Antibacterial activity of basil oil (*Ocimum basilicum* L) and basil oil nanoemulsion

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Abstract. Basil oil contains bioactive compounds with antibacterial activity. One way to increase the antibacterial activity of basil oil is to use a nanoemulsion design. This study aims to obtain a nanoemulsion of basil oil with the best antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* compared to basil oil. Process isolation of basil oils uses the method of steam distillation. The nanoemulsion process uses the ultrasound method. The essential oils and nanoemulsions produced were analyzed by GC-MS. The five components of basil oils with the largest percent area are sabinene (60.01%), myrcene (17.76%), trans-caryophyllene (4.08%), linalool (2.58%), and alpha-pellandrene (2.35%). Whereas in nanoemulsion are sabinene (44.68%), myrcene (17.86%), trans-caryophyllene (8.15%), terpineol-4 (6.65%), and 1.6-octadient-3-ol 3,7-dimethyl (4.89%). The basil oil has a droplet size of 54960 nm while the nanoemulsion of basil oil has a droplet size of 243.4 nm. The concentration of basil oil influences the antibacterial activity. The essential oil of basil, at a concentration of 10% to 25%, has a zone of low to medium protein inhibition at *E. coli* and is strongly directed towards *S. aureus*. The nanoemulsion of essential oils at a concentration of 5% to 25% has a moderate to strong inhibition zone in *E. coli* and a very strong group in *S. aureus*.

1. Introduction

Basil (*Ocimum basilicum* L.) is one of the traditional medicinal plants commonly used by the community because it has properties such as control of bad breath and body, lethargic body, heartburn, decomposition menstrual period and the decay of breast milk [1]. The methanol extract of basil leaves also has the potential for antibacterial, anti-inflammatory, antioxidant and analgesic[2]. Essential oils can inhibit the growth of many types of harmful bacteria such as *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Staphylococcus typhimurium*[3]. Essential oils made by nanoemulsion can increase the antibacterial activity.

This is proven research by Agustinisari et al., 2017, which tested the antibacterial activity of the nanoemulsion of nutmeg oil in *E. coli* and *S. aureus* bacteria. The results obtained are that the nanoemulsion of nutmeg has a larger inhibition zone diameter against *E. coli* and *S. aureus* bacteria which is 12.10 mm and 11.62 mm compared to the nutmeg which is equal to 10.51 mm for *E. coli* and 10.19 for *S. aureus*[4]. The nanoemulsion is an emulsion with a particle size of between 50 and 500 nm, intermediate between the emulsion and the microemulsion [5]. According to other studies, the size of the nanoemulsion droplet is between 20 and 200 nm [6]. The small size of the particles can increase the interactions between the active compounds and the biological membranes, as well as their transfer. Also, the nanoemulsion has good kinetic stability that can be used in a variety of commercial
applications [7]. The nanoemulsion has a high stability against sedimentation because of its small size and is therefore widely used for pharmaceutical and cosmetic applications [8].

In this study, the isolation of essential oils was first performed by steam distillation and then the physical properties were tested. Secondly, the nanoemulsion process of basil oil was ultrasonically performed for one hour. The third was characterization by GC-MS to determine the components of basil oil and basil oil nanoemulsion. Fourth analysis with a particle size analyzer to determine the basil oil and nanoemulsion particle size. Basil oil and basil oil nanoemulsion were tested for their antibacterial activity against *Staphylococcus aureus* (G+) and *Escherichia coli* (G-) bacteria with disk diffusion method. By comparing the antibacterial activity of basil oil and the nanoemulsion, we can see the effect of droplet size of basil oil on the inhibition of bacterial growth. With nanoscale size, it is expected that bioactive compounds in essential oils will penetrate the walls of bacterial cells more easily so that the inhibitory zones produced are larger than those of basil oil.

