



## Phytochemical Profiling, Antibacterial Activity, and Molecular Docking of *Ocimum Basilicum* L. Leaf Extract from Libya: Eugenol and Linalool Interactions with *Staphylococcus Aureus* Targets

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التحليل الكيميائي النباتي، والنشاط المضاد للبكتيريا، والإرساء الجزيئي لمستخلص  
أوراق الريحان (*Ocimum basilicum* L.) في ليبيا: تفاعلات الأوجينول  
واللينالول مع الأهداف البروتينية لبكتيريا المكورات العنقودية الذهبية  
(*Staphylococcus aureus*)

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### Abstract:

This research aimed to analyze the phytochemical constituents of *Ocimum basilicum* L. leaf methanol extract from Libya, assess its antibacterial activity against important bacterial strains, and conduct molecular interactions of its major compounds with four essential *Staphylococcus aureus* protein targets. Gas chromatography-mass spectrometry identified 30 compounds representing 99.36% of the extract, with eugenol (23.08%) and linalool (18.79%) as major constituents. The extract showed stronger activity against Gram-positive strains than against Gram-negative strains. Molecular docking using CB-Dock2 software revealed that eugenol displayed superior binding affinities (range: -6.1 to -6.7 kcal mol<sup>-1</sup>) compared to linalool (-5.7 to -5.9 kcal mol<sup>-1</sup>) against four *S. aureus* targets: ftsA (PDB: 3WQU), tyrosyl-tRNA synthetase (PDB: 1JIJ), clumping factor A (PDB: 1N67), and gyrase B (PDB: 3G75). Eugenol formed hydrogen bonds with key residues including GLU209, SER13, ASP177, SER55, and ASP81. Libyan *O. basilicum* leaf extract is rich in eugenol and linalool, which demonstrate promising antibacterial activity potentially mediated through interactions with essential bacterial proteins. The unique eugenol-rich chemotype distinguishes Libyan basil from previously characterized varieties. This first report on Libyan basil provides a scientific basis for its potential use in developing natural antibacterial agents, with implications for both human and veterinary medicine, particularly against Gram-positive pathogens.

**Key words:** *Ocimum basilicum*, antibacterial activity, eugenol, Linalool, molecular docking.

### الملخص:

هدفت هذه الدراسة إلى تحليل المكونات الكيميائية النباتية لمستخلص الميثانول من أوراق نبات الريحان (*Ocimum basilicum* L.) المزروع في ليبيا، وتقييم نشاطه المضاد للبكتيريا ضد سلالات بكتيرية مهمة، بالإضافة إلى دراسة التفاعلات الجزيئية للمركبات الرئيسية فيه مع أربعة أهداف بروتينية أساسية لبكتيريا المكورات العنقودية الذهبية (*Staphylococcus aureus*). أظهر تحليل كروماتوغرافيا الغاز المقترنة بمطيافية الكتلة (GC-MS) وجود 30 مركبًا كيميائيًا تمثل 99.36% من إجمالي المستخلص، وكان الأوجينول (23.08%) واللينالول (18.79%) من أبرز المكونات الرئيسية. كما أظهر المستخلص فعالية أكبر ضد البكتيريا موجبة الغرام مقارنة بالبكتيريا سالبة الغرام. وكشفت دراسات الإرساء الجزيئي باستخدام برنامج CB-Dock2 أن مركب الأوجينول أظهر ألفة ارتباط أعلى (تراوحت بين 6.1 و 6.7 كيلو كالوري/مول) مقارنة باللينالول (تراوحت بين 5.7 و 5.9 كيلو كالوري/مول) تجاه أربعة أهداف بروتينية لبكتيريا *S. aureus*، وهي: البروتينين FtsA (PDB:3WQU) إنزيم Tyrosyl-tRNA Synthetase (PDB: 1JIJ) البروتين Clumping Factor A (PDB:1N67) إنزيم Gyrase B (PDB: 3G75) كما كَوّن الأوجينول روابط هيدروجينية مع بقايا أحماض أمينية رئيسية، منه SER13 و GLU209 و ASP177 و ASP81. وأظهرت النتائج أن مستخلص أوراق الريحان الليبي غني بمركبي الأوجينول واللينالول، اللذين يمتلكان نشاطًا واعدًا مضادًا للبكتيريا، يُحتمل أن يكون ناتجًا عن تفاعلها مع البروتينات البكتيرية الأساسية. كما يتميز الريحان الليبي بنمط كيميائي غني بالأوجينول يميزه عن الأصناف التي سبق توصيفها في دراسات أخرى. ويُعد هذا البحث أول تقرير علمي عن الريحان الليبي، ويوفر أساسًا علميًا لإمكانية استخدامه في تطوير عوامل طبيعية مضادة للبكتيريا، مع تطبيقات محتملة في الطب البشري والطب البيطري، خاصة في مكافحة مسببات الأمراض موجبة الغرام.

