



Lemon essential oil: molecular docking analysis and investigation of antibacterial and antioxidant activities

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essential oil; limonene; molecular docking; *S. aureus*; antioxidant activities

KEY CONTRIBUTION

Providing a comprehensive analysis of the chemical composition of lemon essential oil (LEO) extracted from fruit peel. Elucidate the potential mechanisms behind its antibacterial activities. Elucidate the potential mechanisms behind its antioxidant activities. By applying molecular docking to the major constituents, the work offers insight into their interactions with relevant biological targets. Potential use of LEO in pharmaceutical or food preservation applications based on the obtained results.



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ABSTRACT

Lemon essential oil is widely used in both commercial and domestic products. It consists of a mixture of volatile, liposoluble compounds responsible for its characteristic fragrance and bioactive properties, including antimicrobial, anti-inflammatory and antioxidant activities. The importance of lemon essential oil is even more significant when considering the increasing interest in natural products that promote human health. In this study, domestically grown lemons were used. The essential oil was extracted from the peel using the hydrodistillation method, an environmentally friendly process. The composition of the resulting essential oil was analysed using gas chromatography coupled with mass spectrometry (GC-MS). The analysis revealed that *d*-limonene, β -pinene, and γ -terpinene are the predominant components found in lemon essential oil. The antimicrobial activity of the essential oil was tested and found to be effective against *Staphylococcus aureus*. Molecular docking analysis revealed that all three principal compounds were able to bind to the DNA gyrase enzyme, with *d*-limonene exhibiting the lowest binding energy. The essential oil also exhibited significant DPPH radical scavenging activity, highlighting its antioxidant potential. The results indicate that both concentration and incubation time affect the antioxidant capacity of the essential oil.

Introduction

Essential oils have been used in folk medicine since ancient times, and their importance has not diminished even to this day. They are naturally synthesised in different parts of plants as part of secondary metabolism (Wani et al., 2021). These are fragrant and volatile substances with a very complex chemical composition and different medicinal properties that vary depending on the specific oil (Aziz et al., 2018). Research has shown that more than 200 different components can be present in these oils (Fisher and Phillips, 2008; Pezantes-Orellana et al., 2024). The composition of an essential oil may vary depending on the geographical origin of the plant. It is believed that each component of the essential oil has its own mechanism of action, and due to their small molecular size, essential oil constituents are typically absorbed quickly and easily. However, uncovering their mechanisms of action remains a challenge for scientists worldwide (Silva et al., 2021).

This study explores lemon essential oil (LEO), which is typically extracted from the peel of the fruit. The lemon (*Citrus limon* or *Citrus limonum*) is a fruit tree belonging to the *Rutaceae* family (Budiarto et al., 2024). It is used worldwide, primarily in the food industry, for products such as juices and flavourings. As a result, the peel is often regarded as a waste. However, both lemon peel and its essential oil are valuable natural resources. Potential applications of essential oils in industries such as cosmetics and pharmaceuticals, due to their positive health effects, and the food industry as natural alternatives to synthetic preservatives and antioxidants are of growing interest. This is particularly significant, given the increasing consumer demand for natural products (Raspo et al., 2020).

Lemon essential oil, obtained through the cold pressing method, is a yellow or yellow-green liquid, while when distilled, it appears as a pale yellow to clear liquid. It has a refreshing, citrusy fragrance that is both pleasant and invigorating. The unique aroma is primarily attributed to a blend of aliphatic aldehydes, terpene alcohols, terpene alcohol-acetic esters, and terpenes (Shan, 2016). The essential oil obtained by distillation mainly contains *d*-limonene, β -pinene, γ -terpinene, geranial and α -pinene. Due to its lipophilicity, it dissolves readily in ethanol and other organic solvents but has limited solubility in water. It also forms stable mixtures with carbon disulfide and glacial acetic acid (*The European Pharmacopeia 11th Edition*, 2023; Jana et al., 2021; Windholz, 1976).

