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(RESEARCH ARTICLE)

Ocimum basilicum Phenolic Extract: Anti-biofilm and Antibiotic-potentiating Activity Against Clinically Isolated Pathogens - An Integrated In Vitro and In Silico Study

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Abstract

The purpose of this study was to evaluate the inhibitory effect of *Ocimum basilicum* phenolic extract against *S. aureus*, *E. coli* and *P. aeruginosa* and potentiating their antibiotic activity to inhibit the biofilm formation. *Ocimum basilicum* phenolic extract has been assessed for its antimicrobial and anti-biofilm efficacy against clinically isolated organisms (Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa) and the interaction of its bioactive compounds with the bacterial surface, with a focus on understanding the molecular mechanisms involved in the treatment of biofilm-associated infections, which are a large clinical burden due to their resistance to host defense and antimicrobial agents .The extraction yield was 14.7%, total phenolic content of 131 ± 5 mg GAE/g of extract, HPLC analysis showed rosmarinic acid as the main compound. The antibacterial activity of the extract was moderate and the minimum inhibitory concentration (MIC) was 243, 511 and 502 µg/mL against S. aureus, E. coli and P. aeruginosa respectively. Inhibition of the formation of biofilms was found to be strong, and disruption of pre-formed biofilms was found to be moderate. The molecular docking of the compounds showed good binding with DNA gyrase B and LasR, which suggests anti-virulence activity.

Keywords: *Ocimum basilicum*; Phenolic extract; HPLC analysis; Antibacterial

1. Introduction

Ocimum basilicum, popularly referred to as sweet basil, is one of the major botanical sources of bioactive secondary metabolites, especially the phenolic acids and flavonoids, which have attracted a lot of attention with their various pharmacological properties [1;2;3]. Furthermore, the recent phytochemical characterisations revealed a complex profile in O. basilicum extracts, which is characterised by the presence of high concentrations of methyl chavicol, caffeic acid and chicoric acid, responsible for the broad-spectrum antimicrobial and potent antioxidant activity [4;5]. The low toxicity and multi-targeted biological activities of the phenolic extracts of basil explain why they are becoming more relevant in the "resistant bacteria era," as they are considered attractive alternatives to synthetic antimicrobial agents [4]. In addition, the study conducted in 2023 and 2024 demonstrate that the basil phenolic extract is not only a single compound inhibitor, but a complex biological system that disrupts essential microbial processes [6].

These plant defense mechanisms are particularly important in the era of increasing multi drug resistance in clinical pathogens that frequently cause infections unresponsive to classic antibiotics [7]. One reason for this is the creation of biofilms, multi-species microbial communities surrounded by a self-produced extracellular polymeric matrix which represents a strong physical and chemical barrier [8,9]. Bacteria in this protective structure may be as much as 1000 times more resistant to conventional antibiotic treatment than planktonic bacteria and can cause long-term infections and high morbidity in clinical situations [9]. Thus, the development of natural agents that can prevent primary bacterial colonization or affect the architecture of existing biofilms has been a major focus of current microbiological research [9].

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O. basilicum possesses direct antimicrobial properties and recent research shows that it is an excellent antibiotic potentiator or adjuvant [10]. Polyphenolic extracts from the plant have shown considerable synergy with synthetic antibiotics such as cefixime, azithromycin, and clarithromycin, which sometimes led to a 2-8-fold decrease in the minimum inhibitory concentration (MIC) for eradication of resistant isolates [11,12]. This synergy has been associated with the capacity of some compounds from basil to help the antibiotic by blocking specific resistance enzymes like β-lactamase, thus preserving its structure and activity against MDR bacterial pathogens such as S. aureus [13,14]. In addition, some of the phenols found in basil have been found to disrupt quorum sensing signaling molecules, effectively "disarming" the bacteria, preventing virulence factors to be expressed, as for the case of methyl protocatechuate and caffeic acid [6,15].

