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# CHEMICAL COMPOSITION, *IN VITRO* WOUND HEALING ACTIVITY, AND *IN SILICO* DOCKING EVALUATION OF ESSENTIAL OILS FROM *CITRUS MAXIMA* (BURM.) MERR LEAVES AND PEELS

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**ABSTRACT**

**Objective:** The purpose of this study was to investigate antibacterial and antioxidant activities, and evaluate *in vitro* wound healing potential via fibroblast proliferation assay of *Citrus maxima* leaves oil (MLEO) and *C. maxima* peels oil (MPEO), determine chemical constituents, and predict the chemical component responsible for antibacterial properties.

**Methods:** This citrus oil was obtained through hydrodistillation. The analysis of chemical composition was obtained using gas chromatography–mass spectrometry (GC-MS) spectroscopy. *In vitro* wound healing using MTT assays against fibroblast cells, antioxidant activities were evaluated through ABTS assay, and antibacterial activities using the broth microdilution method against *Staphylococcus aureus* (ATCC 25423) and *Escherichia coli* (ATCC 25422). The molecular docking of main chemical compounds was evaluated using PBP 1a (3UDI) and PBP 2a (1MWT) proteins.

**Results:** GC-MS revealed that D-limonene was dominant in the peels, while 3-carane was dominant in the leaves. The *in vitro* wound healing activity showed that MLEO and MPEO enhanced the number of fibroblast cells with values of 103.31±3.53% and 100.09±2.28%, respectively, at a concentration of 0.1 μg/mL. MLEO exhibited a lower $IC_{50}$ value than MPEO, with avalue of 251.97±3.34 μg/mL. MLEO also exhibited strong antibacterial activity, with minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of 3.125 mg/mL against *S. aureus*, and MIC of 6.25 mg/mL and MBC of 12.5 mg/mL against *E. coli*. Molecular docking analysis indicated that 3-carene had the strongest binding affinities on PBP 1a protein (−6.222 kcal/mol) and caryophyllene on PBP 2a protein (−6,663 kcal/mol).

**Conclusion:** This study confirms that MLEO showed the potential for wound healing, antioxidant, and antibacterial activity.

**Keywords:** Citrus oils, Wound healing, Antioxidant, Antimicrobial, *In silico.*

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## INTRODUCTION

Effective wound treatment is a significant clinical challenge because wounds can cause acute to chronic complications [1]. Chronic wounds can be infected by microorganisms from the genera *Pasteurella*, *Staphylococcus*, *Corynebacterium*, *Pseudomonas*, *Streptococcus*, and *Enterococcus* [2]. Common local antimicrobials, such as silver, chlorhexidine, and iodine, are used to treat this infection. They were highly effective but cytotoxic at an effective dose [3]. Antibiotics are also used to manage the spread of diseases. Excessive use of antibiotics causes antibiotic resistance, making selecting effective anti-infective drugs for wounds challenging. In addition, other modern treatments in wound healing, such as nanotechnology-based products, growth factor therapies, stem cell therapies, or artificial skin, were expensive and unaffordable for some patients.

Wound healing is a complex process with stages: Coagulation, immune response and inflammation, proliferation, and remodeling [4]. During the proliferation stage, our bodies form new cells that play a role in closing wounds and repairing damaged tissue. Some of the cells involved are keratinocytes, fibroblasts, endothelial cells, and macrophages. Approximately 12 h after an injury occurs, keratinocytes migrate to the wound area and repair the damaged tissue, forming a layer of skin. These cells become more active, producing enzymes and forming new tissue. Hair follicle stem cells will produce new skin cells, especially in minor wounds. Fibroblasts play a significant role in replacing damaged cells with stronger new tissue. Fibroblasts will transform into myofibroblasts, pulling the wound's edges together more quickly [5].

Factors such as reactive oxygen species (ROS) (e.g., hydrogen peroxide, superoxide, and hydroxyl radicals), nitric oxide synthase (nitric oxide, nitrogen dioxide, and peroxynitrite), and chlorine radicals contribute to causing cell damage in wound tissue [6]. Hydrogen peroxide at high concentrations (500 mM) could trigger oxidative stress, damage cells, and induce an inflammatory response [7]. Thus, an antioxidant was needed to reduce oxidative damage and accelerate wound healing [8]. Furthermore, antioxidants had a role in neutralising ROS, inhibiting bacterial growth, and promoting tissue regeneration – three key components essential for effective wound healing [9].

Therefore, effective and safe alternative drugs for wound healing are needed. Natural resources such as essential oils can potentially develop as wound healing agents [10]. The lipophilic properties of essential oils damage the bacterial cell walls and cytoplasmic membranes, releasing and coagulating cytoplasmic content. In addition, essential oils prevent bacterial cells from synthesising proteins, polysaccharides, DNA, and RNA [11]. The ability of essential oils to heal wounds has been the subject of numerous studies. Many studies have been conducted on the wound healing properties of essential oils. Turmeric essential oil has been demonstrated to enhance the quantity of fibroblast cells, which play a role in the proliferation phase of the wound healing process [12]. Another human clinical trial study showed that lavender oil can increase collagen expression and protein activity in wound tissue remodeling [13]. Chamomile essential oil also has wound healing effects in clinical trials [14].