The primary component of LEO is *d*-limonene, whose content can range from 32% to 98% (Mahato et al., 2019). The overall composition of an essential oil directly influences its biological properties, such as antimicrobial and antioxidant activities (Dosoky and Setzer, 2018). Some researchers suggest that limonene plays a key role in determining the biological effects of the oil (Erasto and Viljoen, 2008; Espina et al., 2013). However, other studies argue that these effects result from the combined action of both the major and minor components of the oil (Bakkali et al., 2008).

Many studies have shown various positive effects of LEO on human health. The most notable are its antimicrobial, anti-inflammatory and antioxidant properties (Komiya et al., 2006; Klimek-Szczykutowicz et al., 2020; Masyita et al., 2022). Due to its antimicrobial activity, LEO can be effectively used to treat various skin conditions, including acne and fungal infections (Bungau et al., 2023). Its anti-inflammatory effects are evident in the reduction of pain and swelling associated with arthritis (Uronnachi et al., 2024). Earlier studies have demonstrated that essential lemon oil possesses significant antioxidant properties, which help inhibit free radicals, the primary cause of oxidative cellular stress, thereby reducing the risk of chronic diseases (Calabrese et al., 1999; Budiarto et al., 2024). Lemon essential oil is safe for both inhalation and topical use when properly diluted. It can be diluted with carrier oils such as almond oil, jojoba oil, or coconut oil. Inhalation, typically through a diffuser, can enhance mood, alleviate anxiety, and improve concentration and cognitive function, particularly in patients with Alzheimer's disease.

Additionally, it has been shown to significantly reduce nausea and vomiting in pregnant women (Özer et al., 2022; Sirkeci et al., 2023; Gonçalves et al., 2025).

The objective of this study was to investigate the chemical composition of LEO extracted from the fruit peel and to predict the mechanisms underlying its antibacterial and antioxidant activities by analysing the most abundant compounds using molecular docking. The results of this study suggest promising potential for the application of LEO in therapeutic treatments and the food industry.

Materials and methods

Plant material

The *citrus limon* plant used in this study was collected in the Mostar area (southern Bosnia and Herzegovina). This region is characterised by a Mediterranean climate with warm summers and mild winters. The plant material was manually collected in December 2024 and transported to the laboratory. Freshly grated fruit peel was used for essential oil extraction.

Hydrodistillation

The essential oil was extracted by hydrodistillation using a Clevenger apparatus. A total of 100 grams of freshly grated peel was weighed and transferred to a flask. The plant material was combined with 500 cm³ of distilled water, and the flask was placed on a hot plate with the Clevenger apparatus assembled. Distillation was carried out for two hours. The essential oil collected in a graduated glass tube, was then separated and stored in the dark at a temperature of 4 °C until further analysis.

GC–MS analyses of the essential oil

The semiquantitative chemical characterisation of essential oil was performed using gas chromatography (GC; Agilent 6890B GC-FID instrument, Waldbronn, Germany) coupled with mass spectrometry (MS; Agilent 5977 MSD instrument, Waldbronn, Germany). Essential oil samples (20 µL) were diluted with hexane (1: 49, v/v) and injected in split mode (1:50) at an inlet temperature of 220 °C. The compounds were separated using an HP-5MS capillary column (30 m × 0.25 mm, 0.25 µm; Agilent, Waldbronn, Germany) under the following temperature programme: initial oven temperature (set at 60 °C) was increased at a rate of 3 °C/min until reaching 246 °C. Helium was used the carrier gas, at a flow rate of 1 mL/min. The temperature of the MSD transfer line was set to 230 °C. Spectral data were collected in scan mode ($m/z = 50\text{--}550$). Compound identification was carried out using the NIST Mass Spectral Database (v14), and supported by literature data (Adams, 2017).