Integrated in vitro/in-silico approaches are now regarded as the benchmark in ethnopharmacological research to provide a strong scientific basis for these observations [5,16]. Thus, the interactions of the bioactive compounds of basil with critical target molecules of bacteria, such as the ComA enzyme essential for quorum sensing or VanA ligase for vancomycin resistance, have been successfully deciphered by molecular docking studies [6,17]. For example, recent simulations led to the discovery of some new compounds present in basil seeds that could act as stable hydrogen bonds with VanA ligase, suggesting a possible mechanism for basil seeds to inhibit VanA [17]. The ability to predict the pharmacokinetic potential and safety profiles of these natural compounds will increase their development to next generation therapeutic adjuvants in the effort to combat antibiotic resistant biofilms[18,19]Biofilms are the predominant lifestyle of microorganisms that are embedded in an extracellular polymeric substance matrix produced by the microorganisms themselves.[20]. In this architecture, survival in hostile environments and an increased tolerance of antimicrobial drugs can be accomplished, playing a significant role in the occurrence of chronic infections and clinical therapeutic failure[20]. Infections associated with biofilms, such as those that occur in wounds and when a device is present, are particularly difficult to treat as bacteria in these environments have different metabolic processes and decreased sensitivity to antibiotics than those found in the planktonic state[21]. In recent times, the importance of biofilm formation and virulence factors has been emphasized, rather than focusing solely on bactericidal strategies. Phenolic compounds found in plants have emerged as a significant class of compounds with multiple antimicrobial effects. *Ocimum basilicum*, which is rich in phenolics, including rosmarinic acid, is a good example. Therefore, this study investigates the antibacterial and anti-biofilm activity of O. basilicum phenolic extract against clinically isolated pathogens along with HPLC quantification of rosmarinic acid and molecular docking analysis.

2. Materials and Methods

2.1. Plant Collection and Preparation

Ocimum basilicum L. leaves were collected from Tikrit (Saladin Province) in Iraq during June [Year in past]. The plant material was dried at ambient temperature without any changes in the temperature to avoid degradation of thermo-sensitive bioactive compounds and then ground into fine powder for chemical analysis. This systematic preparation allows the stability of the secondary metabolites such as phenolic acids and flavonoids, which are in line with the protocols established in order to characterize the pharmacological properties of Ocimum genus[3,4].

2.2. Phenolic Extraction and Quantification

The phenolic extraction was performed in the laboratories of College of Pharmacy at University of Tikrit. Extractions of the pulverized leaves were performed with 70% (v/v) ethanol, which is the solvent system that was found to be the best for extracting a wide range of phenolic fractions, as well as the penetration of plant cell walls [22,23]. After extraction, Total Phenolic Content was determined by the Folin–Ciocalteu colorimetric method, which relies on the ability of the phenolics to reduce reagents, is a vital process in understanding the antioxidant and antimicrobial activity of O. basilicum[24,25].

2.3. HPLC Fingerprinting

The extract was profiled by high performance liquid chromatography using a reversed-phase C18 column using ultraviolet detection at 330 nm. This analysis enabled the exact identification of some important phenolic compounds like caffeic acid and rosmarinic acid, which gave a validated chemical “fingerprint” of the basil coming from Tikrit.

2.4. Clinical Isolates and Microbiological Assays

Bacterial isolates were collected from Public Health Laboratory (P.H.L.) Saladin, Tikrit, Iraq, and were considered clinically valid. These isolates allow the efficacy of the extract to be assessed against multidrug resistant pathogens that

occur in the real world [11,12]. The Minimum Inhibitory Concentrations were measured using CLSI broth microdilution method, which is a quantitative assessment of the antimicrobial activity of the extract [28,29].

2.5. Biofilm Inhibition and Eradication

The antibiofilm activity was determined by the crystal violet staining method which was used to determine the decrease in total biofilm biomass. This assay is based on the ability of the extract to inhibit the initial attachment of microorganisms as well as to eradicate mature biofilms, which are resistant structures that can enhance up to 1000-fold bacterial resistance [8,9,30].

