Chemical Components of *Ocimum basilicum* L. and *Ocimum tenuiflorum* L. Stem Essential Oils and Evaluation of Their Antioxidant Activities Using DPPH Method

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**ABSTRACT**

*Ocimum basilicum* L. and *Ocimum tenuiflorum* L. are two types of plants from *Ocimum* (Lamiaceae). One of their chemical compounds is essential oil. Essential oil might have an antioxidant activities. The part of the plants which are often wasted and never used are stem. This study aimed to analyze the chemical components of *O. basilicum* L. and *O. tenuiflorum* L. stem essential oils and evaluate their antioxidant activities. Essential oils were obtained by steam-water distillation. Analysis of chemical component of essential oil was performed using Gas Chromatography-Mass Spectroscopy (GC-MS). Antioxidant activities were evaluated with DPPH method and then the IC$_{50}$ value was determined as 50% inhibition concentration of free radical. The results showed that *O. basilicum* L. stem essential oil had 13 components with the major compounds were methyl eugenol (52.60%), caryophyllene (18.75%), and germacrene-D (9.19%). Whereas, *O. tenuiflorum* L. stem essential oil had 11 components with the major compounds were α-copaene (5.56%), caryophyllene (17.28%), germacrene-D (9.29%) and methyl eugenol (56.72%). IC$_{50}$ value of *O. basilicum* L. stem essential oil was 17.50 µg/mL, whereas IC$_{50}$ value of *O. tenuiflorum* L. stem essential oil was 14.17 µg/mL. It was concluded that both oils might be good natural antioxidant agents.

*Keywords*: antioxidant; essential oils; *Ocimum basilicum* L; *Ocimum tenuiflorum* L; stem

**INTRODUCTION**

Four or five species of *Ocimum* spp. grow in Indonesia. Two of the five species are distinct and should be relatively easy to identify. Whereas, the other two (or three) species are more similar in appearance. *O. basilicum* L. can be identified because it has the largest flowers of any basil found in Indonesia. Stems and leaves are glabrous (smooth, without hairs) or only minutely hairy. *O. tenuiflorum* L. is often encountered under the synonym *O. sanctum* L. It has been described as a short-lived perennial and a dwarf shrub (or subshrub). It is much branched (Mead, 2014).

*Ocimum* tea is efficacious to expel gases, overcome stomach cramps, constipation, diarrhea and vomiting. It is also useful for treating mental fatigue, nervous conditions and hyssop for cough (Marwat et al., 2011). Indonesian people mostly use leaves of *O. basilicum* L. as fresh vegetables. *O. tenuiflorum* L. are also used as a spice in cooking because the aroma can reduce the fishy smell of wet fish. While the stem of this plant is often wasted and not used.

*O. basilicum* L. plant contains essential oils, triterpene, alkaloids, flavonoids, saponins, coumarin, steroids, gycoside and tannins. The essential oils contain monoterpane hydrocarbons, oxygenated monoterpane, sesquiterpene hydrocarbons and oxygenated sesquiterpene (Marwat et al., 2011). Whereas, the main chemical components in *O. tenuiflorum* L. are oleanolic acid, ursolic acid, rosmarinic acid, eugenol, carvacol, linalool and caryophyllene (Ravi et al., 2012).

Essential oils are natural plant products that have various biological properties. Essential oils contain volatile compounds (mainly mono- and sesquiterpenoids, benzenoids, phenylpropanoids, etc.) (Baser and Buchbauer, 2010). This oil is produced from certain parts of plant, such as roots, stems, skin, leaves, flowers and seeds (Gunawan and Mulyani 2004; Lutony and Rahmayati, 1994). Essential oils extracted from *Ocimum* plants have been applied to inhibit growth of microorganisms, in perfumery, in food preservation and also in aromatherapy (Pandey et al., 2014). A large number of studies on the antioxidant potential of essential oils are aimed at obtaining natural non-toxic antioxidants. Several compounds, such as eugenol, α-pinene, terpinene, phellandrene, etc., are example of chemical components of essential oils that are thought to be related to the antioxidant activity (Amorati et al., 2013).

Studying volatile constituent components can be done by profiling the oil metabolites using the gas chromatography-mass spectrometry method. In addition, the process of determining secondary metabolites with certain characteristics is related to the response and mechanism of a drug (Saifudin et al., 2011). Thus, this
study aimed to analyze the chemical components of *O. basilicum* L. and *O. tenuiflorum* L. stem essential oils and evaluate their antioxidant activities.

**METHODS**

**Plant Collection and Identification**

Samples were collected from the Indonesian Spices and Medicinal Crops Research Institute (ISMCRI)/Balai Penelitian Tanaman Rempah dan Obat (BALITTO), Bogor, West Java. All samples then were identified in Herbarium Bogorienne, Biology Research Center, Indonesian Institute of Sciences/Lembaga Ilmu Pengetahuan Indonesia (LIPI), Cibinong.