Antibacterial activity

Antibacterial activity against *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 was determined using the disk diffusion method on Muller-Hinton agar. Bacteria incubated overnight on Columbia blood agar were diluted in sterile 0.9% NaCl to achieve a concentration of 10⁵ CFU/mL. Bacterial inoculum was spread over sterile Muller-Hinton agar using a sterile cotton swab. After 15 minutes, a sterile paper disc (diameter 9 mm) was placed on the agar surface under aseptic conditions and impregnated with 20 µL of essential oil. The plates were incubated for 24 hours at 37 °C. After incubation, the diameter of the clear zone was measured in mm. All tests were performed in triplicate, and average values were calculated. Gentamicin (10 µg) and ciprofloxacin (5 µg) were used as reference standards.

Molecular docking

AutoDock Vina was employed to dock the isolated compounds into the active site of *Escherichia coli* DNA gyrase B. The X-ray crystal structure of the protein was retrieved from the Protein Data Bank (PDB ID: 6KZX), with a resolution of 2.10 Å. Protein preparation was carried out using AutoDockTools following a standard protocol, which included the removal of non-conserved water molecules, addition of polar hydrogens, and the assignment of Gasteiger charges. The chemical structures of the compounds were obtained from PubChem, and their energies were minimised using the Gaussian PM3 semiempirical method integrated within Chem3D Ultra. Partial atomic charges were assigned, and rotatable bonds were identified to allow molecular flexibility during the docking process. The protein and ligands were saved in PDBQT format for further docking analysis.

For the docking simulations, a grid box was defined around the active site based on the coordinates of the co-crystallized ligand: center_x = 40.926, center_y = 50.522, center_z = 34.843, with dimensions of size_x = 42, size_y = 20, and size_z = 20, and a grid point spacing of 1 Å. A maximum of nine conformers were generated for each ligand, and their binding energies (kcal/mol) were calculated. The interactions of the best-ranked conformers (those with the lowest binding energies) with the target receptor were analysed using Biovia Discovery Studio Visualizer.

Antioxidant activity

The antioxidant activity of lemon essential oil was evaluated using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay, one of the most widely used methods for assessing antioxidant activity. It evaluates the ability of antioxidants to neutralise stable DPPH radicals. To prepare the reaction mixture, DPPH radical was dissolved in ethanol to a final concentration of 3×10^{-4} mol/dm³ in a dark glass vial. The solution was then completely dissolved using ultrasonic treatment. Next, 1 cm³ of the DPPH solution was mixed with 2.5 cm³ of an ethanolic solution of lemon essential oil. The antioxidant activity was assessed by measuring the absorbance at 517 nm, which corresponds to the characteristic absorption of the DPPH radical. The experiment was performed using different concentrations of lemon essential oil (10, 50, 100 and 150 mg/cm³). For each concentration, the antioxidant activity was measured at two incubation times (20 and 60 minutes) to observe the time-dependent effect on the DPPH radical scavenging activity. The absorption at 517 nm was measured for the ethanolic solution of the DPPH radical, which was diluted in the previously mentioned ratio (1 cm³ of DPPH radical solution with a given concentration, and 2.5 cm³ of ethanol added). Ethanol served as the blank. The free radical scavenging activity was determined according to Mensor et al. (Mensor et al., 2001; Stanojevic et al., 2017) using the following formula:

$$DPPH \text{ radical scavenging capacity (\%)} = 100 - \left[(A_s - A_B) \times \frac{100}{A_c} \right] \quad \text{Eq. 1}$$

A_s : Absorption of the sample at 517 nm, Sample: ethanolic solution of the lemon essential oil treated with DPPH radical solution, A_B : Absorption of the "blank" at 517 nm, Blank: ethanolic solution of the lemon essential oil which is not treated with DPPH radical solution, A_c : Absorption of the control at 517 nm. Control: ethanolic solution of the DPPH radical (Stanojevic et al., 2017).

All absorbance measurements were performed using a UV-1800 SHIMADZU Spectrophotometer; the experiments were performed in triplicate.

