Chemical Separation and Antibacterial Activity of Nutmeg seed Essential Oil against Shigella sp. and Escherichia coli ATCC 25922

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Abstract. The essential oil of the nutmeg seeds has antibacterial activity and contains some major compounds: sabinene, α-pinene, 2-β-pinene, myristicin, safrole, and 1-4-terpineol. This essential oil was extracted from the nutmeg seeds by separation process using the distillation method. The purpose of this study is to separate compounds in essential oils of nutmeg seed and to evaluate the antibacterial activity against two bacterial species. Nutmeg essential oils compound separation was conducted by distillation at 95 mmHg with different temperature 408, 428, 438, and 503 K. The separation results analyzed physical properties, chemical compounds with GC-MS, and the antibacterial activity test of Shigella sp. and Escherichia coli ATCC 25922 using dilution and diffusion method. The samples antibacterial activity were evaluated by the determination of minimum inhibitory concentration. The GC-MS analysis of nutmeg essential oils shows the presence of 25 compounds. Four fractions of fractional distillation under reduced pressure have 57.12%; 17.00%; 1.40%; and 19.68% of yield with different physical properties as well as the number of different compounds in each fraction. Fraction 1 has 14 compounds with α-pinene (27, 44%), sabinene (23, 78%), dan 2-β-pinene (18, 29%) as major compound. Fraction 2 has 15 compounds with sabinene (16, 18%), 2-β-pinene (14, 27%), β-phellandrene (12, 99%), dan γ-terpinene (14, 48%) as major compound. Fraction 3 has 12 compounds with α-terpinene (11, 24%), para-cymene (23, 74%), limonene (13, 23%), γ-terpinene (23, 46%), dan α-terpinolene (15, 01%) as major compound. Fraction 4 has 12 compounds with 1-4-terpineol (40, 65%), safrol (20, 34%) danmyristicin (16, 06%) as major compound. Results showed that MIC and MBC on Shigella sp. nutmeg essential oils had 12.11mm inhibitory effects at 1.25%; fraction 2 has 5.88mm inhibitory effects at 2.5%, and fraction 4 has 9.67mm inhibitory effects at 1.25%. MIC and MBC in Escherichia coli ATCC 25922 nutmeg essential oils have 10.89mm inhibitory effects at 2.5%; fraction one has 10.22mm inhibitory effects at 10%, and fraction 4 has 10.11mm inhibitory effects at 2.5%.

Keywords—Nutmeg essential oil, Separation compounds of nutmeg essential oil, Antibacterial

1. Introduction
Nutmeg essential oil can be used as an antibacterial [1]. The main contents of nutmeg essential oil are myristicin (22.22%), 4-terpineol (14.45%), safrole (6.94%), sabinene (5.87%), α-pinene (5.45%), γ-limonene (3.88) [2]. Separation of compounds in nutmeg essential oil can be done by fractionation at low pressure [3]. Another method that can be applied is the process of steam distillation [4] [5].
Diarrhea is one of the health problems in the community, and the cause is often found in the field of clinical diarrhea caused by infection and poisoning [6]. *Shigella* sp and *Escherichia coli* ATCC 25922 is a bacterial infection-causing bacteria. Society general treating diseases frequent use of antibiotics synthesis. Overuse of antibiotics and less directed can cause resistance to microorganisms.

One alternative to reduce the consumption of synthetic antibiotics is by consuming natural antibiotic derived from plants. One of the plants that potential as natural antibiotics are nutmeg because it has some terpenoids and phenolic compounds that can be applied as antibacterial [7][8]. Separation of compounds Separation in the essential oil of nutmeg using fractional distillation method under reduced pressure. Analysis of nutmeg essential oil content and results of separation were performed using GC-MS and test the antibacterial activity of essential oils of nutmeg fraction of the bacteria *Shigella* sp. and *Escherichia coli* ATCC 25922 the diffusion and dilution methods.

2. **Method**
   2.1. **Chemical separation**
   Nutmeg essential oil separation performed at 0.125 atm pressure with temperature differences 408, 428, 438, and 503K.