2. Material and methods

2.1. Tools and materials

Materials used in this study include cut and dried basil plants, anhydrous sodium sulfate (Merck), Tween 20 solvent (Merck), distilled water, Whatmann filter paper No. 42, nutrient agar (Merck), peptone (Merck), yeast extract (Merck), amoxiline, 70% alcohol, Gram-positive bacterium *Escherichia coli* and Gram-negative bacterium, *Staphylococcus aureus*.

The tools used in this study include laboratory glassware, a set of distillation equipment, ultrasound, pyrenometers (Pyrex), refractometers (ATAGO), Petri dishes, tweezers, needles for ose, spreaders, aluminum foil, plastic wrap, analytical plastic wrap, Memert IN55 incubator, 1-10 μL micropipette, 10-100 μL micropipette, Laminar Air Flow (LAF), Shidamadzu UV-1280 UV-Vis spectrophotometer (ultraviolet) visible, GC-MS and Particle Size Analyzer (PSA).

2.2. Sample preparation

Research samples of basil obtained in Semarang. The leaves and stems are cleaned with water, cut into small pieces and dried in the air without the sun.

2.3. Isolation of essential oils

Isolation of essential oils by steam distillation. The dried basil plants are first weighed, then placed in a distillation apparatus filled with water up to the filter boundary, and then the distillation equipment is installed and heated. The distillation process is carried out for 5 hours[9]. The essential oil obtained is placed in a bottle while the fraction of water obtained is removed. The application of the essential oil of basil is done by adding anhydrous sodium sulfate to separate the still mixed oil with water, then filtered using a filter paper [10]. The obtained essential oil is stored in the refrigerator in a dark and well-closed bottle to determine the yield, physical properties, characterization and antibacterial activity test [3].

2.4. Physical properties of essential oils

Physical properties determined for basil essential oil (*Ocimum basilicum*L) include specific gravity tests and refractive index[11].

2.5. Test solutions of basil oil and basil oil nanoemulsion

The essential oil and nanoemulsion test solutions were prepared by dissolving 6.25 mL of essential oil with distilled water and adding 1.25 mL of Tween 20 on a 25 mL base at a concentration of 25%, then diluted to 20%, 15%, 10% and 5% [10]. Manufacture of O / W emulsion systems using surfactants with HLB values ranging from 8 to 18[12]. The Tween 20 nonionic surfactant is used to formulate the nanoemulsion with a mean value of 16.7 HLB (hydrophilic-lipophilic balance) which is usually used for the manufacture of oil-in-water nanoemulsion (o/w) [13]. The nanoemulsion test solution was then sonicated using a Volts AC 200-240 V, 50/60 Hz ultrasonic cleaner. The ultrasonication process was performed for 1 hour at 50 ° C [14].
2.6. Characterization of basil oil and basil oil nanoemulsion

Characterization of basil oil and nanoemulsion of basil oil using GC-MS to determine the content of chemical compounds in basil oil and nanoemulsions [3]. Characterization of basil oil and basil oil nanoemulsion using Particle Size Analyzer (PSA) to determine the particle size [13].

2.7. Antibacterial activity test

2.7.1. Making stock of escherichia coli and staphylococcus aureus bacteria. Making the stock of E. coli and S. aureus bacteria is done by making the media to be tilted. 0.6 g of agar-agar, 0.075 g of peptone, and 0.015 g of yeast extract were dissolved in 30 mL of distilled water. A total of 10 mL of media was poured each into 3 test tubes and then UV in the LAF (Laminar Air Flow) and allowed to solidify at room temperature with the test tube position tilted 30 °C for a flat plane. After solidification, the test bacteria will be inoculated on the media so that the slant is taken using a sterile ose needle. The process of bacterial inoculation on the sloping media is carried out in Laminar Air Flow. Incubation of bacterial inoculation on sloping agar media was carried out at 37 °C for 18-24 hours. The same treatment was carried out on E. coli and S. aureus[15].

