Antioxidant activity of volatile compounds from *Syzygium aromaticum* (L.) leaves

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**Abstract.** *Syzygium aromaticum* (L.) leaf essential oil have been identified as antioxidants. This study aimed to determine the volatile compounds and antioxidant activities of *Syzygium aromaticum* (L.) leaf essential oil. *Syzygium aromaticum* (L.) leaves were extracted by steam distillation. The identification of volatile compounds was done by Gas Chromatography Mass Spectroscopy (GC-MS). DPPH test was used to evaluate the sample’s antioxidant activity. The results provide information that the extract have 15 of volatile compounds from *Syzygium aromaticum* (L.) leaf essential oil. Moreover, DPPH test has identified a very strong antioxidant by IC₅₀ values was 8.224 µg/mL.

1. Introduction

Indonesia is named as the world's largest clove producer and consumer. Approximately 95% of clove plants (*Syzygium aromaticum* (L.)) in Indonesia are cultured by smallholder plantations throughout the province. *Syzygium aromaticum* (L.) is a spice plant that has long been used in the tobacco, food and beverage industries [1]. At large, the most commonly used parts of *Syzygium aromaticum* (L.) are leaves, flowers, and stem. The *Syzygium aromaticum* (L.) leaves are used to medicate beriberi, abdominal pain, and impotence. The flowers are used to relieve colds, coughs, and eye diseases. The stem is used as building material, its characteristics are hard but if it comes into contact with the ground it becomes impermanent. *Syzygium aromaticum* (L.) is traditionally used as a medicine for toothache, mouthwash, digestive disorders such as bloating, aromatics, and stimulants [2].

*Syzygium aromaticum* (L.) can also be extracted into essential oils; clove leaf oil, clove stem oil, clove flower oil [3]. According to preceding research, *Syzygium aromaticum* (L.) leaf oil contains approximately 70-96% eugenol [4]. The eugenol compound (C₁₀H₁₅O₂), is a derivative of guaiacol with additional alkyl chain, also known as IUPAC 2-methoxy-4- (2-propenyl) phenol. Yoo Chae-Bin, Han Ki-Tae, reported that *Syzygium aromaticum* contains eugenol compounds and isoeugenol and acetyl eugenol [5]. Eugenol belongs to phenol compounds [6]. Phenolic compounds enclosed in a number of plants are considered as secondary metabolites which play a significant role in antioxidant activities [7].

The *Syzygium aromaticum* (L.) flower essential oil has been identified to have antioxidant activity. However, antioxidant activity has not been testified from the *Syzygium aromaticum* (L.) leaves [8]. Therefore, an identification of the volatile compounds contained in the *Syzygium aromaticum* (L.) leaf
essential oils is imperative to add more data and further studies need to be done on the antioxidant activity of the *Syzygium aromaticum* (L.) leaf essential oils.

2. Methods

2.1. Sample
The samples were clove leaves collected from Manoko Experimental Garden, Lembang, Bandung, which were confirmed as *Syzygium aromaticum* (L.) by Herbarium Bandungense, School of Biological Sciences and Technology, Bandung Institute of Technology. The stages of making simplicia encompassed wet sorting, washing, molding, drying, dry sorting, and simplicia grinding into powder. The *Syzygium aromaticum* (L.) leaf essential oil was then isolated by steam and water distillation methods.

2.2. Chemicals and instrumentation
The tools used were sets of distillation tools, analytic balance, ovens, filter paper, glass vials, Ultraviolet-Visible (UV-Vis) spectrophotometer (Shimadzu UV-120 A12065301569), Gas Chromatography-Mass Spectrophotometer (Shimadzu, ultra GCMS-QP 2010), Infra Red spectrophotometer (Thermo, NICOLET 380) and glass for chemical analysis.

The materials used included simplicia and essential oils from the *Syzygium aromaticum* (L.) leaves, aquadest, methanol pro analysis (p.a), toluene, ethanol 96%, dilute HCl, chlorophome, vitamin C, DPPH (2,2-Diphenyl-1-pyrylhydrazyl).

2.3. Methods

2.3.1. Identifying volatile compounds of essential oils. Identification of volatile compounds in essential oils obtained from the simplicia of *Syzygium aromaticum* (L.) leaves was executed at the Chemistry Education Laboratory (Indonesia University of Education) using a set of GC-MS tools consistent with the following conditions: initial column oven temperature 60 °C; injection temperature 280 °C; the samples were injected with split injection; and the carrier gas was Helium with 1.31 mL/minute flow rate. Infrared spectrum examination was performed on the essential oils from *Syzygium aromaticum* (L.) leaf using infrared spectrophotometer (IR) at the Laboratory of Instrument Chemistry, Faculty of Mathematics and Natural Sciences, University of Garut.