2.2. **Identification of essential oil fractions and fractions nutmeg essential oil nutmeg**
   Essential oils of nutmeg and compound fractions were analyzed by GC-MS with the condition of the columns used is the type of RTX-5ms with programmed column temperature of 70-250°C with an increase of 2 °C / min and using an FID detector [9][10], all sample analyze with the same method.

2.3. **Antibacterial activity test**
   Essential oils of nutmeg and nutmeg essential oil fractions tested the antibacterial activity using diffusion and dilution method at a concentration of 20; 10; 5; 2.5; and 1.25% with aqua dest solvent and addition of tween 80 Starting antibacterial testing as 100μL.

3. **Results and Discussion**
   3.1. **Chemical separation**
   Nutmeg essential oil testing physical properties, the results are as Table 1. Comparison test the physical properties of essential oils of nutmeg with nutmeg oil quality requirements SNI. It tells us that the sample is good enough to use for this research. The main focus in this study remains to dig up information on how to separate chemical compounds in nutmeg essential oils in the best way, so of course, the number of compound components found in nutmeg essential oil becomes the determinant, compared to the physical properties which are focused on SNI.

| type of test          | Nutmeg oil quality requirements | Essential oils of nutmeg |
|----------------------|---------------------------------|--------------------------|
| Color                | Bening-yellow                   | Bening yellowish         |
| Smell                | Typical nutmeg oil             | Typical nutmeg oil       |
| Solubility in ethanol| 1: 1-1: 3                      | 1: 1                     |
| Specific gravity     | 0.880 to 0.910                 | 0.903                    |
| The refractive index | 1.475 to 1.485                 | 1.476                    |
| The content myristicin| At least 10%                   | 16.34%                   |

The comparison showed that the quality of essential oil of nutmeg to meet the standard requirements of ISO nutmeg oil.

Nutmeg essential oil fractionation is done using fractional distillation under reduced pressure. The principle of this separation is the difference in the vapor pressure of each component that causes the boiling point. Pressure reduction used to be the compounds that want to be separated to evaporate at a lower boiling point and to avoid the use of heating is too high.
At pressures 0.125 atm, four fractions were obtained as shown in Table 2. It was observed that fraction 1 has a high yield, as predicted by the author, it could cause a major compound composition with a lower boiling point like pinene and sabinene.

![Figure 1. Results GC essential oils of nutmeg, fraction 1, fraction 2, fraction 3, and fraction 4.](image)

The separation process which is carried out starting from low to high temperatures also allows this compound to still be present in the second fraction, and this can also be caused by unstable heating temperatures that use cooking oil as a conductor of heat. The author suggests that refractive index get better results than this.

| No. | Fraction | Heating temperature (K) | The yield (%) |
|-----|----------|--------------------------|---------------|
| 1.  | fraction 1 | 408                      | 57.12         |
| 2.  | fraction 2 | 428                      | 17:00         |
| 3.  | fraction 3 | 438                      | 1:40          |
| 4.  | fraction 4 | 503                      | 1968          |

3.2. Identification of essential oil fractions and fractions nutmeg essential oil nutmeg.

The results of the analysis of physical properties testing compared with the test results of Nutmeg essential oils is as follows:
Table 3. Comparative testing physical properties of the fraction 1 to fraction 4 with essential oils of nutmeg

| Essential oils of nutmeg | fraction 1 | fraction 2 | fraction 3 | fraction 4 |
|--------------------------|-----------|-----------|-----------|-----------|
| Color                    | Bening    | Clear     | Clear     | Bening    |
|                          | yellowish | Typical   | Typical   | yellowish |
| Smell                    | Typical   | Typical   | Typical   | Typical   |
|                          | nutmeg oil| nutmeg oil| nutmeg oil| nutmeg oil|
| Solubility in ethanol    | 1:1       | 1:1       | 1:1       | 1:1       |
| Specific gravity         | .903      | 0.841     | .842      | -         |
| The refractive index     | 1,476     | 1,471     | 1,470     | 1,475     |
|                          |           |           |           | 1,220     |