2.7.2. Making bacterial suspension test. Preparation of the bacterial suspension was carried out by dissolving 0,125 g peptone and 0,025 g of yeast extract into 50 mL of distilled water. The bacterial stock that has been incubated for 24 hours is taken one scratch using a sterile ose needle and then suspended in a sterilized liquid media. The bacterial suspension was incubated in a shaker incubator for 3 hours then measured at a wavelength of 600 nm to obtain the same turbidity as a standard solution of 0,5 Mc scale. Farland ie with an absorbance of 0,08 1. The turbidity standard of Mc. Farland is intended to replace the bacterial calculations one by one to estimate cell density that will be used in testing [15].

2.7.3. Making test media. The nutrient medium agar was prepared by dissolving 2 g of agar nutrient, 0,25 g of peptone and 0,05 g of yeast extract in 100 ml of distilled water. The mixture is homogenized by stirring and then sterilized by autoclaving for 45 minutes. A total of 20 ml of the nutrient medium should be poured into a Petri dish and allowed to condense. Then, not less than 75 μL of the bacterial suspension having reached the turbidity of a standard solution of a concentration of 0,5 Mc. Farland was then inoculated into a nutrient agar medium that had solidified. Test media are used to test in vitro antibacterial activity[15].

2.7.4. Testing antibacterial activity. A total of 5 μL of the test solution consists of essential oil of basil; nanoemulsion of essential oil solution; Tween 20 as a negative control; The amoxicillin solution as a positive control was dripped onto a paper disk which had been placed on the surface of the test medium and allowed to stand for about 15 minutes until the solution of test diffuse completely. The essential oils of basil and nanoemulsion used are concentrations of 5%, 10%, 15%, 20%, and 25%. Test media were incubated at 37 °C for 24 hours. Antibacterial activity was observed at 12 and 24 hours of incubation by measuring the clear area formed around the disc paper. A null inhibition zone means that no inhibition zone has formed around the paper of the disk. Partial inhibition zone means that the inhibition zone formed is always a visible bacterial growth. The zone of total resistance means that the zone of resistance formed around the paper of the disk is disengaged. The diameters of the inhibition zones are measured using a ruler in millimeters [15].

3. Results and discussion

3.1. Isolation of essential oil

In the distillation process, it can simultaneously produce volatile water vapor and oil, even if the differences in the boiling point of oil and water are high. The distillation is carried out for 5 hours. The resulting oil will be transported by steam and condensed in a cooler, then the results of distillation as water and essential oils will be stored in the filtrate, then the water will be separated from the essential
oil by the valve from denstrat slowly. The resulting oil is dark yellow and has a distinctive aroma. The essential oil obtained is then stored in a bottle covered with aluminum foil and placed in the refrigerator. The distillation is carried out fifteen times with the total of the ingredients used and the results obtained are presented in Table 1.

| Material Weight (grams) | The volume of essential oil(mL) | Weight of essential oil (gram) | Oil yield (%) |
|-------------------------|--------------------------------|--------------------------------|---------------|
| 5297.24                 | 14                             | 12.154                         | 0.22          |

In previous research, the isolation of essential oils from basil leaves and stems from Muscat, Oman, yielded 0.6% [3]. The isolation of Omani basil oil by other studies has yielded an essential oil yield of 0.171% [9]. Boyolali basil oil yielded 0.0967% [16]. There is a difference in yield of basil essential oil produced due to differences in geographical conditions and the environment in which basil plants grow so that the essential oil obtained is different [3].

3.2. Determination of the physical properties of essential oil

The test results are presented in Table 2.

| Parameter                     | The isolate of basil oil                     |
|-------------------------------|----------------------------------------------|
| Color                         | Dark yellow                                  |
| Smell                         | Typical basil plants                         |
| Spesific gravity, 25 °C (gram / mL) | 0.9                                          |
| Revractive index, 25 °C       | 1.487                                        |

In another study, essential oils with a refractive index of 1.486 and a specific gravity of 0.926 g / mL at a temperature of 20 ° C were produced [17]. The results of the analysis do not differ much from previous studies.