2.3.2. Antioxidant activity by DPPH radical scavenging assay. The antioxidant activity test was carried out by using the DPPH (2,2-Diphenyl-1-pyrylhydrazyl) method on the *Syzygium aromaticum* (L.) leaf essential oils and vitamin C was used as a positive control. Essential oils were prepared in a number of concentrations (7, 8, 9, 10 and 11 ppm). A blank solution was prepared by dissolving DPPH in methanol p.a without additional solution test. The test solution (2 mL) was added with 2 mL DPPH 0.1 mM. The mixture was strongly shaken and incubated for 30 minutes, then the absorbance was measured using a UV/Vis spectrophotometer at a wavelength of 516 nm. The Vitamin C was given the same treatment with various concentrations (1, 1.5, 2, 2.5, and 3 ppm). The absorbance value of DPPH solution before and after the addition of the samples was calculated as a percent of DPPH radical inhibition (% inhibition).

3. Results and discussion
This study used *Syzygium aromaticum* (L.) leaves taken from Manoko Experimental Garden in Lembang, Bandung, West Java. The leaves were then distilled to acquire the essential oils by steam distillation method. This process resulted in 70 mL of *Syzygium aromaticum* (L.) leaf essential oil. The distillation process yield is presented in Table 1.
Table 1. The *Syzygium aromaticum* (L.) leaf essential oil yield.

| Simplicia (gr) | Essential Oil (gr) | Yield % |
|---------------|-------------------|---------|
| 6000          | 72.548            | 1.2091  |

3.1. *Volatile compounds of essential oils*

The examination of the *Syzygium aromaticum* (L.) leaf essential oil component was carried out through the Chromatography Gas Mass Spectrometry (GC-MS) method. Table 2. shows the results of the examination of 15 volatile compounds in *Syzygium aromaticum* (L.) leaf essential oil. There are 4 dominant components including phenol, 2-methoxy-4- (2-propenyl) - (CAS) Eugenol (73.25%), trans-Caryophyllene, (19.43%), 3-Allyl-6-methoxyphenol (2.92%), and alpha.-Humulene (2.32%). The main compounds with the highest output are phenol, 2-methoxy-4- (2-propenyl) - (CAS) Eugenol (73.25%). The retention time required is 10.705 minutes and the the peak area is 73.25% with molecular formula C_{10}H_{12}O_{2}.

Table 2. Volatile compounds of *Syzygium aromaticum* (L.) leaf essential oils.

| Peak | Compounds | Retention Time | Area (%) |
|------|-----------|----------------|----------|
| 1    | 2-beta-Pinene | 4.252          | 0.02     |
| 2    | 6-Methyl-5-hepten-2-one | 4.349          | 0.05     |
| 3    | 3-Allyl-6-methoxyphenol | 10.522         | 2.92     |
| 4    | Phenol, 2-methoxy-4-(2-propenyl)-(CAS) Eugenol | 10.705         | 73.25    |
| 5    | beta.Elemene | 11.045         | 0.02     |
| 6    | Trans-Caryophyllene | 11.561         | 19.43    |
| 7    | Germacrene-D   | 11.655         | 0.03     |
| 8    | Alpha-Humulene | 12.058         | 2.32     |
| 9    | Cis-Caryophyllene | 12.556         | 0.04     |
| 10   | Farnesene     | 12.765         | 0.36     |
| 11   | 1-Cyclohepten, 1,4-dimethyl-3-(2-methyl-1-propene | 12.895         | 0.02     |
| 12   | delta-Cadinene | 13.070         | 0.04     |
| 13   | Phenol, 2-methoxy-4-(2-propenyl)-acetate (CAS) Aceteugenol | 13.131         | 0.74     |
| 14   | (-)-Caryophyllene oxide | 14.052         | 0.70     |
| 15   | Humulene oxide | 14.437         | 0.06     |

Based on the data, phenol, 2-methoxy-4- (2-propenyl)-(CAS) Eugenol mass spectrum (Figure 1.) are similar to the peak mass spectrum (SI) 93. It was displayed in Figure 2.