Citrus species contain essential oils extensively used in the pharmaceutical, food, fragrance, and cosmetic industries. Citrus oil has anticancer, antiviral, antibacterial, antioxidant, antifungal, antiparasitic, and insecticidal activities [15,16].

The previous research showed that citrus oils derived from *Citrus aurantiifolia* (Christm) Swingle peels had antibacterial activity [17]. In addition, citrus oils derived from the peels and leaves of *Citrus × microcarpa* Bunge and *Citrus medica* L. had *in vitro* wound healing activity, enhancing fibroblast proliferation and migration [18]. The other research on the leaves and peels of *C. aurantiifolia* and *Citrus × aurantifolia* showed antibacterial and wound healing activity *in vitro* against fibroblast cells [9]. *Citrus maxima* also showed good antibacterial activity against skin bacteria [19].

The study on the wound healing activity of *C. maxima* is still limited. Therefore, the purpose of this study was to investigate antibacterial, antioxidant, and wound healing activity of citrus oils, namely *C. maxima* leaves oil (MLEO), and *C. maxima* peels essential oil (MPEO), as well as to determine metabolite profiling and predict the chemical component that is responsible for the antibacterial activity. This comprehensive approach provides novel insights into the therapeutic potential of Citrus oils and supports their development as an alternative medicine for the healing of wounds.

## METHODS

### Plant material

The leaves and peels of *C. maxima* (Burm.) Merr was obtained from Padang Pariaman District, West Sumatra Province, Indonesia. Herbarium specimens were classified in the Herbarium of Andalas, Biology Division, Mathematics and Natural Sciences Faculty, Universitas Andalas, with the voucher specimen number of K-350.

### Citrus oil extraction

Citrus leaves and fruit were thoroughly washed, then leaves and peels were finely chopped and placed in a flask. The distillation procedure was performed in 5 h. The essential oil was obtained in a bottle. An addition of sodium sulfate ($Na_2SO_4$) was employed to isolate the citrus oil from the water. The citrus oils were tagged MLEO and MPEO. The citrus oil was kept at 4°C until further testing. Physical characterization of the essential oil included determining the yield, color, density, and refractive index.

### Gas chromatography coupled with mass spectrometry analysis of citrus oils

The chemical compounds of MLEO and MPEO were analyzed using gas chromatography–mass spectrometry (GC-MS) Agilent® 7890A. The analytical conditions of the GC-MS are based on the method by Husni *et al*. [18]. Compound identification was performed by comparing mass spectra with the NIST 11 database, using a quality match threshold with a minimum value of 90%. Compound identification was performed by matching fragmentation patterns against data from literature and the NIST Chemistry WebBook. Meanwhile, the relative quantification of compounds was determined according to the percentage of peak area in the chromatogram.

### Antioxidant activity using the ABTS method

Antioxidant capacity was measured using (2, 2'-Azinobis (3-ethylbenzothiazoline-6-sulfonic acid)) ABTS test in a 96-well microplate. Citrus oils were prepared at 15.625, 31.25, 62.5, 125, and 250 µg/mL concentrations, then 20 µL of each concentration was diluted in the wells. Each well received a solution of 180 µL of ABTS. The absorbance was measured with a Microplate Reader (BiochromAsys UVM 340) at a wavelength of 734 nm. Trolox served as the positive control. The tests were replicated 3 times, along with inhibition percentage and $IC_{50}$ values.

### *In vitro* fibroblast proliferation activity using MTT assay

The MTT assay used [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] to analyze fibroblast cell proliferation. Fibroblast cells ($10^4$ cells/well) were suspended in 180 µL of culture medium and then put onto a 96-well plate, where they were incubated for 24 h at 37°C with 5% $CO_2$ in a humidified environment. Following incubation, each well received 20 µL of citrus oil dissolved in dimethyl sulfoxide (DMSO) at concentrations of 0.1, 1, 10, and 100 mg/mL. Control wells contained RPMI medium containing only DMSO. After that, the plates were incubated under the same conditions. To give live cells time to transform MTT into insoluble purple formazan crystals, 100 µL of MTT solution (0.5 mg/mL) was added to each well and incubated for 4–6 h. 100 µL of DMSO was added to dissolve the resulting formazan, and the absorbance was measured at 550 nm. The test was replicated 5 times.