3.3. Making basil oil nanoemulsion

Producing nanoemulsions using a type of oil-in-water (O / W) emulsion, this type of emulsion is used to protect various types of lipophilic bioactive components as antibacterial, antitumor and anti-inflammatory agents [18]. The O / W emulsion system comprises an internal oily phase (up to 55%) and an external aqueous phase. This system must use emulsifying agents that are hydrophilic and can be ionic or nonionic [19]. The results of making a nanoemulsion of basil essential oil with five concentration variations (5%, 10%, 15%, 20%, and 25%) using the ultrasound method gave a more hazy solution because the particles have dispersed in colloidal conditions concerning basil essential oil. The results of the nanoemulsion of essential oil of basil are illustrated in Figure 1.
During the manufacture of an ultrasonic nanoemulsion, a cavitation process occurs, namely the formation of thermal energy bubbles and ultrasonic waves propagating in the aqueous phase. Fine droplets of oil form spontaneously when the organic phase containing the surfactants is mixed with the aqueous phase. The hydrophilic surfactant passes from the organic phase to the aqueous phase. When bubbles form, a collection of essential oil particles of basil adheres to the surface of the bubble. When the bubbles reach their maximum size, they burst and disperse the particles into smaller particles. They disperse completely in the presence of Tween 20 as a stabilizer. The Tween 20 molecule binds to the surface of the colloidal particles and protects the surface of the junction with other precursor particles.

3.4. The particle size of basil oil and basil oil nanoemulsion

The results of the particle size of basil oil and basil oil nanoemulsion with PSA are shown in table 2.

| Particle size results of basil oil and basil oil nanoemulsion |
|---------------------------------------------------------------|
| Basil oil                        | Particle Size (nm) |
| Before ultrasonication          | 54960              |
| After ultrasonication           | 243.4              |

Table 2 shows the size distribution of basil essential oil is 54960 nm and the size of the nanoemulsions is 243.4 nm. The resulting measurements indicate that basil essential oil has become a nanoemulsion of basil essential oil. The nanoemulsion particle size is in the range of 50-500 nm. According to other studies, the size of the nanoemulsion droplet is 20-200 nm.

3.5. Identification of basil oil components and basil oil nanoemulsion

The results of the analysis of basil oil obtained GC chromatograms as in Figure 2 below.
Figure 2. GC Chromatogram of basil oils

The resulting chromatogram showed 27 peaks that showed that in the essential oil of basil 27 chemical compounds had been identified. Based on the chromatogram, the five basil oil compounds with the highest percentage were found to be sabinene (60.01%), myrcene (17.76%), trans-caryophyllene (4.08%), linalool (2.58%), and alpha-pellandrene (2.35%). The results of the basil oil nanoemulsion chromatogram differ slightly from those of the chromatogram of basil oils before nanoemulsion. The results of the analysis of the nanoemulsion components obtained by chromatograms are illustrated in Figure 3 below.

Figure 3. Chromatogram GC of basil oil nanoemulsion

The resulting chromatogram showed 18 peaks, which shows that in the basil oil nanoemulsion, there were still 18 chemical compounds. Based on the chromatogram, there were five essential oil nanoemulsion compounds of basil which had the highest percentage of compounds of sabinene (44.68%), myrcene (17.86%), trans-caryophyllene (8.15%) and terpineol-4 (6.65%), and linalool (4.89%). The component is the main component that was previously present in basil essential oil. Components of compounds that were previously present in basil essential oil with small presentations, after becoming nanoemulsion, the compound content was not identified. This is because the application of ultrasonic waves to a solution causes the molecules to oscillate in the solution to their average position. The solution is extensive and dense. When the ultrasonic energy supplied is sufficiently large, the wave deformation can break the molecular bonds between the solutions [21].