**Extraction of Essential Oils**

The extraction process was carried out in the BALITTO, Bogor, West Java. 12 kg of fresh *O. basilicum* L. stem and 20 kg of fresh *O. tenuiflorum* L. stem were extracted separately using the steam-water distillation for 6 hours. Essential oils were obtained then added with anhydride Na2SO4 to obtain pure essential oils. Essential oils were then analyzed for their characteristics including organoleptic parameters, yield percentages, specific gravity, refractive index and solubility in absolute alcohol.

**Analysis of Essential Oils**

Analysis of essential oils was carried out in the BALITTO, Bogor, West Java. 10 μL of the *Ocimum* essential oil were mixed with 1 mL of GC grade n-hexane. One μL of the mixture was analyzed with the Gas Chromatography with Auto Sampler (Agilent Technologies 7890), 5975 Mass Selective Detector and Chemstation data system. Separation was carried out using the capillary column (Innowax HP) with length of 30 m x 0.25 mm and film thickness of 0.25 μm. The carrier gas was helium gas at a constant flow rate of 0.6 mL/min. The injector temperature was set at 250 °C. The initial temperature of the oven was set at 60 °C then hold for 0 min, rising at 2 °C/min to 150 °C then hold for 1 min and finally rising at 20 °C/min to 210 °C then hold for 10 min. According to Herebian et al. (2009), the main compounds in the GC-MS chromatogram have criteria for percent area >5% of essential oils. The similarity of mass spectra of sample with a library was determined at a qualifier value of at least 80%. The components was identified based on compatibility with an authentic mass spectrum in Wiley electronic library.

**DPPH Radical Scavenging Activity**

The free radical scavenging activity assay using the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) reagent was determined according to Brand-Williams et al. (1995). 0.3 mL of 100 μg/mL of essential oil was reacted with 0.5 mL of DPPH 0.1 mM solution in ethanol and 1.5 mL of absolute ethanol. The solution was incubated in the dark at room temperature for 30 min. The decolorizing (from deep violet to light yellow) process was read at 516 nm using UV-VIS Spectrophotometer (Shimadzu) and compared with a blank control. For comparison, activities of Vitamin E standard was evaluated.

In the analytical procedure, the measurements were performed at four points of concentration, including control. The measurement of the DPPH radical scavenging activity of sample solution was repeated three times at each concentration. The results were expressed as percentage of decrease of DPPH radical. DPPH DPPH inhibition percentage is calculated using the following formula:

\[
\text{Inhibition ratio(%) = (absorbance of control - absorbance of sample) / absorbance of control) x 100%}
\]

The sample concentrations (x) were plotted against the inhibition ratios (y) and then the regression line equation \(y = bx + a\) was obtained. The calculation of 50% of the inhibition concentration of DPPH radical is obtained by entering 50 on y in the regression line equation, so x is the \(IC_{50}\) value.

**RESULTS AND DISCUSSION**

Extraction yields of *O. basilicum* L. and *O. tenuiflorum* L. stem essential oils are shown in Table 1. Oil contents (% v/w) in fresh stems of *O. basilicum* L. and *O. tenuiflorum* L. were 0.183% and 0.200%, respectively. Characteristics of essential oils are shown in Table 2. Chromatograms of *O. basilicum* L. and *O. tenuiflorum* L. stem essential oils are shown in Figure 1 and Figure 2. *O. basilicum* L. stem essential oil had 13 components. The major chemical constituents (>5%) from this oil were methyl eugenol (52.60%), carvophyllene (18.75%) and germacrene-D (9.19%). Whereas, *O. tenuiflorum* L. stem essential oil had 11 components with the major compounds were α-copaene (5.56%), carvophyllene (17.28%), germacrene-D (9.29%) and methyl eugenol (56.72%). Chemical constituents of the essential oils are shown in Table 3. *IC_{50}* values of DPPH radical from *O. basilicum* L. and *O. tenuiflorum* L. stem essential oils were 17.50 μg/mL and 14.17 μg/mL, respectively. Whereas, the *IC_{50}* value of Vitamin E was obtained at 30.89 μg/mL.

In term of composition, essential oil of the stem is different from essential oil of aerial parts. Essential oils from *O. basilicum* L. aerial parts from Clanjur, West Java were reported to contain 4.88% methyl eugenol. On the other hand, the essential oil of *O. tenuiflorum* L. has a content of methyl eugenol of 8.69% (Sulianti, 2008). *O. basilicum* essential oil from China, Croatia, Israel, Republic of Guinea, Nigeria, Egypt, Pakistan and Malaysia was reported having a major component
Table 1. Extraction yield of *O. basilicum* L. and *O. tenuiflorum* L. stem essential oils

| Sample           | Weight of fresh stem (Kg) | Volume of essential oil (mL) |
|------------------|---------------------------|-----------------------------|
| *O. basilicum* L | 12                        | 22                          |
| *O. tenuiflorum* L | 10                       | 20                          |