Results and discussion

GC–MS analyses of the essential oil

GC-MS identified the presence of 18 constituents in LEO, with monoterpene olefins being the most abundant, accounting for approximately a 95-97% of the total composition (Figure 1). *d*-limonene was the major component, present at 63,48%, followed by β -pinene and γ -terpinene, with contents of 13.3% and 10.42%, respectively. Non-monoterpene components such as camphene, nerol-acetate, geranyl-acetate and caryophyllene were also detected. It was previously reported that monoterpenes account for 97% of the citrus essential oil content, with the remainder consisting of alcohols, aldehydes and esters (Djenane, 2015). Our results show a very similar monoterpene, with *d*-limonene as the dominant component, classifying this sample as a limonene chemotype. It is important to note that previous studies have also identified limonene as the major constituent of lemon essential oil (Espina et al., 2011; Himed et al., 2019). The limonene content can vary depending on the method of extraction, maturity stage, climatic conditions, geographical location, and other factors.

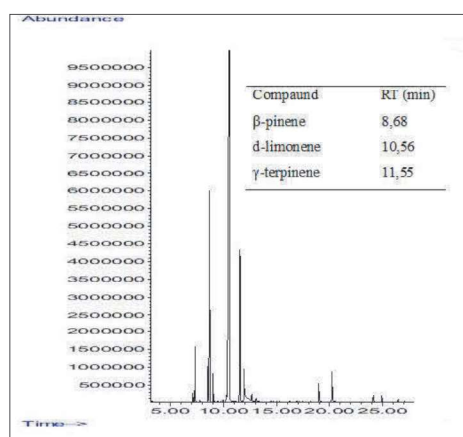


Figure 1 GC-MS Chromatogram of lemon essential oil

Antibacterial activity

After overnight incubation, antibacterial activity was noticed only against Gram-positive bacteria *S. aureus*. A clear inhibitory zone with a diameter of 21 mm was detected around *S. aureus*, while *E. coli* was completely resistant to the testing substance without any zone of inhibition.

The efficient antibacterial activity of plant substances is very important and remains a key focus for the pharmaceutical industry, mainly due to their safety for human use and uncontrolled and widespread bacterial resistance to antibiotics. Our results against *S. aureus* are very promising, as some authors consider a diameter zone above 7 mm as a positive result, indicating bacterial sensitivity to the test substance (Prabuseenivasan et al., 2006). Song et al. (2020) discovered that mandarin essential oil could inhibit *S. aureus* growth and replication by affecting cell membrane stability, permeability and integrity, leading to leakage of cell contents. On the other hand, gram-negative bacterium *E. coli* was completely resistant, without any clear zone of inhibition. This can be explained by differences in cell wall structure, which does not allow the penetration of hydrophobic EO molecules (Gomez-Sequeda et al., 2020). These results are consistent not only for lemon essential oils, but for many other types of essential oils when comparing their activity on gram-positive and gram-negative bacteria (Galgano et al., 2022).

Molecular docking

Bacterial DNA gyrase is a critical enzyme involved in DNA replication and supercoiling, and it is absent in humans, making it an attractive target for selective inhibition in antimicrobial therapy (Barančoková et al., 2018; Fois et al., 2020). DNA gyrase inhibitors, such as fluoroquinolones, have been widely used in clinical settings; however, the overuse and misuse of these drugs have led to the emergence of resistance in many pathogens (Bhatt and Chatterjee, 2022; Shariati et al., 2022). Lemon essential oil has demonstrated antibacterial activity in our study, particularly against *S. aureus*, prompting further investigation into whether this effect may be mediated through the inhibition of DNA gyrase.