Table 4. The result of the separation of compounds

| No. | Compound name         | Retention Time (Minutes) | Nutmeg Seed Essential Oil Composition (%) |
|-----|-----------------------|--------------------------|------------------------------------------|
|     |                       |                          | fraction 1 | fraction 2 | fraction 3 | fraction 4 |
| 1.  | 2-pentene             | 1.52                     | 0.23       | 0.22       | 0.23       |
| 2.  | α-thujene             | 3.52                     | 6.45       | 0.91       |
| 3.  | α-pinene              | 3.69                     | 17.60      | 27.44      | 7.15       |
| 4.  | Camphene              | 3.96                     | 0.47       |            |
| 5.  | Sabinene              | 4.44                     | 16:39      | 23.78      | 16:18      |
| 6.  | 2-β-pinene            | 4.56                     | 11.89      | 18.69      | 14:27      |
| 7.  | β-myrcene             | 4.74                     | 2:08       | 2:14       |
| 8.  | 1-phellandrene        | 5.16                     | 1.49       | 2.87       | 1:46       |
| 9.  | Delta-3-Caren         | 5.31                     | 2.13       | 3.42       | 0.77       |
| 10. | α-terpinene           | 5.44                     | 4.71       | 9:31       | 11:24      |
| 11. | para-cymene           | 5.61                     | 1.71       | 7:03       | 23.74      |
| 12. | Limonene              | 5.72                     | 5:24       | 13:23      |
| 13. | β-phellandrene        | 5.74                     |            | 12.99      |
| 14. | γ-terpinene           | 6.32                     | 14:48      | 23:46      |
| 15. | Trans-sabinene        | 6.47                     | 0.45       | 0.76       |
| 16. | α-terpinolene         | 6.84                     | 5.94       | 15:01      |
| 17. | Bisiklo [2.2.1] heptane-2-ol | 8.05   |            |            | 0.96       |
| 18. | 1-4-terpinolene       | 8.17                     | 6:16       | 2.63       | 8.91       | 40.65      |
| 19. | 3-cyclohexane-1-methanol | 8.34   |            |            | 0.63       | 9.95       |
| 20. | Safrole               | 9.44                     |            | 0.57       | 20.34      |
| 21. | Citronellyl Acetate   | 9.96                     |            |            | 0.62       |
| 22. | Eugenol               | 10.01                    |            |            | 1.92       |
| 23. | Geraniol acetate      | 10.23                    |            |            | 0.95       |
| 24. | α-copaene             | 10.29                    |            |            | 5:49       |
| 25. | Methyl eugenol        | 10.44                    |            |            | 0.81       |
| 26. | Isoleuugenol          | 10.86                    |            |            | 1.60       |
| 27. | Myristicin            | 11.43                    | 16:34      |            |            |
| 28. | Cis-asaron            | 11.58                    |            |            | 0.65       |

**INFORMATION:** FIGURES RETENTION TIME TO TWO BEHIND THE DIFFERENCES IN EACH SAMPLE.

The results of the analysis of the nutmeg essential oil content and fractions, as in figure 1, the same retention time shows the same compound, because the analysis is done by the same method for all samples.
3.3. **Antibacterial Activity Testing**

Antibacterial activity test conducted by diffusion and dilution methods. The bacteria used are *Shigella* sp with SSA media and *Escherichia coli* ATCC 25 922 with EA media.