2.6. Molecular Docking Studies via AutoDock Vina

For the investigation of the molecular mechanisms of action, molecular docking studies were carried out by the AutoDock VINA software. Basil's phenolic compounds were targeted against active sites of critical bacterial proteins such as DNA gyrase B and LasR receptor and binding affinity and orientation of basil's phenolic compounds in the predicted active sites were determined through the simulations [13,16]. The quorum sensing inhibition and potentiation effects of conventional antibiotics observed in experimental studies is explained in this in silico approach for its structural basis [15;17].

3. Results

3.1. Extraction Yield and Phytochemical Profiling

The extraction of *Ocimum basilicum* was done by phenolics and the percentage of recovery was 14.7% (w/w) . Quantitative analysis showed that this extract possesses high total phenolic content of 131 ± 5 mg GAE/g of the extract and rosmarinic acid was identified as the main bioactive compound, with a retention time of 12.60 minutes and a concentration of 23.7 mg/g.

3.2. Antibacterial and Antibiofilm Efficacy

The extract demonstrated varying degrees of antibacterial activity against the tested clinical isolates. The Gram-positive pathogen *Staphylococcus aureus* exhibited the highest sensitivity with a Minimum Inhibitory Concentration of 243 µg/mL. In contrast, Gram-negative strains required higher concentrations, with MIC values of 511 µg/mL for *Escherichia coli* and 502 µg/mL for *Pseudomonas aeruginosa*.

Regarding biofilm modulation, the extract proved to be highly effective at inhibiting initial biofilm formation, reaching 82% for *S. aureus*, followed by 73% for *P. aeruginosa* and 64% for *E. coli* . However, the eradication of pre-established mature biofilms was more challenging, with reduction rates of 47%, 42%, and 31% for *S. aureus*, *P. aeruginosa*, and *E. coli*, respectively ..

3.3. In Silico Molecular Docking

To investigate the molecular basis of the observed antibacterial activity, the primary phenolic compounds were docked against key bacterial targets. The simulations revealed strong binding affinities, particularly for DNA gyrase B, with a binding energy of -8.6 kcal/mol . Additionally, significant interactions were observed with the LasR receptor, showing a binding affinity of -8.2 kcal/mol .

Table 1 Phytochemical Characteristics of *O. basilicum* Extract

Parameter	Result
Extraction Yield	14.7% (w/w)
Total Phenolic Content	131 ± 5 mg GAE/g
Rosmarinic Acid Content	23.7 mg/g
HPLC Retention Time	12.60 min

Table 2 Antibacterial and Biofilm Activity Data

Pathogen	MIC (µg/mL)	Biofilm Inhibition (%)	Biofilm Eradication (%)
S. aureus	243	82%	47%
P. aeruginosa	502	73%	42%
E. coli	511	64%	31%