To assess this, we performed molecular docking of the principal compounds of the lemon essential oil, *d*-limonene, γ -terpinene, and β -pinene, into the active site of DNA gyrase B (PDB ID: 6KZX). The molecular docking analysis revealed that all three principal compounds were able to bind to the DNA gyrase enzyme (Table 1). Among them, *d*-limonene exhibited the lowest binding energy (-5.9 kcal/mol), which was comparable to that of ciprofloxacin (-7.0 kcal/mol), a well-known DNA gyrase inhibitor. For each ligand, nine different conformations were generated and evaluated based on their binding energies, with the conformation displaying the lowest atomic energy selected for further interaction analysis using Biovia Discovery Studio Visualizer.

Given its hydrophobic nature, it is unsurprising that *d*-limonene primarily engaged in alkyl interactions with several amino acid residues at the active site of the receptor, specifically Ile78, Met95, Val120, and Val167 (Figure 2). These interactions are consistent with *d*-limonene's nonpolar structure, which facilitates the formation of hydrophobic interactions with the receptor's interior residues.

Table 1 Binding energy of principal compounds of lemon essential oil and DNA gyrase inhibitor ciprofloxacin

Compound	Binding energy (kcal/mol)
<i>d</i> -limonene	-5.9
γ -terpinene	-5.8
β -pinene	-4.9
ciprofloxacin	-7.0

The structurally similar compound γ -terpinene also interacted with these same residues – Met95, Val120, and Val167 – through alkyl interactions, much like *d*-limonene. However, a notable difference was observed at Ile78. While *d*-limonene formed a typical alkyl interaction with Ile78, γ -terpinene engaged in a pi-sigma interaction with this residue (Figure 3). This shift in interaction type reflects subtle differences in the molecular properties of the two compounds. Despite this, binding affinity was not significantly affected, as both compounds showed almost identical binding energies (Table 1). As shown in Figure 4, β -pinene, which exhibited the weakest binding affinity (-4.9kcal/mol), formed only two alkyl interactions with Ile78.

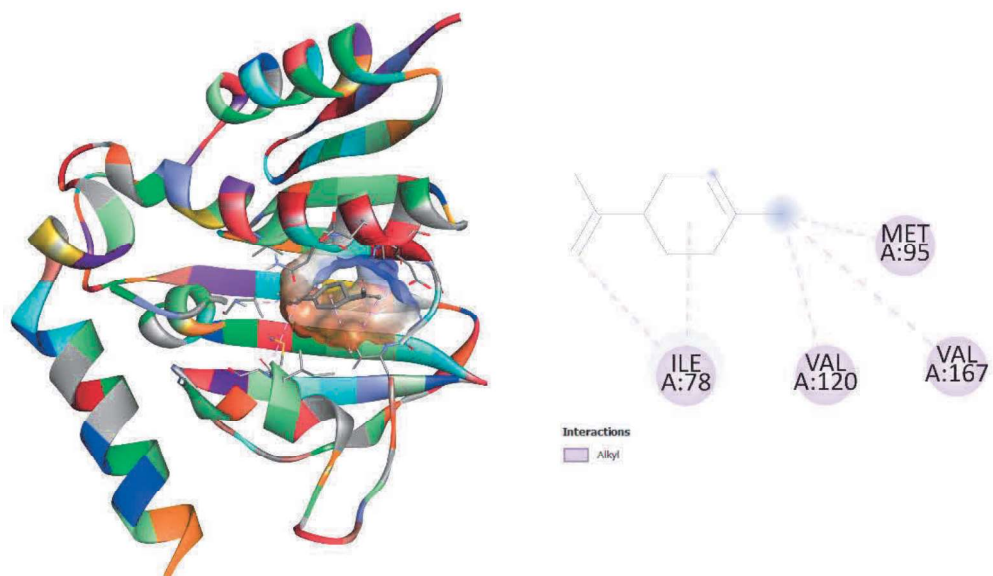


Figure 2 2D and 3D representation of *d*-limonene interactions within the active site of DNA gyrase B (PDB ID: 6KZX)