**الكلمات المفتاحية:** الريحان (*Ocimum basilicum*) النشاط المضاد للبكتيريا الأوجينول (Eugenol) اللينالول (Linalool) الإرساء الجزيئي (Molecular Docking).

### INTRODUCTION:

Antimicrobial resistance (AMR) is one of the most serious risks to public health in the 21st century. The World Health Organization has declared AMR as one of the top ten global health threats, with multidrug-resistant *Staphylococcus aureus* and gram-negative pathogens causing millions of infections annually [1]. In both veterinary and human medicine, the diminishing efficacy of conventional antibiotics has necessitated the discovery of alternative medical strategies [2]. Plant-derived natural products have garnered considerable attention as potential raw materials for novel antibacterial agents. Unlike conventional antibiotics, which typically target a single bacterial component, many phytochemicals have demonstrated multi-target interactions, potentially lowering the likelihood of rapid resistance development [3-5]. Furthermore, medicinal plants are more affordable and accessible in low-resource settings, making them particularly valuable for veterinary applications in developing countries.

*Ocimum basilicum* L. has been extensively used in traditional medicine systems across Asia, Africa, and the Mediterranean region [6]. Ethnobotanical studies have documented its use for treating malaria among the Tetun people in West Timor [7], rheumatism and hypertension among the Batak Karo people in North Sumatra [8] and as an anthelmintic remedy by the Muna tribe in Southeast Sulawesi [9]. These traditional applications are attributed to the plant's essential oil composition, which includes methyl eugenol, eugenol, linalool, 1,8-cineole, and geraniol [10]. The phytochemical profile of *O. basilicum* exhibits considerable variation depending on geographical origin, chemotype diversity, cultivation conditions, and genetic factors. Previous studies have characterized basil varieties from Turkey, where methyl chavicol predominates (62.4–86.9%) [11], Iraq, where linalool is the major constituent (34.2%) [12], and India, where limonene is dominant (28.6%) [13]. However, the phytochemical profile and antibacterial potential of Libyan *O. basilicum* have remained unexplored prior to this investigation.

*S. aureus* possesses several essential proteins that represent promising targets for antibacterial agents. The filamentous temperature-sensitive protein A (ftsA) anchors the FtsZ ring during bacterial cell division [14]. Tyrosyl-tRNA synthetase (tyrRS) is critical for protein translation [15]. Clumping factor, A mediates fibrinogen binding and contributes to virulence [16]. Gyrase B provides energy for DNA supercoiling and is the target of fluoroquinolone antibiotics [17]. The current research aimed to: (1) characterize the phytochemical composition of *O. basilicum* leaf methanol extract from Libya using GC-MS analysis; (2) evaluate its antibacterial efficacy; and (3) investigate the molecular interactions of its major compounds (eugenol and linalool) with these four essential *S. aureus* target proteins through molecular docking simulations.

## Materials and Methods:

### Plant material:

Fresh leaves of *O. basilicum* were obtained from a local market in Al-Bayda, Libya in April 2025. The plant material was authenticated by the Pharmacognosy Department at the Faculty of Pharmacy, Omar Al-Mukhtar University, and a voucher specimen (number 6623) was deposited. Approximately 50 g of fresh leaves were dried in the shade at room temperature for ten days, ground into a fine powder, and stored at 4°C.

### Extract preparation:

Approximately 20 g of dried leaf powder was macerated in methanol (200 mL) with continuous stirring for 30 min. The mixture was then filtered using filter paper (Whatman No. 1). The resulting filtrate was concentrated using a Büchi rotary evaporator (Switzerland). The concentrated extract was further dried in open air, resulting in 490 mg of dried methanolic extract (extraction yield: 2.45% weight/weight).

### GC-MS analysis:

The leaf methanol extract of *Ocimum basilicum* was screened for phytochemical compounds using GC-MS analysis on an Agilent 7890A instrument with a DB-1 GC column (30 m × 250 µm × 0.25 µm). Helium gas was used as the carrier gas at a constant flow rate of 1.5 ml/min (split ratio of 1:100). With a maximum temperature of 280°C, the oven temperature was adjusted to rise at a rate of 4°C per minute to 220°C (hold time 3 min) and 260°C (hold time 5 min).

The total runtime was 40 min. The constituents were characterized by comparing the mass spectra with those in the NIST database [18].

### Bacterial strains:

The antibacterial properties of *O. basilicum* leaf extract were assessed against *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Bacillus subtilis* (NCTC 8236), and *Staphylococcus aureus* (ATCC 25923). All microorganisms were provided by the Department of Biomedical Science, Omar Al-Mukhtar University.

