxidant evaluation demonstrated consistent concentration-dependent activity across DPPH, ABTS, FRAP, and TAC assays. DPPH and ABTS produced IC_{50} values of 58.3 ± 1.4 and 49.7 ± 1.2 µg/mL, respectively, while the aqueous extract exhibited the highest FRAP and TAC values. The strong statistical effect sizes observed for DPPH and ABTS further support the reproducibility and concentration dependence of the antioxidant response. Similarly, antimicrobial assessment demonstrated substantial activity, particularly for the aqueous extract against Proteus vulgaris, with an MIC of 1.56 mg/mL and MBC of 3.12 mg/mL and an MBC/MIC ratio of 2.0, indicating bactericidal activity.

The computational component provided additional mechanistic insight. Dehydrodieugenol demonstrated a favorable docking score of −7.2 kcal/mol against DNA gyrase B, supported by hydrogen-bond and hydrophobic interactions within the ATP-binding region. Limonene Dioxide 1 showed a docking score of −5.8 kcal/mol against E. coli topoisomerase IV, with hydrogen bonds involving ILE A:878 and LYS A:879. ADMET analysis further indicated favorable drug-likeness for both compounds, although CYP inhibition associated with Dehydrodieugenol and the predicted AMES toxicity and skin

sensitization of Limonene Dioxide 1 represent important liabilities requiring further investigation. Overall, *O. americanum* represents a valuable source of natural antioxidant and antimicrobial constituents with potential for pharmaceutical and nutraceutical development. However, the present computational findings remain predictive, and the selected compounds were based on literature precedent rather than direct characterization of the investigated extracts. Future studies should therefore prioritize GC-MS/LC-MS/MS confirmation, bioactivity-guided fractionation, purified-compound testing, direct enzyme inhibition assays, cytotoxicity and genotoxicity evaluation, and in vivo validation before therapeutic applications can be established.

6. Disclosure Statements

6.1. Author Contribution

A.M.A. contributed to Conceptualization, Methodology, Investigation, Formal Analysis, Data Curation, Software, Visualization, and Writing—Original Draft. **S.V.** contributed to Supervision, Conceptualization, Methodology, Validation, and Writing—Review & Editing. **E.A.** contributed to Validation, Interpretation, and Writing—Review & Editing. All authors reviewed and approved the final manuscript. **A.M.A.** served as the corresponding author and was responsible for overall coordination of the study and manuscript.

6.2. Declaration of Generative AI

The authors used generative artificial intelligence tools solely for language editing and grammatical correction. The AI tools did not contribute to the study design, data collection, analysis, interpretation, or generation of scientific content. All content has been reviewed and approved by the authors, who take full responsibility for the integrity and accuracy of the work.

6.3. Ethics approval (for clinical/animal studies)

Ethics approval was not required for this study, as it did not involve human participants, clinical samples, or experimental animals. The study was limited to plant material, in vitro antimicrobial and antioxidant assays, and in silico computational analyses.

6.4. Informed Consent Statement

Not applicable. This study did not involve human participants, human-derived samples, or clinical data; therefore, informed consent was not required.

6.5. Data Availability Statement

The data generated and analyzed during the present study are provided in the Supplementary Materials, including the relevant experimental results and supporting computational data. Additional data and information related to the findings of this study are available from the corresponding author upon reasonable request.

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6.8. Conflicts of Interest

The authors declare that they have no known financial, personal, academic, or other relationships that could inappropriately influence, or be perceived to influence, the work reported in this manuscript. All authors confirm that there are no competing interests to declare.

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6.10. Supplementary Information

Supplementary material is available for this article at https://jomi.aayvu.com/SuppFile/225259/1/

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