### Antimicrobial activity

A 50% citrus oil stock in DMSO (w/v) was prepared and mixed with 450 µL of MHB, or Mueller–Hinton Broth, to achieve a 5% concentration (50 mg/mL). The bacterial strains were *Escherichia coli* (ATCC 25422) and *Staphylococcus aureus* (ATCC 25423). The sterile 0.9% sodium chloride (NaCl) solution was used to suspend the bacterial subculture, and the McFarland standard of 5% was applied to adjust their turbidity. The final concentration of bacteria, about $1\times10^6$ colony-forming units per milliliter (CFU/mL), was derived from the standard suspension, which was then diluted 1:150 in MHB, Mueller–Hinton Broth. Then, a 96-well microplate was added with 50 µL of MHB, Mueller–Hinton Broth. Subsequently, 50 µL of the sample solution (50 mg/mL) was poured into the wells and serially diluted to yield final concentrations of 0.781, 1.562, 3.125, 6.25, 12.5, and 25 mg/mL. Ofloxacin was used as a positive control. Then, 50 µL of standard bacterial suspension was dispensed to every well, excluding the sterility and growth control wells. The microplates were incubated at 37°C for 18–24 h.

After incubation, each well received 40 µL of MTT solution to reach a 0.5 mg/mL concentration, and the incubation process was continued for 30 min at 37°C. A shift in color from colorless to purple indicated bacterial viability. The lowest concentration at which there was no change in the solution's color was determined to be minimum inhibitory concentration (MIC), MIC. Each experiment was carried out 3 times. After streaking medium from each well onto nutrient agar plates, the plates were incubated at 37°C for 18–24 h. The absence of visible bacterial colonies on the agar surface indicated the minimum bactericidal concentration (MBC) value.

### Molecular docking

Molecular docking was conducted to investigate the binding interactions between seven dominant citrus oil compounds with penicillin-binding protein 1a (3UDI) and PBP 2a (1MWT). RCSB Protein Data Bank (https://www.rcsb.org) provided the protein's three-dimensional structures. The three-dimensional structures of the active compounds were obtained in SDF format from the PubChem database and then changed into PDB format. The molecular preparation process included optimising the geometric structure of ligands and applying the MMFF94 force field with the help of Avogadro software. Meanwhile, protein preparation was carried out by removing water molecules, adding hydrogen atoms, and setting Gasteiger partial charges to ensure the accuracy of the docking simulation. The molecular docking protocol was verified by comparing the native and docked ligand. Docking was performed using AutoDockTools 1.5.7 with a grid box defined based on the protein's active site. Validation was performed by redocking the native ligand (root mean square deviation [RMSD] <2 Å). Binding interactions were evaluated using the calculated bond free energy (ΔG, kcal/mol). Molecular visualization and interaction analysis were performed using Discovery Studio Visualizer.

## RESULTS AND DISCUSSION

### Physical properties of *C. maxima* essential oils

Table 1 presents the physical properties of essential oils obtained from *C. maxima* (Burm.) Merr peels and leaves include yield, color, density, and refractive index. Fig. 1 shows the physical form of the *C. maxima* essential oil.

The physical characterization analysis revealed that the citrus oils extracted from *C. maxima* exhibited a colorless to pale yellow color. The yield analysis showed that MLEO had a lower yield rate at 0.04% than MPEO at 0.12%. Other research stated the yield of *C. maxima* peels and leaves was 0.12% and 0.07%, respectively [20]. The yields may vary based on the edaphic conditions, extraction techniques, intrinsic plant characteristics, and environmental factors [21,22].

The density values showed 0.714 and 0.926 g/mL for MPEO and MLEO, respectively. Essential oils exhibited densities lower than one, except those containing oxygenated aromatic compounds. This study recorded that MPEO (1.472) had a lower refractive index value than MLEO (1.485). The presence of water, long-chain hydrocarbons, or oxygenated compounds may cause differences in refractive index values. A higher water content decreased the refractive index due to water's ability to alter the path of light. Furthermore, the findings demonstrated a correlation between the refractive index and density, suggesting a potential relationship between these physical parameters [17].

**Chemical composition of the essential oil of *C. maxima***

Terpenoids are one of the main compounds frequently present in essential oils. The chemical compositions for the two essential oils are shown in Table 2. GC-MS study showed that essential oil composition varied depending on the plant part. D-limonene was the most dominant compound, especially in the citrus peels; meanwhile, 3-carene was the dominant compound in citrus leaves. In both samples, compounds such as Germacrene D, β-Myrcene, and L-β-Pinene were also detected in significant amounts. These results indicated that *C. maxima* peel and leaves had distinct chemical profiles, influencing their potential bioactivity.

The major constituents in the MPEO included D-limonene, β-myrcene, and L-β-pinene, while the MLEO were rich in 3-Carene, L-β-Pinene, and Caryophyllene. Citrus Peels contained D-limonene as a significant constituent. Previous research stated that D-Limonene dominated the main compound of *C. maxima* peels at 89.04%, and *C. maxima* leaves dominantly contained Citronellol (28.26%), Caryophyllene (16.89%) [20,23]. The Grouping of terpene compounds in Table 3 showed that the monoterpene hydrocarbon was the most abundant, which can be explained by the existence of D-Limonene, 3-carene, β-myrcene, β-Ocimene, and L-β-pinene. The differences in chemical composition were impacted by edaphoclimatic factors such as soil types, humidity, temperature, altitude, and UV radiation [24].