### Minimum inhibitory concentration (MIC) determination:

The broth microdilution technique was utilized in accordance with standard protocols. Bacterial cultures were kept on nutrient agar at 4°C and refreshed every month. Overnight cultures were generated by inoculating a single colony into 10 mL of nutrient broth and incubating at 37°C for 18-20 hours, after which the bacterial suspension was standardized to 0.5 McFarland (~1.5 × 10<sup>8</sup> CFU mL<sup>-1</sup>) and diluted to a final concentration of approximately 5 × 10<sup>5</sup> CFU mL<sup>-1</sup> in the test wells [19]. Sterile nutrient broth was used to create a two-fold serial dilution series of basil leaf extract ranging from 5.0 to 0.0195 mg mL<sup>-1</sup>, which was then distributed into 96-well microtiter plates. Every well, holding a total volume of 200 µL, was filled with 100 µL of diluted extract combined with 100 µL of bacterial suspension. Ciprofloxacin at concentrations ranging from 0.5 to 0.0078 µg mL<sup>-1</sup> served as the positive control, whereas the negative control consisted of sterile broth and extract devoid of bacteria. The plates were sealed and maintained at 37°C for a 24-hour period. Following incubation, 20 µL of 0.02% resazurin solution was added to each well, and the plates were re-incubated for 2-4 hours, with bacterial growth signaled by a color shift from blue (non-fluorescent resazurin) to pink (fluorescent resorufin). MIC was determined as the minimum extract concentration that halted the color shift. Each experiment was conducted in triplicate.

### Molecular docking experiments:

#### Ligand and protein selection:

The three-dimensional structures of eugenol (PubChem CID: 3314), linalool (PubChem CID: 6549), and ciprofloxacin (PubChem CID: 2764) were obtained from PubChem database. Four target proteins of *S. aureus* were retrieved from the Protein Data Bank. The proteins included ftsA (PDB ID: 3WQU, 2.80 Å), tyrosyl-tRNA synthetase (PDB ID: 1JIJ, 3.20 Å), clumping factor A (PDB ID: 1N67, 1.90 Å), and gyrase B (PDB ID: 3G75, 2.30 Å).

#### Protein preparation and docking protocol:

Protein structures were prepared using BIOVIA Discovery Studio software (version 25.1.0.20284, Dassault Systèmes, San Diego, CA, USA). All heteroatoms (water molecules, co-crystallized ligands, and buffer components) were removed, and missing hydrogen atoms were added. Molecular docking was performed using the online tool CB-Dock2 [20]. Docking calculations were performed with default parameters, and binding energies were expressed in kcal mol<sup>-1</sup>. Visualization of ligand-protein interactions (hydrogen bonds and hydrophobic contacts) was conducted using BIOVIA Discovery Studio.

## RESULTS AND DISCUSSION:

### GC-MS profiling:

GC-MS analysis identified 30 distinct compounds, which accounted for 99.36% of the total extract composition (Table 1). The predominant compound was eugenol (23.08%), followed by linalool (18.79%) and eucalyptol (1,8-cineole, 9.16%). Other notable constituents included caryophyllene (7.61%), pulegone (5.35%), methylcinnamate (5.04%), fenchol (4.50%), and tau-cadinol (3.97%).

**Table (1):** Compounds identified in *O. basilicum* leaf methanolic extract by GC-MS

No.	Compound	Retention time (min)	Area (%)
1	$\alpha$ -Pinene	3.359	0.27
2	Sabinene	3.842	0.35
3	$\beta$ -Pinene	3.912	0.53
4	$\beta$ -Myrcene	4.012	0.59
5	D-Limonene	4.574	0.81
6	Eucalyptol (1,8-Cineole)	4.640	9.16
7	Pulegone	5.439	5.35
8	Fenchol	5.816	4.50
9	Camphor	6.257	2.07
10	L- $\alpha$ -Terpineol	6.529	1.00
11	Terpinen-4-ol	6.678	0.68
12	$\alpha$ -Terpineol	6.862	3.44
13	(-)-Myrtenol	6.951	0.58
14	Nerol	7.325	0.50
15	Linalool	7.897	18.79
16	Methyl cinnamate	8.431	1.65
17	Methyleugenol	9.132	1.50
18	Geranyl acetate	9.392	0.81
19	Methyl cinnamate (isomer)	9.497	5.04
20	$\beta$ -Elemene	9.779	0.95
21	Eugenol	9.779	23.08
22	Caryophyllene	10.172	7.61
23	$\alpha$ -Guaiene	10.235	0.38
24	Bicyclogermacrene	10.575	0.43
25	$\alpha$ -Farnesene	10.799	0.95
26	$\delta$ -Cadinene	11.197	2.70
27	Calamene	11.297	0.84
28	Globulol	11.996	0.86
29	Epicubanol	12.419	0.63
30	$\tau$ -Cadinol	12.708	3.97
	Total identified		99.36

### Antibacterial activity:

The methanolic extract exhibited potent antibacterial activity against all tested bacterial strains, with MIC values ranging from 0.19–0.39 mg mL<sup>-1</sup> (Table 2). The extract demonstrated stronger activity against gram-positive bacteria, with MIC values of 0.19 mg mL<sup>-1</sup> for both *S. aureus* and *B. subtilis*. Gram-negative bacteria were less susceptible, requiring 0.39 mg mL<sup>-1</sup> to inhibit *E. coli* and *ps. aeruginosa*.