**Figure 1.** 2-methoxy-4-(2-propenyl)-(CAS) eugenol mass spectrum.

**Figure 2.** Peak mass spectrum (SI) 93.
The eugenol is the main component contained in the *Syzygium aromaticum* (L.) oil by 70-96%. Although *Syzygium aromaticum* (L.) oil contains some other components but the main one is eugenol compound, so the quality of clove oil is determined by the content of the compound. Higher eugenol content ensures better clove quality. Based on SNI 06-2387-2006, the minimum content of eugenol compounds is 78% [9]. The eugenol compound with molecular formula C_{10}H_{12}O_{2} contains several allyl functional groups (CH_2-CH = CH_2), phenol (-OH) and methoxy (OCH_3) as presented in Table 3.

| Wave Number (cm\(^{-1}\)) | Functional Group Prediction |
|-----------------------------|----------------------------|
| 3500-3200                   | O-H                        |
| 2270-1950                   | X=C=Y                      |
| 1680-1600                   | C=C                        |

The functional groups of the compounds found in the *Syzygium aromaticum* (L.) leaf essential oils were predicted from the IR spectrum examination results as shown in Figure 3.

![Infrared spectrum examination results of (*Syzygium aromaticum* (L.)) leaf essential oil.](image)

3.2. Antioxidant activity

Previous studies revealed that eugenol has a variation of biological activities, such as: antifungal, anticancer, and anti-inflammatory. Another use of eugenol is that it can be used as an antioxidant [11,12]. The antioxidant activity test was carried out by using the 2,2-Diphenyl-1-pyrylhydrazyl (DPPH) method and vitamin C was used as a positive control. The principle of this method is decolorization to measure antioxidant capacity that reacts with DPPH free radicals by observing the decrease in color absorbance which shows the free DPPH amount due to the reduction reaction of antioxidants [13]. Test results on the standard of vitamin C test with concentrations of 1, 1.5, 2, 2.5 and 3 ppm resulted in % of DPPH radical inhibition by 33.078%, 40.025%, 46.526%, 54.111%, and 61.249%. The results of *Syzygium aromaticum* (L.) leaf essential oils with various concentrations of 7, 8, 9, 10, and 11 ppm discovered the % of DPPH radical inhibition by 44.094%, 48.547%, 54.174%, 58.936%, and 62.771%. The IC\(_{50}\) value of the standard vitamin C was 2,216 \(\mu\)g / mL while the test for *Syzygium aromaticum* (L.) essential oil was 8.224 \(\mu\)g / mL. The results of test on the antioxidant potential of Vitamin C and *Syzygium aromaticum* (L.) leaf essential oils is displayed in Table 4.
Table 4. Antioxidant Activities of *Syzygium aromaticum* (L.) leaf essential oils and vitamin C.

| Sample              | Concentration (ppm) | Absorbance (A) | Absorbance Control (A) | Inhibition Percentage (%) | IC₅₀ (µg/mL) |
|---------------------|---------------------|----------------|------------------------|---------------------------|-------------|
| Clove Leaf Essential Oils | 7                   | 0.301          |                        |                           |             |
|                     | 8                   | 0.277          | 0.539                  |                           | 2,2165       |
|                     | 9                   | 0.247          |                        |                           |             |
|                     | 10                  | 0.221          |                        |                           |             |
|                     | 11                  | 0.201          |                        |                           |             |
| Vitamin C           | 1                   | 0.350          |                        |                           |             |
|                     | 1,5                 | 0.314          | 0.523                  |                           | 8,2240       |
|                     | 2                   | 0.279          |                        |                           |             |
|                     | 2,5                 | 0.240          |                        |                           |             |
|                     | 3                   | 0.203          |                        |                           |             |

The antioxidant activity of *Syzygium aromaticum* (L.) leaf essential oil is very strong because the level of antioxidant is deemed strong if IC₅₀ < 50 ppm, strong (IC₅₀ = 50-100 ppm), moderate (IC₅₀ = 100-150 ppm), and weak (IC₅₀ = 150-200 ppm) [13].

4. Conclusions

The results showed that there are 15 volatile compounds of *Syzygium aromaticum* (L.) leaf essential oils wherein phenol, 2-methoxy-4-(2-propenyl) - (CAS) Eugenol is the primary compound with 10.705 minute retention and 73.25% peak area.

The results of the antioxidant activity by using the DPPH assay on the antioxidant potential of *Syzygium aromaticum* (L.) leaf essential oils has revealed that there is a very strong antioxidant activity indicated by IC₅₀ values by 8.224 µg/mL.

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