3.6. Test of antibacterial activity
Antibacterial activity test of basil oils and basil oil nanoemulsion on *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive) bacteria. In this research, a disk diffusion method was
carried out, paper discs containing basil oils and basil oil nanoemulsion placed on the surface of a solid medium previously inoculated by test bacteria on the surface of the medium. In the diffusion method, if the antibacterial can inhibit bacterial growth, a clear area will appear around the disc paper. The samples used are essential oils and nanoemulsion of essential oils with five concentration variations, namely 5%, 10%, 15%, 20%, and 25%. The positive and negative controls used are amoxiline and tween 20. The results of the antibacterial activity test for essential oils and the nanoemulsion of essential oils are presented by measuring the diameter of the inhibitory zone of the compound (mm) shown in Table 3.

**Table 4. Inhibition zone diameter of antibacterial activity of basil oils and basil oil nanoemulsions**

| Inhibitor zone (mm) | Basil oil | Basil oil nanoemulsion |
|---------------------|-----------|------------------------|
|                     | S.aureus  | E.coli                 | S.aureus  | E.coli |
| Positive control    | 8         | 22                     | 8         | 22     |
| Negative control    | -         | -                      | -         | -      |
| 5%                  | 5         | -                      | 7         | 2      |
| 10%                 | 8         | 2                      | 9         | 4      |
| 15%                 | 12        | 4                      | 16        | 6      |
| 20%                 | 14        | 5                      | 19        | 7      |
| 25%                 | 21        | 7                      | 28        | 11     |

Table 3 shows that the highest antibacterial activity of *S.aureus* and *E.coli* bacteria at 25% concentrations for basil oil or basil oil nanoemulsion. This is because the higher the concentration, the higher the content of the essential oil compound. Inhibition zones formed on the media can be categorized as presented in table 4 below.

**Table 5. Categories of bacterial growth obstacles response based on inhibition zone diameter**

| Inhibition zone diameter | Obstacle Response |
|--------------------------|-------------------|
| ≤ 5 mm                   | weak              |
| 6-10 mm                  | moderate          |
| 11-20 mm                 | strong            |
| ≥ 21 mm                  | very strong       |

When linked to Table 4, the bacterium *Escherichia coli*, the diameter of the zone of inhibition of essential oils at concentrations of 10% and 15% are classified as low and the concentrations of 20% and 25% at concentrations moderate. Zones of inhibition of nanoemulsions of essential oils at concentrations of 5% and 10% were classified as low and concentrations of 15%, 20%, and 25% were classified as being strong. In *Staphylococcus aureus* bacteria, the diameter of the zone of inhibition of essential oils at a concentration of 5% is classified in the low category, for a concentration of 10% in the moderate category, 15% and 20%, and 25% in the strong category. Inhibition zone diameters in basil essential oil nanoemulsions at concentrations of 5% and 10% were classified as moderate, while 15% and 20% were categorized as strong, 25% very strong category. The basil oil nanoemulsion showed good physical stability, with few signs of phase separation. Due to the small size of the droplets, the nanoemulsions containing the essential oils penetrate the cell membrane of the bacteria more easily. This allows the hydrophobic molecules contained in the essential oils to damage the cell membranes by altering the integrity of the phospholipid bilayer or by interfering with the active transport proteins included in the phospholipid bilayer [7]. Changes in the permeability of the disrupted cell membrane cause leakage of nucleic acids, proteins, and potassium from within the bacterial cell, rendering the cell membrane unstable, thereby preventing the growth of the bacterial...
cells [4]. The comparison of the antibacterial activity of essential oils and nanoemulsions shows that nanoemulsions are much more effective. Small nanoemulsion particles can bring essential oils to the surface of bacterial cell membranes, while essential oils (which have low water solubility) can not easily interact with cell membranes because of their larger size than that of nanoemulsions [7]. The content of sabinene, myrcene, and trans-caryophyllene in oils and nanoemulsions of basil essential oil, which is a group of terpenoid compounds, plays a role in antibacterial activity. The chemical structure of the compound is illustrated in Figure 4.