**Table (2):** MICs of *O. basilicum* leaf extract and ciprofloxacin

Bacteria Strain	Gram classification	Extract MIC (mg mL <sup>-1</sup> )	Ciprofloxacin MIC ( $\mu$ g mL <sup>-1</sup> )
<i>S. aureus</i> (ATCC 25923)	Positive	0.19	0.125
<i>B. subtilis</i> (NCTC 8236)	Positive	0.19	0.125
<i>E. coli</i> (ATCC 25922)	Negative	0.39	0.25
<i>Ps. aeruginosa</i> (ATCC 27853)	Negative	0.39	0.25

### Molecular Docking of the Selected Compounds:

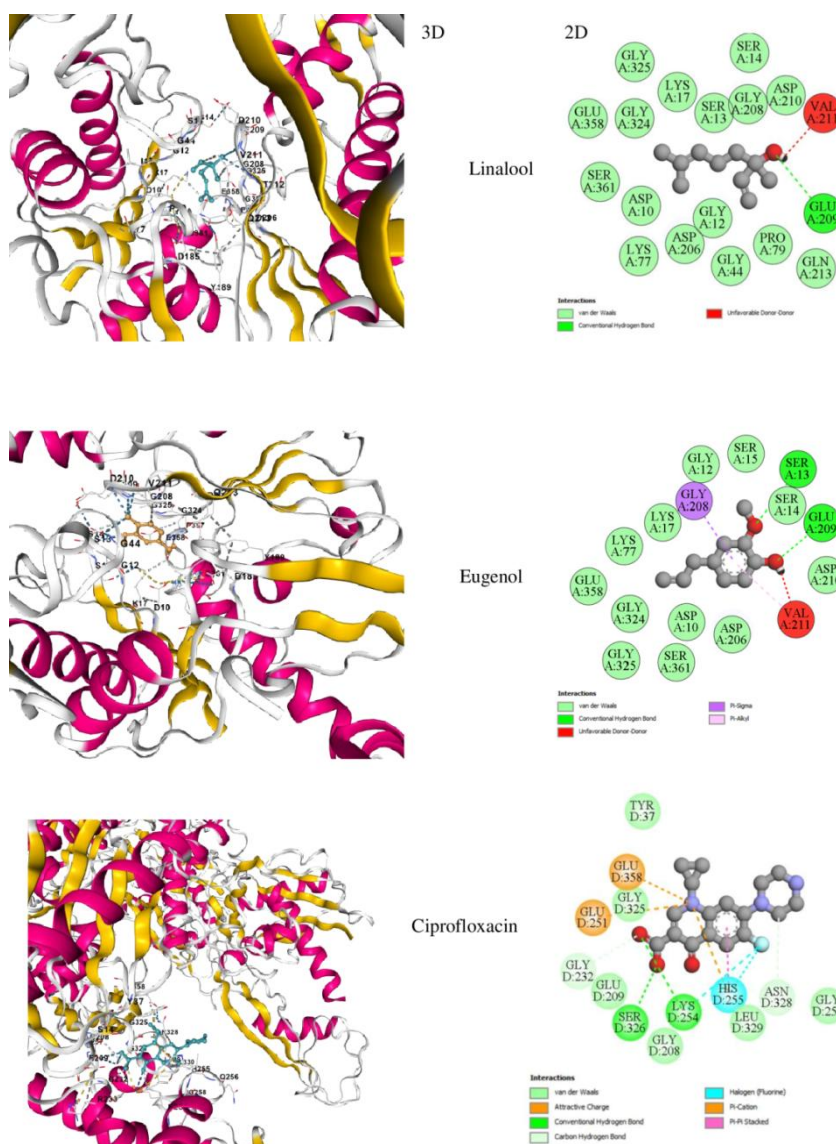
GC-MS analysis of the basil leaf extract revealed the presence of 30 distinct compounds. Linalool and eugenol were selected for docking studies because of their superior performance compared to the four *S. aureus* targets: FtsA, TyrRS, clumping factor A, and gyrase. Ciprofloxacin was used as a positive control to compare the results of the molecules with their target receptors. The findings, as outlined below, show the minimum binding energies for each molecule.

#### Fts A receptor (PDB: 3WQU):

Eugenol exhibited a binding energy of  $-6.1 \text{ kcal mol}^{-1}$ , outperforming linalool ( $-5.7 \text{ kcal mol}^{-1}$ ). Eugenol formed two hydrogen bonds with GLU209 and SER13, along with hydrophobic interactions involving GLY208 and VAL211. Linalool formed a single hydrogen bond with GLU209 and one hydrophobic contact with VAL211 (Table 3, Figure 1).