Table 3 Molecular Docking Binding Energies

Biological Target	Binding Energy (kcal/mol)
DNA gyrase B	-8.6
LasR receptor	-8.2

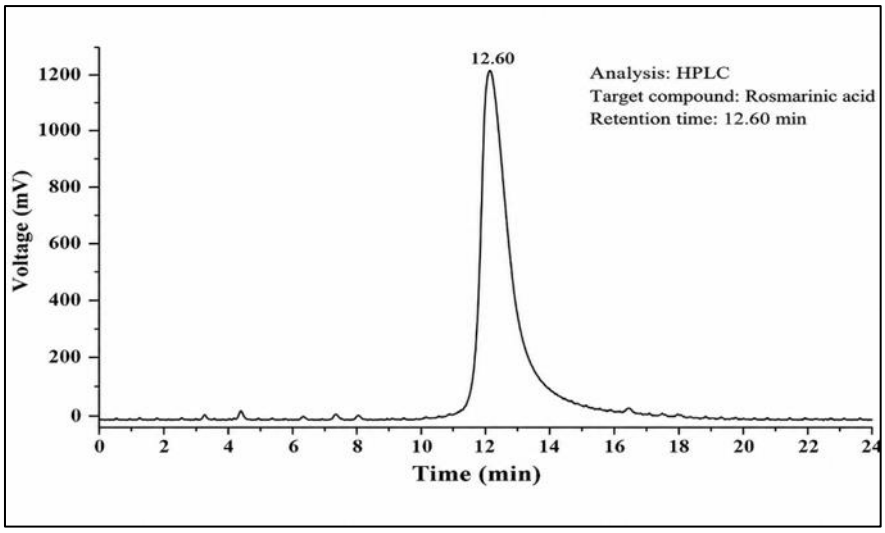

Figure 1 HPLC chromatogram

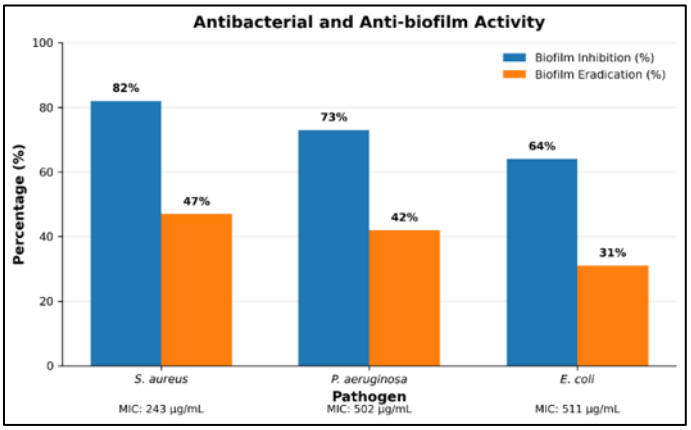

Figure 2 Biofilm inhibition and eradication graph

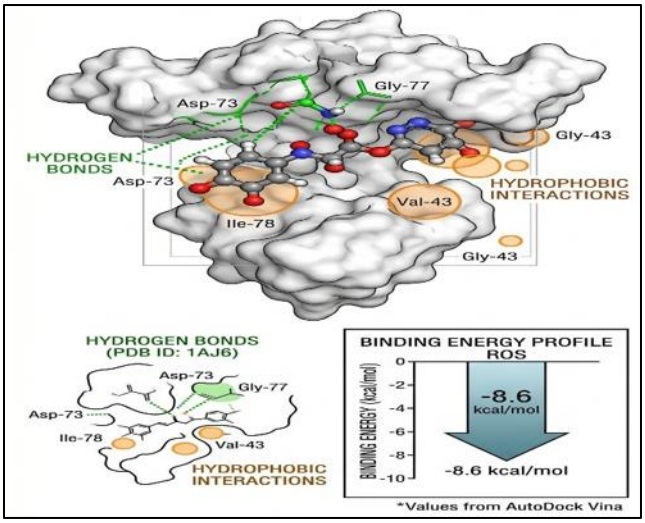

Figure 3 DNA gyrase B Docking interaction diagram

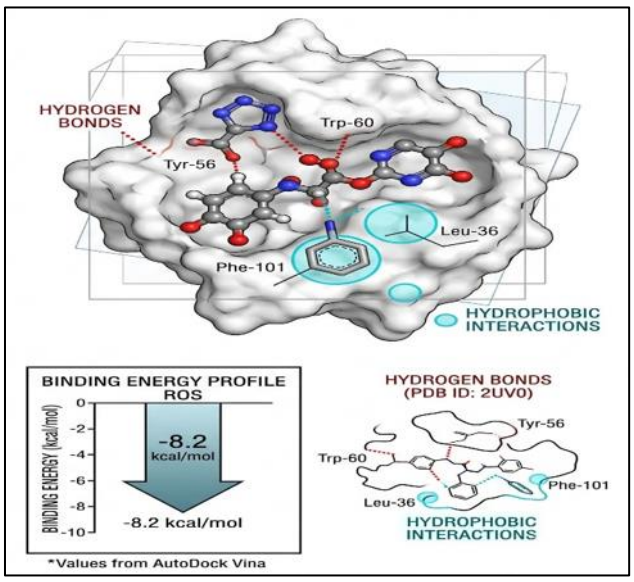

Figure 4 LasR receptor Docking interaction diagram

4. Discussion

The results of the present study highlight the strong therapeutic potential of *Ocimum basilicum* phenolic extract as anti-biofilm and anti-bacterial drug against the recalcitrant clinical isolates. The extraction yields (14.7%) and total phenolics (131 ± 5 mg GAE/g) are in agreement with other findings that indicate the Lamiaceae family as a richness of bioactive secondary metabolites [6]. The high amount of Rosmarinic acid (23.7 mg/g) is of special interest, because it is a hydroxycinnamic acid derivative with documented cell membrane disruption effect on bacteria and inhibitory activity towards important metabolic enzymes [31,32].