![Chemical structure terpenoids of basil oil](image)

**Figure 4.** Chemical structure terpenoids of basil oil

The hydrophobic nature of the compounds contained in the essential oils can damage the cell wall and the cytoplasm of the bacteria due to increased permeability, which makes it difficult to separate the essential oils from the membranes of the bacterial cells. The hydrophobic nature of essential oils can penetrate bacterial cells and change their structure and function. The integrity of the cell membrane is very important for bacterial survival because it is a key element of biological activities that take place inside the cell. The membrane provides an effective barrier between the cytoplasm and the external environment when importing and exporting essential metabolites and ions for all microbial cell activities across cell membranes [23]. The inhibition zone diameter at 24 hours of E. coli essential oil bacteria and nanoemulsion can be seen in Figure 5.

![Inhibitor zone E.coli bacteria](image)

**Figure 5.** Inhibitor zone *E.coli* bacteria a. basil oil, b.basil oil nanoemulsion

Inhibition zone diameter of *S.aureus* bacteria is greater than *E.coli* bacteria both in essential oils and nanoemulsion of essential oils. Inhibition zones of *S. aureus* bacteria can be seen in figures 6 and 7.
Figure 6. a. inhibitory zone basil essential oil concentration of 5% and 25%, b. inhibition zone of nanoemulsion of essential oils of basil 5% and 25%

Figure 7. *S.aureus* inhibition zone diameter at concentrations of 10%, 15%, and 20%, a. basil oil, b. basil oil nanoemulsion
The inhibitory zones of positive controls for *E.coli* bacteria and *S.aureus* bacteria are shown in figure 9 as followed.

![Inhibitory zones](image)

**Figure 8.** a. inhibitory zones of positive controls for *E.coli*, b. inhibitory zones of negative controls for *S.aureus*

The difference in diameter of the zone of inhibition shows that the essential oils and the nanoemulsion of essential oils more effectively inhibit the growth of *S. aureus* (gram-positive) compared to *E. coli* (gram-negative). Gram-negative bacteria have a more complex cell wall structure than Gram-positive bacteria. The cell wall of the bacterium *E. coli* contains three layers, namely the outer layer of lipoprotein, the intermediate layer of lipopolysaccharide and the inner layer of peptidoglycan and the outer membrane in the form of a bilayer (better resistance to the compounds that enter and leave the cells and have toxic effects) [24]. This double cell wall prevents the diffusion of hydrophobic compounds such as essential oils of basil through lipopolysaccharides. The hydrophobicity of essential oils can degrade the lipids of bacterial cell membranes, damage the membrane structure, cause leakage of the cell membrane and ultimately inhibit bacterial growth [25].

4. Conclusion

Based on research that has been done it can be concluded that the essential oil obtained is dark yellow with a refractive index of 1.487, a specific gravity of 0.9 g / mL and a yield of 0.22% the chemical components of essential oils have 27 compounds identified with the largest components are sabinene (60.01%), myrcene (17.76%), trans-caryophyllene (4.08%), linalool (2.58%), and alpha-pellandrene (2.35%). Nanoemulsion of basil essential oil has 18 components, with the largest components being sabinene (44.68%), myrcene (17.86%), trans-caryophyllene (8.15%), terpineol-4 (6.65%), and linalool (4.89%), the particle size of nanoemulsion of basil essential oil is 243.4 nm and the polydispersity index is 0.335, the essential oil of basil, at a concentration of 10% to 25%, has a zone of low to medium protein inhibition at *E. coli* and is strongly directed towards *S. aureus*. The nanoemulsion of essential oils at a concentration of 5% to 25% has a moderate to strong inhibition zone in *E. coli* and a moderate to a very strong group in *S. aureus*. Need further research related to the application of basil essential oil nanoemulsion as an antibacterial.

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