**Table (3):** Docking results for ftsA (PDB: 3WQU)

Compound	Binding energy ( $\text{kcal mol}^{-1}$ )	Hydrogen bonds	Hydrophobic integrations
Linalool	-5.7	GLU209	Val211
Eugenol	-6.1	GLU209, SER13	GLY208, VAL211
Ciprofloxacin	-8.7	SER326, LYS254	HIS255, GLU358, GLU251



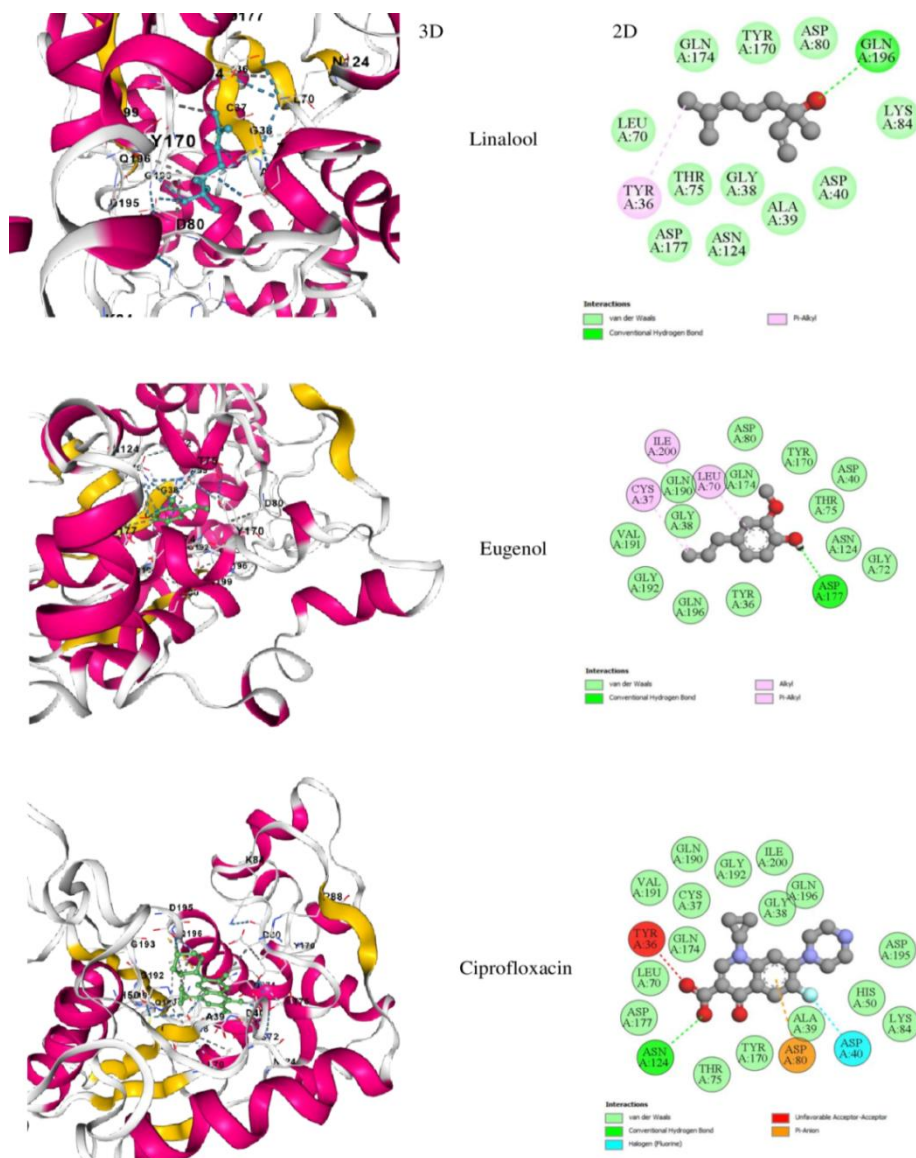
**Figure (1):** 3D and 2D visualizations of ligand interactions with the active site of *S. aureus* ftsA receptor.

### Tyrosyl-tRNA synthetase (PDB: 1JIJ):

Eugenol showed stronger binding ( $-6.2$  kcal/mol) than linalool ( $-5.7$  kcal/mol). Eugenol formed one hydrogen bond with ASP177 and three hydrophobic interactions (LEU70, ILE200, and CYS37). Linalool formed a single hydrogen bond with GLN196 and one hydrophobic contact with TYR136 (Table 4, Figure 2).

**Table (4):** Docking results for tyrosyl-tRNA synthetase (PDB: 1JIJ)

Compound	Binding energy (kcal mol <sup>-1</sup> )	Hydrogen bonds	Hydrophobic integrations
Linalool	-5.7	GLN196	TYR136
Eugenol	-6.2	ASP177	LEU70, ILE200, CYS37
Ciprofloxacin	-9.0	ASN124	ASP80, 1SP40, TYR36