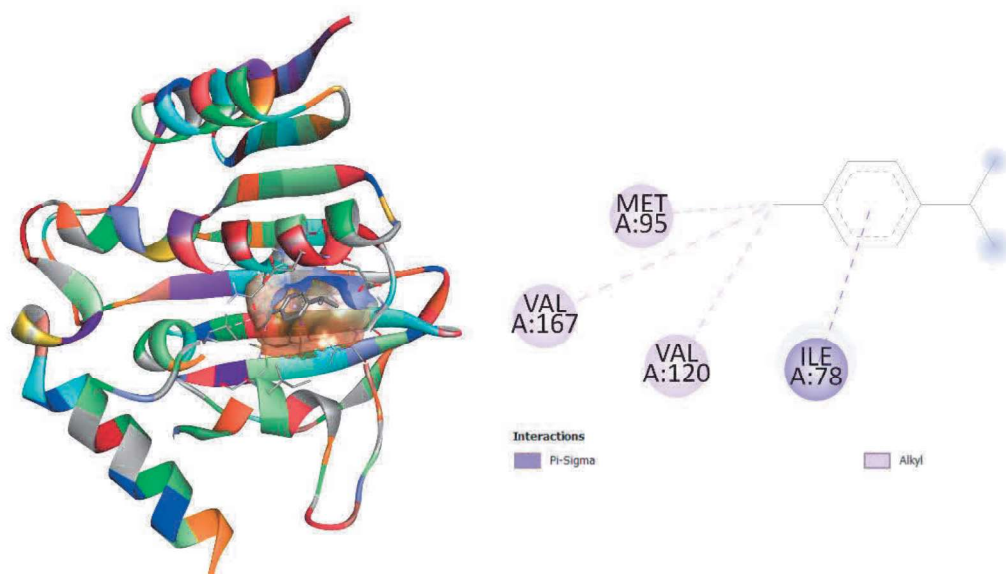


Figure 3 2D and 3D representation of γ -terpinene interactions within the active site of DNA gyrase B (PDB ID: 6KZX)

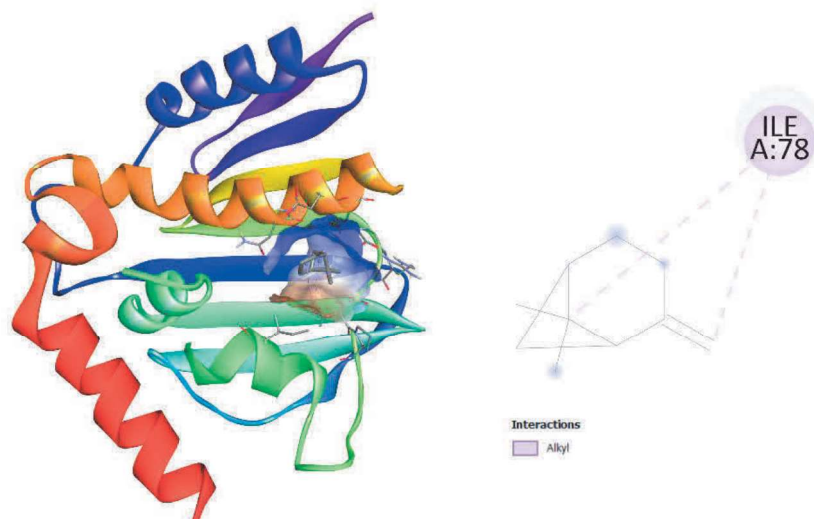


Figure 4 2D and 3D representation of β -pinene interactions within the active site of DNA gyrase B (PDB ID: 6KZX)

These findings suggest that the antibacterial activity of lemon essential oil may be, at least in part, due to the inhibition of DNA gyrase by its principal components, especially *d*-limonene and γ -terpinene. This highlights the potential of natural compounds, such as those found in essential oils, to serve as alternative or adjunct therapeutic agents against bacterial pathogens by targeting critical enzymes like DNA gyrase.