**Table 1: Physical properties of *Citrus maxima* leaves and peels oils**

| Physical characteristics | *Citrus maxima* | |
|---|---|---|
| | **MLEO** | **MPEO** |
| Percentage yield (% v/w) | 0.04 | 0.12 |
| Color | Pale yellow | Colorless |
| Density (g/mL) | 0.926 | 0.714 |
| Index of refraction | 1.485 | 1.472 |

Data are presented as triplicate measurements, MLEO: *Citrus maxima* leaf essential oil, MPEO: *Citrus maxima* peel essential oil

**Antioxidant activity**

The $IC_{50}$ values for MLEO and MPEO are 251.97±3.34 μg/mL and 784.86±8.59 μg/mL, respectively, as shown in Table 4. These values indicate that both oils have weak antioxidant capacity ($IC_{50}$ above 250 μg/mL). Trolox, a reference compound, showed a moderate antioxidant activity with an $IC_{50}$ at 125.03±2.11 μg/mL. These results were the same as a previous study, where the antioxidant activity of the essential oil of *C. maxima* peels and leaves was weak, as indicated by an $IC_{50}$ value above 500 μg/mL [25]. Conjugated double bonds on the antioxidant significantly affect the antioxidant activity of terpenoid molecules [26]. Antioxidant activity of MLEO is stronger than that of MPEO. It may be due to a higher concentration of β-Ocimene (conjugated sesquiterpenes) in Fig. 2. Phytochemical compounds can work through complementary or overlapping mechanisms of action in the body, such as antioxidant activity [27].

The findings are displayed as mean±standard deviation (n=3), where n indicates the number of replications, with trolox as a positive control.

***In vitro* wound healing activity with the MTT assay method**

Wound healing activity showed that MLEO and MPEO increase cell proliferation with values of 103.31±3.53% and 100.09±2.28%, respectively, compared to curcumin with a 104.71±1.11% value, as shown in Table 5. These oils exhibited cytotoxic effects at higher concentrations toward fibroblast cells. Previous research revealed that citrus oils derived from *Citrus aurantifolia* and *Citrus x aurantifolia* enhanced fibroblast cell proliferation at low concentrations [9]. The monoterpene compound in essential oils such as terpineol, borneol, genipin, thymol, and aucubin has been reported to influence wound healing properties [28]. Fig. 3 shows fibroblast cell proliferation of MLEO at 0.1, 1, 10, and 100 μg/mL concentrations. MLEO had better proliferation activity than MPEO. This might be due to differences in the chemical content of these essential oils. MLEO was rich in caryophyllene. A previous study stated that caryophyllene accelerated the re-epithelialization process through increased proliferation and migration of cells [29].

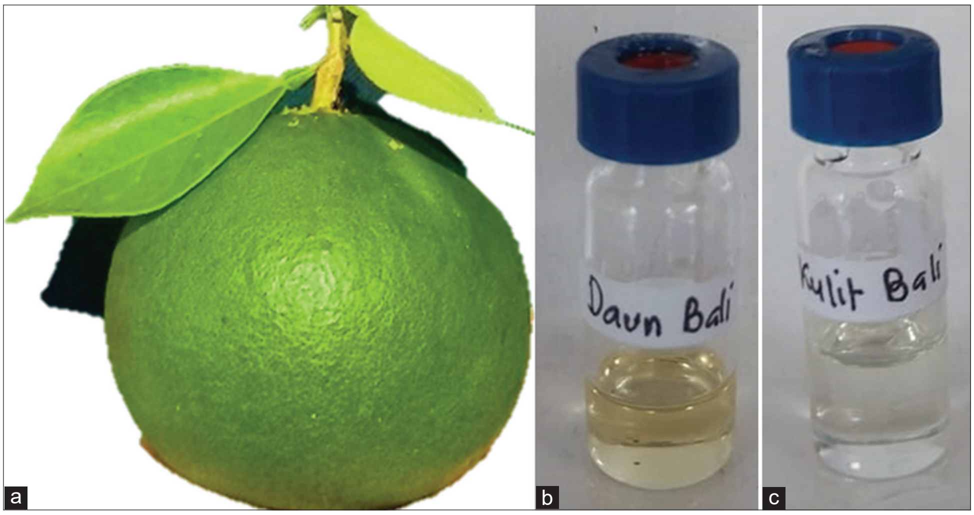


**Fig. 1: (a) Fruits of *Citrus maxima* (Burm.) Merr.; (b) Essential oil extracted from leaves (MLEO); (c) Essential oil extracted from peels (MPEO)**