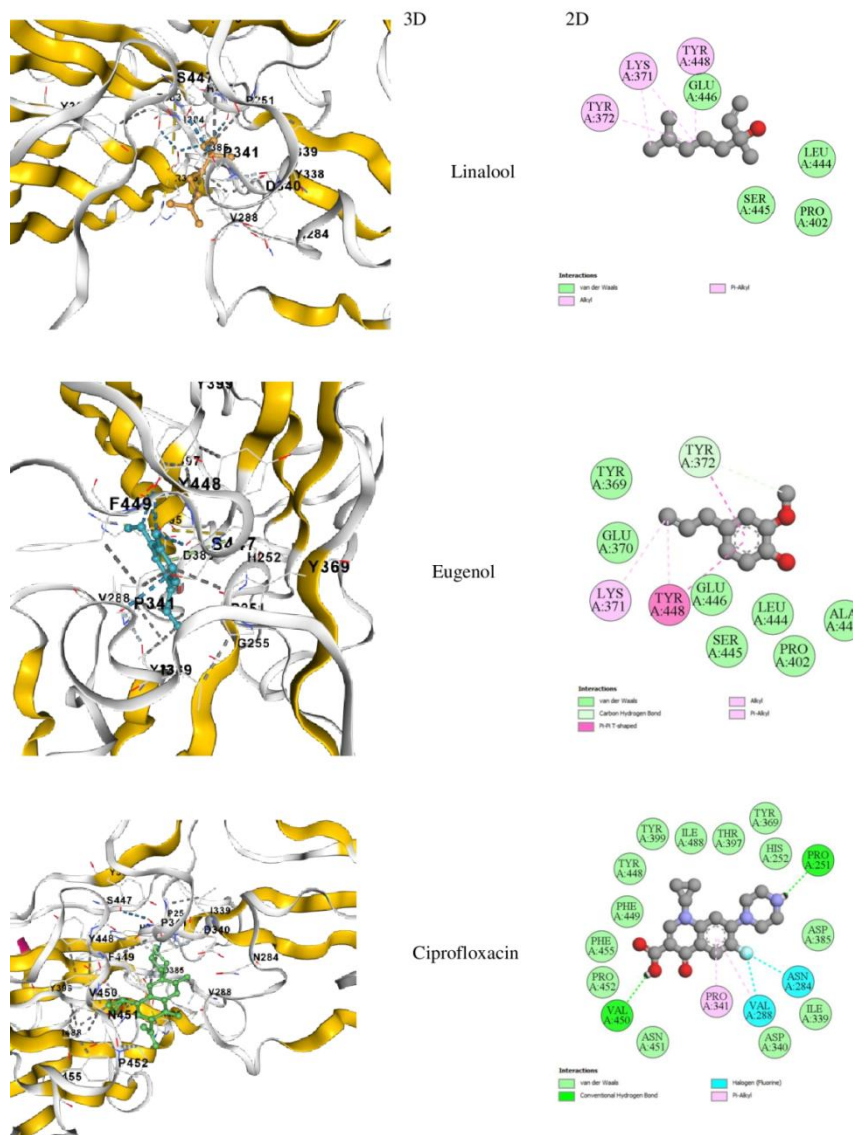
**Figure (2):** 3D and 2D visualizations of ligand interactions with the active site of *S. aureus* tyrosyl-tRNA synthetase receptor.

### Clumping factor A (PDB: 1N67)

Eugenol exhibited the highest binding affinity among the plant compounds for this target ( $-6.4$  kcal mol<sup>-1</sup>), compared to linalool ( $-5.8$  kcal mol<sup>-1</sup>). Neither compound formed hydrogen bonds; binding was mediated through hydrophobic interactions. Eugenol interacted with TYR369, TYR372, TYR448, and LYS371, while linalool engaged LYS371, TYR372, TYR448, and GLU446 (Table 5, Figure 3).

**Table (5):** Docking results for clumping factor A (PDB: 1N67)

Compound	Binding energy (kcal mol <sup>-1</sup> )	Hydrogen bonds	Hydrophobic integrations
Linalool	-5.8	–	LYS 371, TYR372, TYR448, GLU446.
Eugenol	-6.4	–	TYR369, TYR372, TYR, 448, LYS371.
Ciprofloxacin	-8.0	PRO251, VAL450	ASN284, VAL288, PRO321.