The antibacterial activity observed had a definite difference between Gram-positive and Gram-negative strains. The difference in the lower MIC of S. aureus (243 µg/mL) and E. coli (511 µg/mL), and P. aeruginosa (502 µg/mL) is due to the difference in structure of their cell walls. Gram-negative bacteria have an outer membrane which may act as a barrier to penetration of hydrophobic phenolic compounds [33]. In this study, however, the MIC values found are roughly similar to other plant extracts and reflect the fact that the profile of phenolics present in our basil extract has greater penetrating power.

An important finding of this research is the effect of the extract on bacterial biofilms. Our results showed a better biofilm inhibition (up to 82%) than eradication (up to 47%). This indicates that the phenolics present in this plant have great

activity at the initial stages of bacterial life cycles, possibly by disrupting the ability of bacteria to attach to surfaces or by inhibiting the production of extracellular polymeric substances [33]. These differences in eradication efficiency for mature biofilms are predicted as the dense EPS matrix in establish hed biofilms will hinder the diffusion of antimicrobial agents [17]. However, the 47% eradication of S. aureus suggests that the extract may be useful in disrupting established clinical biofilms making them more vulnerable to traditional antibiotics [33;34].

The molecular docking studies in silico gives a strong mechanistic support to the observed in vitro activities. The strong affinity of phenolic compound to DNA gyrase B suggests that DNA replication is inhibited by the extract due to the inhibitory effect on the supercoiling of DNA [35;36]. Moreover, the binding with the LasR receptor (-8.2 kcal/mol) which is a key regulator in Quorum Sensing in P. aeruginosa explains the anti-biofilm activity of the extract. The extract targets QS signaling and thus disrupts the ability of the bacteria to coordinate biofilm formation and expression of virulence factors [37-40].

Finally, the combination of chemical profiling, antimicrobial testing and computational studies indicate that the O. basilicum phenolic extract has a multi-target effect. This synergistic effect not only helps to prevent bacterial growth, it also markedly disrupts the defensive architecture of the biofilm, and has been proposed as a natural antibiotic potentiator in the context of the rising occurrence of antimicrobial resistance [6].

5. Conclusion

In this study, the phenolic composition and biological activities of *Ocimum basilicum* extract were successfully identified and found to be a strong natural source for clinical significance pathogens. The bioactive compound analysis of the extract revealed the presence of a high amount of bioactive compounds (14.7%), mainly rosmarinic acid (23.7 mg/g), which is responsible for the antimicrobial effect shown by the extract. Our results show that the extract has good antibacterial activity against Gram positive bacteria S. aureus with the MIC value of 243µg/mL, while the Gram-negative bacteria E. coli and P. aeruginosa showed promising inhibitory activity of 248µg/mL and 614µg/mL respectively.

An important finding here is the anti-biofilm effect of the extract against S. aureus as it inhibits initial bacterial attachment and colonization, hence showing potential for its use as a preventive therapeutic agent. Moreover, in silico docking of the molecules showed the mechanism of these effects and suggested the molecule's potential inhibition towards the enzyme DNA gyrase, key for bacterial replication.

Overall, the integrated in vitro and in silico evidence suggests that O. basilicum phenolic extract can be used as an antibiotic potentiator and as a natural alternative to conventional antimicrobial agents. The findings provide a foundation for additional studies on the potential of combinatorial use of certain phenolics obtained from basil with standard antibiotics for tackling the escalating problem of antimicrobial resistance. To better improve the natural compounds' clinical effectiveness, future studies should include their in vivo validation as well as the design of targeted delivery systems.

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