**Table 2: Chemical composition of *Citrus maxima* essential oils**

| No. | Compound | Area (%) | |
|---|---|---|---|
| | | *Citrus maxima* | |
| | | MLEO | MPEO |
| 1 | D-Limonene | 4.32 | 62.47 |
| 2 | β mircene | 2.34 | 7.06 |
| 3 | L-β-Pinene | 16.05 | 5.73 |
| 4 | Caryophyllene | 13.91 | - |
| 5 | 3-Carene | 19.47 | - |
| 6 | β ocimene | 12.94 | - |
| 7 | β cyclogermacrane | 5.81 | - |
| 8 | Germacrene D | 1.77 | 2.29 |
| 9 | Terpinolene | 4.01 | - |
| 10 | Terpineol | - | 2 |
| 11 | (R)-(+)-Citronellal | 0.91 | - |
| 12 | Citral | - | 1.77 |
| 13 | Terpinen-4-ol | - | 0.72 |
| 14 | 1S-α-Pinene | - | 2.48 |
| 15 | 1-Isopropyl-4,7-dimethyl-1,2,3,5,6,8a-hexahydronaphthalene | 0.87 | - |
| 16 | Carveol | - | 1.15 |
| 17 | 1,3,7,11-Tridecatetraene, 4,8,12- trimethyl-, (3E,7E) | 0.78 | - |
| 18 | Citronellol | 0.56 | - |
| 19 | 2-(5-ethenyl-5-methyloxolan-2-yl) propan-2-yl ethyl carbonate | - | 8.24 |
| 20 | Geraniol | 0.44 | - |
| 21 | Geranyl acetate | 1.38 | - |
| 22 | Humulene | 1.94 | - |
| 23 | Isospathulenol | - | - |
| 24 | Isoterpinolene | 0.47 | |
| 25 | Linalool | - | 2.39 |
| 26 | Longifolene | 0.62 | - |
| 27 | Nerol acetate | 0.85 | - |
| 28 | Phytol | 1.8 | 0.58 |
| 29 | Sabinen | - | 1.5 |
| 30 | trans-β-Ocimene | 1.32 | - |
| 31 | α Citral | 1.41 | - |
| 32 | α Farnesene | 1.21 | - |
| 33 | α Phellandrene | 0.54 | - |
| 34 | α Pinene | 1.88 | - |
| 35 | β Citral | 1.04 | 1.63 |
| 36 | β Cymene | 0.46 | - |
| 37 | β Elemene, | 0.41 | - |
| 38 | δ Elemene | 0.5 | - |

Compounds were identified by GC-MS based on comparison with the NIST 11 library (similarity index >90%). Relative percentages are based on peak area normalization. "-": Not detected

**Table 3: Classification of terpenoid compounds identified in the essential oils of *Citrus maxima* leaves (MLEO) and peels (MPEO)**

| No | Group of compounds | Area (%) | |
|---|---|---|---|
| | | MLEO | MPEO |
| 1 | Monoterpene hydrocarbon | 63.8 | 79.24 |
| 2 | Sesquiterpene hydrocarbon | 27.82 | 2.29 |
| 3 | Oxygenated monoterpene | 6.59 | 9.66 |
| 4 | Others | 1.8 | 8.82 |
| | Total | 100 | 100 |

### Antibacterial activity

Table 6 shows that MLEO had the lower MIC values for *E. coli* (6.25 mg/mL) and *S. aureus* (3.125 mg/mL). MLEO also presented the lower MBC values for *S. aureus* (3.125 mg/mL) and *E. coli* (12.5 mg/mL). According to earlier studies, *C. maxima* essential oil had vigorous antibacterial activity against six bacterial strains, with MIC values ranging from 0.475 to 1.104 mg/mL and MBC values between 1 and 2 mg/mL [30].

**Table 4: Antioxidant activity of *Citrus maxima* oils (the findings are displayed as mean±SD (n=3) where n indicates the number of replications, with trolox as a positive control)**

| No | Sampel | | $IC_{50}$ (μg/mL) |
|---|---|---|---|
| 1. | *Citrus maxima* | MPEO | 617.23±41.46 |
| | | MLEO | 251.97±3.34 |
| 2. | Trolox | | 125.03±2.11 |

SD: Standard deviation

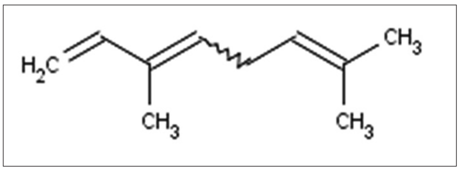


**Fig. 2: Structure of beta-ocimene**

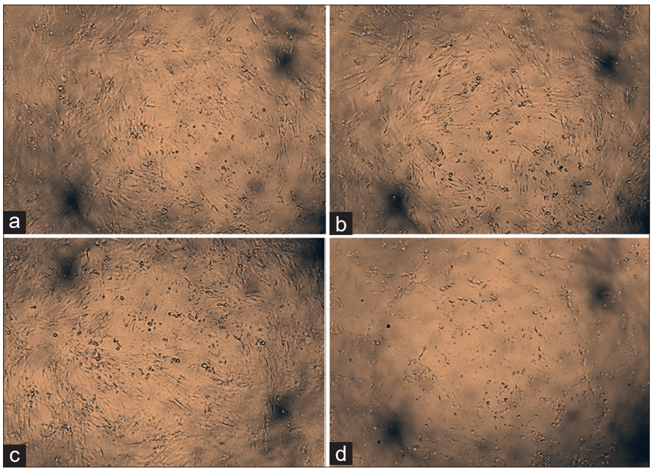


**Fig. 3: Representative microscopy images (likely phase-contrast, specify) showing fibroblast cell morphology after treatment with MLEO at concentrations of (a) 0.1 μg/mL, (b) 1 μg/mL, (c) 10 μg/mL, and (d) 100 μg/mL for 48 h. Scale bar: 100 μm**