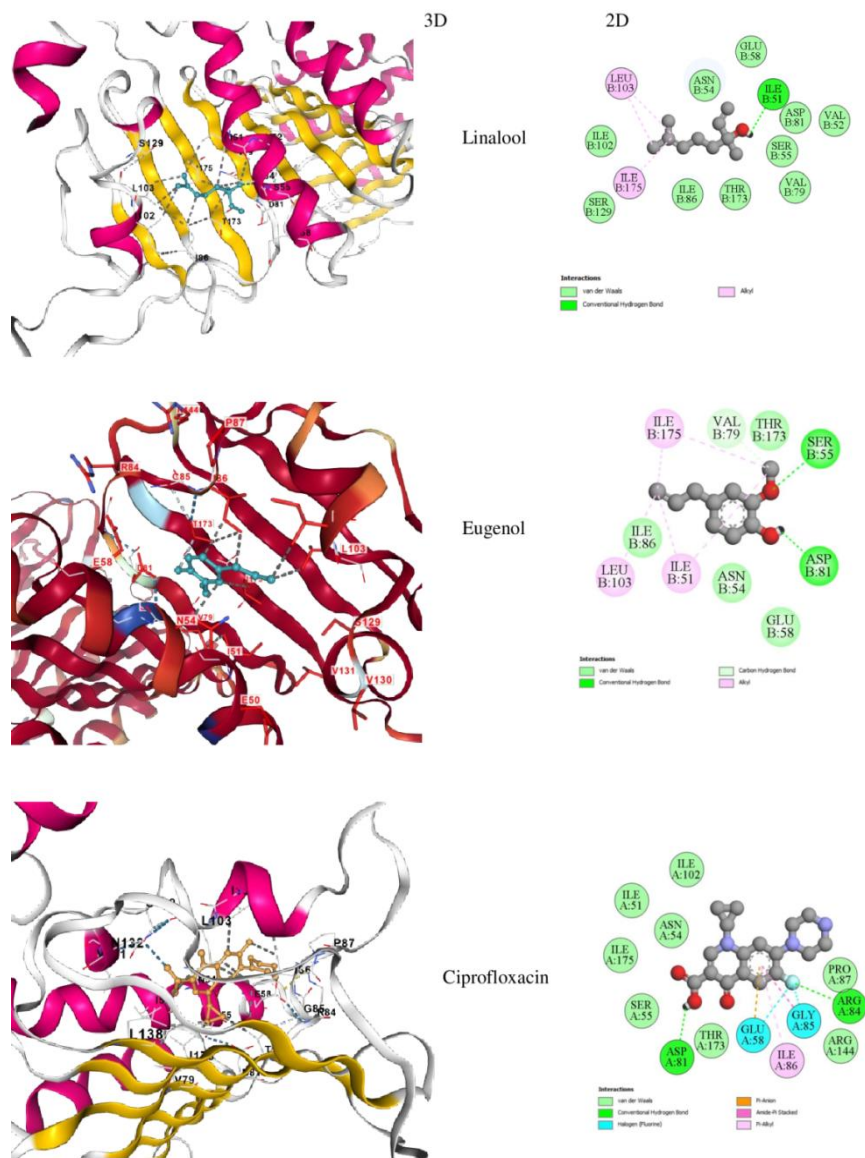
**Figure (3):** 3D and 2D visualizations of ligand interactions with the active site of *S. aureus* clumping factor A receptor.

**Gyrase B (PDB: 3G75):**

Both compounds showed the strongest binding energies for gyrase B, with eugenol achieving  $-6.7$  kcal mol<sup>-1</sup> and linalool achieving  $-5.9$  kcal mol<sup>-1</sup>. Eugenol formed two hydrogen bonds (SER55 and ASP81) and four hydrophobic interactions (ILE51, LEU13, ILE175, and VAL79). Linalool formed one hydrogen bond (ILE51) and two hydrophobic interactions (LEU103 and ILE175) (Table 6, Figure 4).

**Table (6):** Docking results for gyrase B (PDB: 3G75)

Compound	Binding energy (kcal mol <sup>-1</sup> )	Hydrogen bonds	Hydrophobic integrations
Linalool	-5.9	ILE51	LEU103, ILE175
Eugenol	-6.7	SER55, ASP81	ILE51, LEU13, ILE175, VAL79
Ciprofloxacin	-7.6	ASP81, ARG84	GLU58, GLY85, ILE86



**Figure (4):** 3D and 2D visualizations of ligand interactions with the active site of *S. aureus* gyrase B receptor.

This study is the first to comprehensively identify the phytochemical profile and antibacterial potential of *O. basilicum* growing in Libya. The findings revealed that Libyan basil possesses a unique chemotype dominated by eugenol (23.08%), linalool (18.79%), and eucalyptol (9.16%), distinguishing it from previously characterized basil varieties from Turkey, Iraq and India [11-13]. This geographical variation underscores the profound influence of environmental factors, soil composition, and genetic drift on the production of secondary metabolites in medicinal plants.

The MIC values obtained (0.19–0.39 mg mL<sup>-1</sup>) are particularly noteworthy. In phytopharmacological research, plant extracts with MIC values below 1 mg mL<sup>-1</sup> are generally considered highly active, while those below 0.5 mg mL<sup>-1</sup> are classified as exceptionally potent [21]. The Libyan basil extract falls into this top category, with activity against *S. aureus* (MIC 0.19 mg mL<sup>-1</sup>) comparable to that of some purified antibiotics. The stronger activity against gram-positive bacteria is expected due to the absence of an outer membrane in these organisms, which in gram-negative bacteria serves as an additional permeability barrier containing lipopolysaccharides and porin proteins [22]. Of particular interest is the activity against *Ps. aeruginosa* (MIC 0.39 mg mL<sup>-1</sup>), a pathogen notoriously resistant to multiple antibiotic classes due to constitutive expression of efflux pumps, low outer membrane permeability, and biofilm formation. The observed activity suggests that the extract contains compounds capable of circumventing these resistance mechanisms, possibly through membrane disruption or efflux pump inhibition.