**Table 5.** The test results diffusion and dilution *Shigella* sp and *Escherichia coli* ATCC 25 922

| Compound     | Trial | Diffusion Test | Dilution Test |
|--------------|-------|----------------|---------------|
|              |       | *Shigella* sp  | *Escherichia coli* ATCC 25 992 | *Shigella* sp  | *Escherichia coli* ATCC 25 992 |
| Essential oils of nutmeg | 20%  | 11.67          | 9.00          | -              | -              |
|              | 10%  | 11.00          | 8.56          | -              | -              |
|              | 5%   | 12.22          | 10.56         | -              | -              |
|              | 2.5% | 11.00          | 10.89         | -              | -              |
|              | 1.25%| 12.11          | 0             | +              | +              |
| control +    | 15.55| 14.11          | +             | +              | +              |
| control -    | 0    | 0              | -             | -              | -              |
| Fraction 1   | 10%  | 0              | 10.22         | +              | -              |
|              | 5%   | 0              | 0             | +              | +              |
|              | 2.5% | 0              | 0             | +              | +              |
|              | 1.25%| 0              | 0             | +              | +              |
| control +    | 15.22| 16.45          | +             | +              | +              |
| control -    | 0    | 0              | -             | -              | -              |
| Fraction 2   | 10%  | 8.55           | 0             | -              | +              |
|              | 5%   | 7.22           | 0             | -              | +              |
|              | 2.5% | 7.89           | 0             | -              | +              |
|              | 1.25%| 5.88           | 0             | +              | +              |
| control +    | 15.22| 15.89          | +             | +              | +              |
| control -    | 0    | 0              | -             | -              | -              |
| Fraction 4   | 10%  | 9.89           | 10.01         | -              | -              |
|              | 5%   | 7.89           | 9.78          | -              | -              |
|              | 2.5% | 8.56           | 10.11         | -              | -              |
|              | 1.25%| 9.67           | 0             | -              | +              |

**INFORMATION:**

**DIFFUSION:**
- **CONTROL +**: DISC DISCS GENTAMICIN
- **CONTROL -**: DISC DISK + SOLVENT

**DIFFUSION:**
- **POSITIVE CONTROL**: BACTERIAL CULTURE
- **NEGATIVE CONTROL**: SOLVENTS
- **+: NO BACTERIAL GROWTH**
- **-**: NO BACTERIAL GROWTH

The result of dilution and diffusion addressed that there is an excellent antibacterial activity in fraction 4 containing compound propanoierterpenoids and phenyl which is the phenol group, as compared with the fraction one and fraction two containing always compounds terpenoids. The results of the best antibacterial activities contained in the essential oils of nutmeg compared with the fraction four due to their compound and phenylpropanoidterpenoids which are groups of phenols and terpenoids more complex compounds. Terpenoids as an antibacterial mechanism are reacted with Porin (transmembrane proteins) on the outer membrane of the bacterial cell wall, forming an active bond polymer destroying Porin. Damage Porin which is the entry and exit of the compounds will reduce the permeability of the bacterial cell wall which will result in a bacterial cell would be a lack of nutrients,
thereby inhibited bacterial growth or death [11]. Phenolic compounds may function as an antimicrobial for their OH groups that are toxic to microbes. The mechanism of antibacterial phenolic compounds at low concentrations in the cytoplasmic membrane damage and can lead to leakage of the cell nucleus, whereas at high concentrations of phenolic compounds with cellular proteins coagulate. Activity is beneficial when the bacteria in the cleavage stage wherein the phospholipid layer around the cell is in a state that is so thin that it can easily damage the phenol content of the cell [12]. Essential oils of nutmeg and Fraction have better antibacterial activity shown in the bacteria Shigella sp. compared with Escherichia coli ATCC 25 922.

4. Conclusion
Based on the research conclusions can be drawn with the dominant compound in the fraction 1 contained three compounds, namely α-pinene (27.44%), sabinene (23.78%), and 2-β-pinene (18.69%); 2 there are 4 compound fraction that is sabinene(16.18%), γ-terpinene (14.48%), 2-β-pinene (14.27), and β-phellandrene (12.99%); 3 fractions which contained the compound 5 cymene (23.74%), γ-terpinene (23.46%), α-terpinolene (15.01%), limonene (13.23%), and α-terpinene (11, 24%); 4 fractions contained 3 compound is 1-4-terpineol (40.65%), safrole (20.34%), and myristicin (16.06%). Essential oils of nutmeg possess antibacterial activity in bacteria Shigella sp. and Escherichia coli ATCC 25 922IM ore fraction better than the target because the essential oil compounds are much more complicated so much better against bacterial.

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