The antimicrobial properties of terpenoids are linked to their functional groups, the hydroxyl groups of phenolic terpenoids, and the presence of delocalized electrons in disrupting cell wall permeability and bacterial activity [31]. Hydrophobic essential oils crossed the lipid membrane of bacterial cells, disrupted the structure of the cell wall, and made it more permeable. Then these oils caused ions and other cellular materials to leak, resulting in cell death [32]. The antibacterial activity of MLEO is associated with its chemical content, which contains a large number of monoterpenes and sesquiterpenes such as L-β-Pinene, Caryophyllene, 3-Carene, β-Ocimene, and β-Cyclogermacrane, where these compounds might work synergistically to destroy bacterial activity [33]. The previous research has indicated that antimicrobial properties do not always depend on the most abundant chemical compound, but are also supported by synergistic effects of compounds [34].

The *C. maxima* oils showed stronger antibacterial properties against gram-positive bacteria than Gram-negative ones. Gram-positive bacteria had walls composed of peptidoglycan and teichoic acid layers and no outer membrane, allowing essential oils to penetrate the cells and lyse the bacteria. However, the cell wall structure of Gram-negative bacteria is more complex, consisting of peptidoglycan, lipopolysaccharides, porins, and outer membrane, making it difficult for active compounds to penetrate [35].

**Table 5: The proportion of fibroblast cell proliferation induced by *Citrus maxima* oils and curcumin**

| No. | Sample | Percenatge cell proliferation | | | |
|---|---|---|---|---|---|
| | | 0.1 (μg/mL) | 1 (μg/mL) | 10 (μg/mL) | 100 (μg/mL) |
| 1 | MLEO | 103.31±3.53 | 101.96±1.43 | 99.25±1.22 | 3.45±0.45 |
| 2. | MPEO | 100.09±2.28 | 98.93±2.36 | 95.24±1.75 | 3.92±1.49 |
| 3 | Cucurmin | 104.71±1.11 | 100.75±1.62 | 92.91±0.78 | 13.29±1.20 |
| 4. | Negative control | 100 | 100 | 100 | 100 |

(The results are shown as mean±SD (n=5), where n indicates the number of replications, with curcumin as a positive control)

**Table 6: MIC and MBC values of *Citrus maxima* essential oils**

| No | Sample | *Staphylococcus aureus* | | *Escherichia coli* | |
|---|---|---|---|---|---|
| | | MIC (mg/mL) | MBC (mg/mL) | MIC (mg/mL) | MBC (mg/mL) |
| 1 | MLEO | 3.125 | 3.125 | 6.25 | 12.5 |
| 2 | MPEO | 6.25 | 12.5 | 12.5 | 12.5 |
| 3 | *Ofloxacin* | 0.0014 | 0.0014 | 0.0014 | 0.0014 |

MIC: Minimum inhibitory concentration, MBC: Minimum bactericidal concentration. Ofloxacin was used as a positive control. Values are derived from four independent experiments (n=4)