The molecular docking results provide mechanistic insights into how eugenol and linalool might exert their antibacterial effects. Eugenol consistently outperformed linalool across all four target proteins, with binding energies ranging from  $-6.1$  to  $-6.7$  kcal mol<sup>-1</sup> compared to  $-5.7$  to  $-5.9$  kcal mol<sup>-1</sup> for linalool. This difference can be attributed to the phenolic hydroxyl group in eugenol, which facilitates the formation of hydrogen bonds with key amino acid residues. Hydrogen bonding is a critical determinant of ligand-receptor affinity, and the ability of eugenol to form two hydrogen bonds with ftsA (GLU209, SER13) and gyrase B (SER55, ASP81) likely contributes to its superior binding. The target proteins in this study were selected based on their essential roles in bacterial physiology. FtsA is an actin-like protein that anchors the FtsZ ring to the cell membrane during division, and its inhibition blocks septation and bacterial proliferation [14]. Tyrosyl-tRNA synthetase is a validated antibacterial target, as its inhibition halts protein synthesis by preventing tRNA charging [15]. Clumping factor A is a virulence factor that mediates adherence to fibrinogen, and its disruption can attenuate *S. aureus* pathogenicity [16]. Gyrase B is the ATPase subunit of DNA gyrase, and its inhibition is the mechanism of action of fluoroquinolone antibiotics, such as ciprofloxacin [17].

The stronger binding of eugenol to gyrase B ( $-6.7$  kcal mol<sup>-1</sup>) than to the other targets suggests that this may be the primary mechanism of action, consistent with the known susceptibility of bacterial DNA replication to phenolic compounds. Notably, both eugenol and linalool bound to gyrase B at residues (ASP81, ILE51, SER55) that are distinct from the ciprofloxacin-binding site, suggesting a potential synergistic activity or activity against ciprofloxacin-resistant mutants. The dominance of eugenol in Libyan basil contrasts with the findings from other geographical regions. Telci et al. (2006) reported that Turkish basil chemotypes are dominated by methyl chavicol (estragole), with concentrations ranging from 62.4% to 86.9% in the essential oil [11]. Ahmed et al. (2019) identified linalool (34.2%) as the major constituent in Iraqi basil [12], whereas Kishan et al. (2024) found limonene (28.6%) to be predominant in Indian basil [13]. These differences highlight the importance of chemotype characterization for quality control in herbal medicine and for the selection of plant materials with desired bioactive profiles.

The MIC values obtained in this study were lower (indicating higher potency) than those reported for basil extracts from other regions. For example, Rehab and Zeinab (2016) reported MIC values of 0.5-1.0 mg mL<sup>-1</sup> for basil essential oil against *S. aureus* [17]. This enhanced activity may be attributed to the higher eugenol content in Libyan basil, as eugenol has been shown to be more potent than linalool in several antibacterial assays. This study has several limitations. First, the *in vitro* nature of the antibacterial assays does not account for pharmacokinetic factors, such as absorption, distribution, metabolism, and excretion, which influence *in vivo* efficacy. Second, the use of a crude methanolic extract, although relevant for traditional medicine applications, complicates the attribution of the observed activity to specific compounds. Third, the molecular docking results are computational predictions that require experimental validation using enzymatic inhibition assays. Fourth, the study only tested reference bacterial strains; the activity against multidrug-resistant clinical isolates requires further investigation. Finally, cytotoxicity assessment of mammalian cells was not performed.

This study establishes Libyan *O. basilicum* as a rich source of eugenol, a compound with well-documented antimicrobial activity [23-25]. The unique eugenol-rich chemotype identified here has potential applications in the development of natural antibacterial agents for human and veterinary medicine. The potent activity against gram-positive pathogens, including *S. aureus*, suggests possible applications in treating skin and soft tissue infections, whereas the activity against *Ps. aeruginosa*, indicating a broader potential. Future research should focus on *in vivo* efficacy studies, isolation of individual compounds to identify synergistic effects, and evaluation against multidrug-resistant clinical isolates.

#### **CONCLUSION:**

This study comprehensively characterized the phytochemical profile of the methanolic extract of *O. basilicum* L. leaves from Libya and demonstrated its potent antibacterial activity against clinically relevant bacterial pathogens. The extract was rich in phenolic compounds, with eugenol (23.08%), linalool (18.79%), and eucalyptol (9.16%) as the major constituents. The extract exhibited considerable antibacterial activity, particularly against gram-positive bacteria. Molecular docking revealed that eugenol binds with superior affinity to four essential *S. aureus* targets (ftsA, tyrRS, clumping factor A, and gyrase B) compared with linalool. The unique eugenol-rich chemotype of Libyan basil distinguishes it from previously characterized varieties of basil. These findings position *O. basilicum* as a promising source of natural antibacterial agents, warranting further *in vivo* investigations.

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**Conflict of interest:**

The authors declare no conflict of interest.

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