Activity Test And Inhibitory Mechanism Of Essential Oil Of Lime Leaf (Citrus aurantifolia, Swingle) Against Some Of Bacterial Pathogens

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ACTIVITY TEST AND INHIBITORY MECHANISM OF ESSENTIAL OIL OF LIME LEAF (Citrus aurantifolia, Swingle) AGAINST SOME OF BACTERIAL PATHOGENS

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ABSTRACT

Lime fruit (*Citrus aurantifolia* Swingle) is traditionally known to be useful for the treatment of various infectious diseases like fever, cough, toothache, sore throat, diphtheria, body odor, ringworm etc as folk medicine. Objectives: This study aims to determine the activity and mechanism of inhibition of the growth of some bacterial pathogens by the essential oil of lime leaves (*Citrus aurantifolia*, swingle). Materials and Methods: The lime leaves was obtained from Balitro Bagor, distilled by steam distillation process. The essential oil of lime leaf that obtained was analyzed by Gas Chromatography-Mass Spectroscopy. The activity test of anti-bacterial of essential oil of lime leaves was carried out by using the method mikrodilution against the bacterium *Staphylococcus epidermidis, Staphylococcus aureus, Escherichia coli, Micrococcus luteus and Bacillus subtilis*. For detect the mechanism of cell damage process of bacteria was observed leakage of proteins and nucleic acids by using Ultra Violet-Visible spectrophotometer and the leakage of cations K+ and Ca2+ was observed by using Atomic Absorbtion Spectrum. While bacterial cell morphology change was observed by using Scanning Electron Microscope (SEM). Results and Conclusion: From the research was obtained, Lime leaf contains about 0.4% (v/w) essential oils with the main components are geranial (10.39%), limonene (10.2%), neral (8.94%), kariofilena (5.72%) and citronellal (5.41%). The essential oil of lime leaf has the highest antibacterial activity on the bacteria tested was on *Bacillus subtilis*, with a minimum inhibitory concentration (MIC) of 0.125% (v/v). Test results nucleic acid and protein leakage and cations leakage of K+ and Ca2+ greatly increased from 1 MIC to 2 MIC concentration of the tested bacteria compared with the normal controls. While the observations that was detected by using Scanning Electron Microscope (SEM) showed worse cell damage by giving the higher doses to bacteria.

Keyword: Essential oil of lime leaf, Antibacterial, Staphylococcus epidermidis, Staphylococcus aureus, Escherichia coli, Micrococcus luteus, Bacillus subtilis.
**BACKGROUND**

- Indonesia's richest biodiversity
- **Medicinal plants**
  - **Citrus aurantifolia**
    - Food flavoring, refreshment drinks
    - Antibacterial
    - Fever, cough, toothache, sore throat, diphtheria, body odor, ringworm etc
Flow of Research

Collecting lime leaves

Determination of plants

Distillation with water vapor

Determination components of essential oil

Herbarium Bogoriensis LIPI Puslit Biologi

Rendemen of essential oil

GC-MS
Testing of antibacterial activity

Testing for cell membrane leakage

Analysis of bacterial cell morphology

With Scanning Electron Microscope (SEM)

Determination of inhibitory diameter

Determination MIC

Analysis of leakage of proteins and nucleic acids with Spektrophotometer UV

Analysis of metal ion leakage with AAS
Material:

Essential oil of lime leaves
Medium (NA, MHA, MHB)
Paper discs
Tween 80
Ethanol 95%

Bacteria test:

- Staphylococcus aureus
- Staphylococcus epidermidis
- Bacillus subtilis
- Micrococcus luteus
- Escherichia coli
Isolation of Essential Oils

1. Fresh lime leaves
2. Weighed 3.1 kg
3. Washed clean
4. Distillation with water vapor for 5 hours
5. Destilat + Natrium sulfat anhidrat
6. Obtained essential oil
7. Calculated rendemen Essential oil
Determination of Essential Oil Components

Essential oil components were analyzed using GC-MS with an injection volume of 5 μl. The column used is VF-17 MS, and the carrier gas is Helium with a flow rate of 1.3 ml/min.

The column temperatures are programmed from 50 °C to 250 °C with 2 increment stages.
Preparation of Test Solution

ESSENTIAL OIL OF LIME LEAF

Determination of diameter area inhibition

Concentration 50% (v/v)

Determination of MIC value

Concentration (v/v):
1.25 %
0.63 %
0.32 %
0.16 %
0.08 %
0.04 %
0.02 %
RESULTS

Isolation Essential oil of lime leaves
The essential oil produced is yellow with a distinctive aroma

• Identification of essential oil components
• Essential oils have 40 chemical components, with 5 chemical components having content above 5%:
  - Geranial
  - Limonena
  - Neral
  - Kariofilena
  - Sitronellal
### Classification of essential oil components

| No. | Group of compounds     | Quantity  |
|-----|-----------------------|-----------|
| 1.  | Monoterpene           | 14.63 %   |
| 2.  | Monoterpene alcohol   | 2.99%     |
| 3.  | Monoterpene aldehyde  | 24.74 %   |
| 4.  | Sesquiterpene         | 22.04 %   |
| 5.  | Sesquiterpene alcohol | 5.22 %    |
| 6.  | Etc                   | 24.56 %   |
| 7.  | Not identified        | 5.82 %    |
|     | Total                 | 100 %     |
## Determination of diameter of inhibition area

| No. | Bacterial test         | Diameter of Inhibition (mm) |
|-----|------------------------|-----------------------------|
| 1.  | *Staphylococcus epidermidis* | 10 mm                      |
| 2.  | *Staphylococcus aureus*  | 26 mm                      |
| 3.  | *Escherichia coli*       | 8 mm                       |
| 4.  | *Micrococcus luteus*     | 17 mm                      |
| 5.  | *Bacillus subtilis*      | 31 mm                      |
Determination of MIC value

0.25 % → Suspect bact + med + solvent
0.125 % → Control of medium
0.06 % → medium + solvent
0.03 % → Bacteria control
0.0016 %
0.008 %
0.004 %
- Analysis of leakage of proteins and nucleic acids
Analisis kebocoran ion logam K dan Ca
Cells with 1 MIC treatment
• Cells with 2 MIC treatment
CONCLUSION

- The essential oil of lime leaves obtained is about 0.4% (v/w) with 40 chemical components. The five main components are geranial, limonene, neral, kariofilena and sitronellal.

- The essential oil of lime leaves has antibacterial activity against *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Escherichia coli*, *Micrococcus luteus* and *Bacillus subtilis*. The value of MIC obtained from essential oils to B.subtilis is 0.125%.

- The mechanism of inhibition of essential oils against B.subtilis occurs through cell membrane destruction, in the presence of leakage of proteins, nucleic acids, metal ions and cell morphological changes.
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