**Table 7: Docking results of major compounds of citrus oil with PBP protein 1a (3UDI) and PBP protein 2a (1MWT)**

| No | Protein | Compound | Docking score (kcal/mol) | Interaction | Type of interaction |
|---|---|---|---|---|---|
| | | β ocimene | −5.362 | ARG 482, ASP 648, GLY 653, HIS 652, ILE 645, VAL 649, TYR 485, ARG 481 | Van der Waals, Alkyl, Pi Alkyl |
| | | β mircene | −5.138 | ASP 648, HIS 652, GLY 653, ARG 481, VAL 649, ILE 645, ARG 482, TYR 485 | Van der Waals, Alkyl, Pi Alkyl |
| | | L-β-Pinene | −5.51 | ILE 645, VAL 649, ASP 648, HIS 652, TYR 485, ARG 481, ARG 482 | Alkyl, Pi Sigma, Pi Alkyl, Van der Waals |
| 1 | 3UDI | Caryophyllene | −5.897 | ILE 710, THR 654, GLY 709, SER 487, THR 672, SER 470, LEU 486, TYR 707 | Van der waals, Pi- Alkyl |
| | | β Cyclogermacrane | −6.154 | SER 487, LEU 486, TYR 485, THR 672, SER 470, THR 654, THR 670, GLY 709, ILE 710, TYR 707 | Van der Waals, pi- Sigma |
| | | D-Limonene | −6.212 | ARG 482, GLY 653, HIS 652, ASP 648, TYR 485, ILE 645, ARG 481, VAL 649 | Van der Waals, Pi-Sigma, Alkyl |
| | | 3-Carene | −6.222 | ILE 645, VAL 649, ARG 481, ARG 482, TYR 485, ASP 648, HIS 652 | Van der Waals, Alkyl, Pi Alkyl |
| | | Native ligand (Penicillin G) | −7.048 | ASP 675, THR 673, LYS 437, LEU 486, GLY 708, TYR 707, ASN 674, GLY 671, LYS 669, ASN 489, SER 470, THR 670, SER 434, THR 672, SER 487 | Conventional Hydrogen Bond, Salt Bridge, Pi-Alkyl, Van der Waals, Carbon Hydrogen Bond |
| 2 | 1 MWT | β mircene | −4.593 | ASN 464, LYS 406, SER 403, SER 461, SER 462, THR 600, HIS 583, SER 583, GLY599, MET 641, TYR 446, ALA 642 | Van der Waals, Alkyl, Pi-Alkyl |
| | | β Ocimene | −4.635 | THR 600, MET 641, SER 598, THR 582, HIS 583, GLU 447, SER 461, SER 462, SER 403, TYR 446 | VAn der Waals, Pi-Alkyl |
| | | L-β-Pinene | −5.145 | THR 582, SER 461, HIS 583, SER 462, THR 600, GLY 599, SER 598, SER 403, LYS 597, MET 641, TYR 446, ALA 642 | VAn der Waals, Alkyl, Pi-Alkyl |
| | | D-Limonene | −5.231 | THR 582, GLU 447, SER 461, GLU 460, SER 598, SER 462, HIS 583, TYR 446, MET 641, THR 600 | Van der Waals, Alkyl, Pi-Alkyl |
| | | 3-Carene | −5.292 | SER 461, SER 462, GLY 599, SER 598, SER 403, ASN 464, THR 600 | VAn der Waals, Alkyl, Pi-Alkyl |
| | | Caryophyllene | −6.663 | MET 641, THR 600, GLU 602, GLN 521, ASN 464, SER 403, LYS 406, GLY 599, SER 462, TYR 446 | Van der Waals, Pi-Alkyl |
| | | Native ligand (Penicillin G) | −7.881 | TYR 519, ASN 464, LYS 406, GLU 602, GLN 613, MET 641, ALA 601, GLY 599, ALA 642, SER 598, LYS 597, HIS 583, GLN 521, THR 600, SER 403, SER 462, TYR 446 | Van der Waals, Conventional Hydrogen Bond, Attractive Charge, Pi-Sigma, Carbon Hydrogen Bond |

**Molecular docking**

The target protein of the molecular docking study was Penicillin-binding proteins (PBPs). PBPs were essential proteins in synthesising peptidoglycan in the cell wall of bacteria. PBPs are *serine acyltransferases* that play a role in synthesising cross-linked peptidoglycan and are the target of β-lactam antibiotics. Bacteria generally have four endogenous PBP enzymes, namely PBP1, PBP2, PBP3, and PBP4 [36].

This molecular docking was carried out on seven major compounds in citrus oil, namely: D-limonene, β-myrcene, L-β-pinene, caryophyllene, 3-carene, β-ocimene, and β-cyclogermacrene. The specific proteins used in the molecular docking procedure were Penicillin Binding Protein PBP 1a (3UDI) and PBP 2a (1MWT). The RMSD value was valid from the data, namely, <2 Å, where the RMSD data for PBP 1a (3UDI) was 1.662 Å and PBP 2a (1MWT) was 1.597 Å. This supports the idea

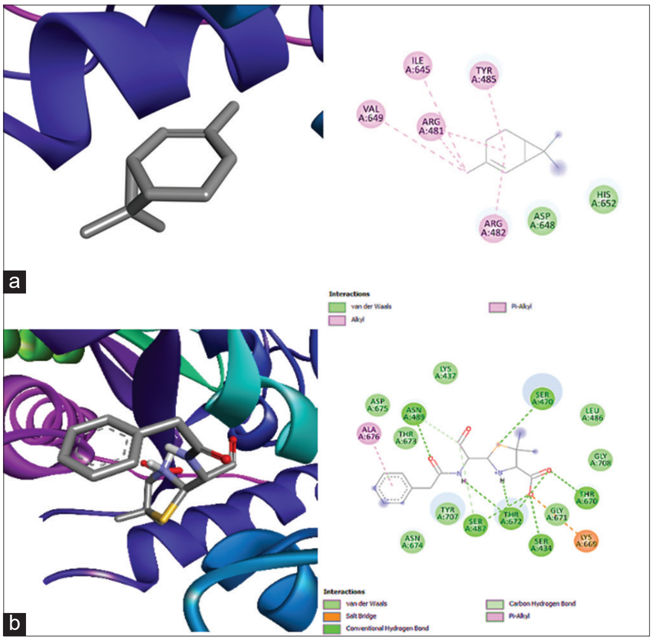

**Fig. 4: Three-dimensional binding poses (left) and Two-dimensional interaction diagrams (right) and within the active site of PBP1a (3UDI). (a) 3-Carene (green); (b) Native ligand Penicillin G (yellow). Key interactions: Van der Waals (green dashed lines), Pi alkyl (pink spokes/arcs). Generated using discovery studio visualizer**