Antioxidant activity

Previous studies have demonstrated that lemon essential oil possesses antioxidant properties, as it can effectively neutralise stable free radicals and reduce oxidative cellular stress (Bora et al., 2020; Li et al., 2022). The degree of DPPH radical neutralisation depends on both the oil concentration and the incubation time. Our study results indicate that the antioxidant activity of lemon essential oil increases with concentration, both after 20 minutes and 60 minutes of incubation (Figure 5). At the highest concentration of 150 mg/mL, LEO exhibited a strong DPPH free radical scavenging rate (76.0% after 20 min incubation time; 82.1% after 60 min incubation time). Previous studies have reported that essential oils showed a dose-dependent increase in free radical scavenging activity, with maximum activity observed at the highest concentrations (Li et al., 2022; Stanojevic et al., 2017). Some authors suggest that LEO exhibits stronger antioxidant activity compared to essential oils from other citrus fruits, such as orange and tangerine (Frassinetti et al., 2011). The antioxidant properties of LEO are influenced by its overall composition, not only the primary component, *d*-limonene, but also secondary compounds. It is challenging to attribute biological activity to a single component. Some researchers believe that the potent antioxidant effects of LEO can be attributed to the combined action of γ -terpinene, limonene, and other components (Li et al., 2022; Teneva et al., 2019). In our study, γ -terpinene was present at a concentration of 10.42%. Based on the results obtained for the antioxidant activity, we believe that our essential oil could be used as a green antioxidant in industry.

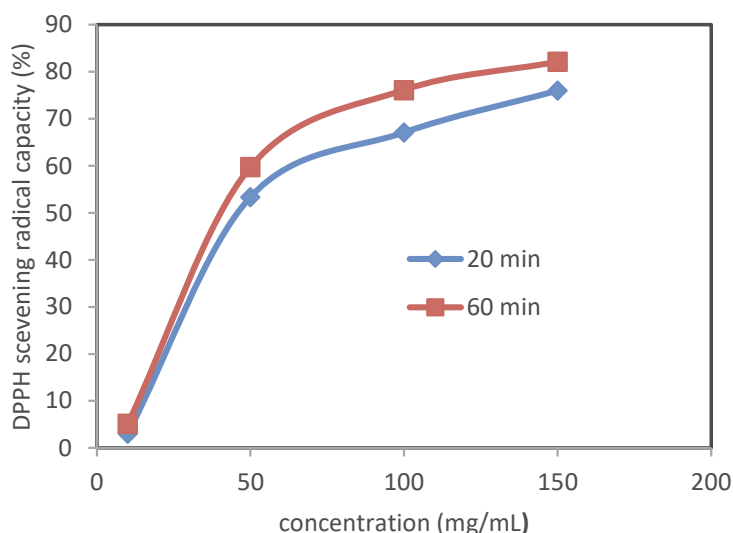


Figure 5 Antioxidant activity of LEO

Conclusion

Our study aims to present the chemical composition and biological activity of essential oil obtained by hydrodistillation from the fresh peel of lemons grown in the Mostar area. GC-MS analysis revealed three principal components: *d*-limonene, β -pinene, and γ -terpinene. The lemon essential oil demonstrated antibacterial activity against *Staphylococcus aureus*. Molecular docking analysis revealed that all three principal components have close binding energies comparable to ciprofloxacin, suggesting their potential as effective inhibitors of DNA gyrase. The radical scavenging activity displayed by the lemon essential oil suggests its potential as an antioxidant. Due to its significant biological properties, this oil can play a key role in combating infections and protecting cells from damage caused by free radicals.

Author Contributions: V.A. project conceptualisation, laboratory work, formal analysis, manuscript preparation, review and editing. Ž.M.B. project conceptualisation, laboratory work, review. A.Š. laboratory work, contributed to manuscript preparation. Ž.G. molecular docking analysis, contributed to manuscript preparation. N.K. laboratory work, formal analysis. V.G.C. laboratory work.

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Conflicts of Interest: The authors declare no conflict of interest.

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