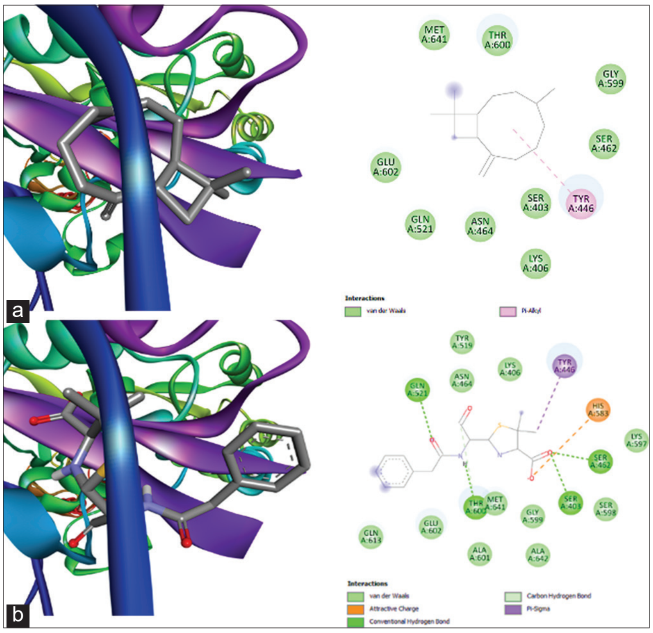

**Fig. 5: Three-dimensional binding poses (left) andTwo-dimensional interaction diagrams (right) within the active site of PBP2a (1MWT). (a) Caryophyllene (green); (b) Native ligand Penicillin G (yellow). Key interactions: Van der Waals (green dashed lines), Pi alkyl (pink spokes/arcs). Generated using discovery studio visualizer**

that molecular docking can provide accurate results about the binding interactions between proteins and ligands [37].

Docking analysis showed that 3-Carene had the strongest binding affinities with PBP 1a (−6.222 kcal/mol) and caryophyllene with PBP 2a (−6,663 kcal/mol). This strong affinity is given by the specific molecular structure and interactions with the protein's binding site. 3-Carene showed significant interactions with residues such as ILE 645, VAL 649, ARG 481, ARG 482, TYR 485, ASP 648, and HIS 652, as detailed in Table 7. Caryophyllene formed stable interactions with MET641, THR600, GLU602, GLN521, ASN464, SER403, LYS406, GLY599, and SER462 through van der Waals forces, and with TYR446 through a pi-alkyl interaction. Meanwhile, the native ligand Penicillin G had binding affinities with PBP 1a (−7.048 kcal/mol) and PBP 2a (−7.881 kcal/mol).

These molecular docking results were in line with the *in vitro* antibacterial activity. MLEO contained a higher concentration of 3-carene and caryophyllene compared to MPEO. The interactions between PBP 2a Protein (1 MWT) and caryophyllene showed that about 90% of the amino acid residues bound were the same as in the interaction between Penicillin G (Native Ligand) and protein PBP2a, as detailed in Figs. 4 and 5. This docking approach allows for the exploration of high-dimensional space while effectively assessing functional utilization, resulting in a ranking of acceptable docking candidates. In addition, this method can be used to screen large molecular libraries and provide a basis for structural hypotheses that support understanding of the processes involved [38].

Molecular docking scores (binding affinity, ΔG in kcal/mol) and key interacting residues for the major compounds of *C. maxima* essential oils against *S. aureus* PBPs PBP1a (3UDI) and PBP2a (1MWT). The native ligand Penicillin G is included for comparison. Docking was performed using AutoDockTools 1.5.7.

## CONCLUSION

The citrus oil derived from MLEO was found to have stronger antioxidant and antibacterial activity, as well as enhanced fibroblast cell proliferation, than MPEO. MLEO contained a higher concentration of caryophyllene and 3-carene, supporting high binding affinity scores with the PBP 1a (3UDI) and PBP 2a proteins (1MWT). It can be concluded that MLEO demonstrates potential to develop MLEO as a natural agent for wound healing.

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## AUTHORS CONTRIBUTIONS

FauziaHusna: formal analysis, investigation, data curation, writing - original draft, visualization. Suryati Syafri: supervision, methodology, validation, writing, review, and editing. M. Alfariocky Syahroni: investigation, software. Elidahanum Husni: supervision, funding acquisition, resources, and conceptualization.

## CONFLICT OF INTEREST

The authors have declared that no competing interests exist.

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