Table 2. Characteristics of *O. basilicum* L. and *O. tenuiflorum* L. stem essential oils

| Parameters          | Essential Oils | Essential Oils | Essential Oil Association of *O. basilicum* (Hadipoentyanti and Sri, 2018) |
|---------------------|----------------|----------------|-------------------------------------------------------------------------|
|                     | *O. basilicum* L. stem | *O. tenuiflorum* L. stem |                                                                           |
| Organoleptic        |                 |                 |                                                                          |
| Shape               | Liquid          | Liquid          | -                                                                       |
| Colour              | Brownish yellow | Pale yellow     | Light yellow                                                            |
| Odor                | Specific        | Specific        | -                                                                       |
| Taste               | Tighten         | Bitter          | -                                                                       |
| Yield percentage    | 0.183% (v/w)    | 0.200% (v/w)    | -                                                                       |
| Refractive index    | 1.2906          | 1.3493          | 1.510-1.5165                                                           |
| Specific gravity (g/mL) | 0.98054       | 1.0108          | 0.952-0.973                                                            |
| Solubility in absolute alcohol | 1:1 (soluble) | 1:1 (soluble) | 4:1 (soluble)                                                         |

Figure 1. Chromatogram of *O. basilicum* L. stem essential oil

Figure 2. Chromatogram of *O. tenuiflorum* L. stem essential oil
Table 3. Chemical components of O. basilicum L. and O. tenuiflorum L. stem essential oils

| Samples                        | Rt   | Quality | Compounds       | Percentage (%) |
|--------------------------------|------|---------|-----------------|----------------|
| **O. basilicum L. Stem Essential Oils** |      |         |                 |                |
|                                 | 20.230 | 99      | α-copaene       | 4.93           |
|                                 | 21.559 | 99      | β-bourbonene    | 1.58           |
|                                 | 23.492 | 95      | linalool        | 1.58           |
|                                 | 25.573 | 99      | Caryophyllene   | 18.75          |
|                                 | 29.340 | 99      | β-selinene      | 0.81           |
|                                 | 30.192 | 97      | z-citral        | 2.37           |
|                                 | 31.464 | 99      | Germacrene-D    | 9.19           |
|                                 | 32.930 | 96      | e-citral        | 3.39           |
|                                 | 34.178 | 91      | d-gurjunene     | 3.16           |
|                                 | 44.959 | 94      | Caryophyllene oxide | 0.64   |
|                                 | 47.126 | 98      | Methyl eugenol  | 52.6           |
|                                 | 49.659 | 98      | Eugenol         | 2.4            |
|                                 | 50.983 | 98      | 1,3-benzodioxole| 0.75           |
| **O. tenuiflorum L. Stem Essential Oils** |      |         |                 |                |
|                                 | 20.239 | 99      | α-copaene       | 5.56           |
|                                 | 21.568 | 99      | β-bourbonene    | 1.64           |
|                                 | 25.582 | 99      | Caryophyllene   | 17.28          |
|                                 | 29.340 | 99      | α-Humulene (CAS)| 0.76           |
|                                 | 31.473 | 99      | Germacrene-D    | 9.29           |
|                                 | 34.187 | 94      | Germacrene-A    | 3.52           |
|                                 | 44.968 | 93      | Caryophyllene oxide | 0.86   |
|                                 | 47.126 | 99      | Methyl eugenol  | 56.72          |
|                                 | 49.664 | 98      | Eugenol         | 3.17           |
|                                 | 50.511 | 97      | t-Murolol       | 0.41           |
|                                 | 50.983 | 98      | 1,3-benzodioxole| 0.79           |

Note: The percentage of components is the relative percentage calculated based on the area of the peak.

Comparison of components of essential oils from stems of these two plants shows a slight similarity, both contain high amounts of methyl eugenol. Methyl eugenol is an oxygenated compound. Macchia et al. (2006) reported that methyl eugenol, produced up to 8% in the vegetative stage, was only found in some of the cultivars. Methyl eugenol is formed through cyclic acid pathways, where phenylalanine is the precursor. Through a few reaction stages, eugenol will be formed. With methyl donor from Sadenosylmethionine (SAM) and enzymatic activity from O-methyltransferase (OMT), eugenol is then converted to methyl eugenol (Murningsih et al., 2009).

The intensity of antioxidant activities of essential oils from O. basilicum L. and O. tenuiflorum L. stem was classified as very strong antioxidant (<50 µg/mL). Ocimum plants contain large amounts of antioxidants,
such as flavonoids, carotenoids, vitamin C, and vitamin E. The presence of antioxidant compounds provides protection against free radical which can cause oxidative damage of cellular component (Pandey et al., 2014). Natural essential oils contain several components. When they are used to protect some material, their activity is thought to be due to the dominance of the effective content of certain components therein. This is true in some cases. However, there are some exceptions. The antioxidant activity of essential oil, in fact, is also the result of the complex interplay among components and material to be protected from oxidation (Amorati, 2013).

CONCLUSION

Based on our results, there is a similarity in the chemical composition between O. basilicum L. and O. tenuiflorum L. stem essential oils. Methyl eugenol is a major compound that is produced by Ocimum and it might have a role in their antioxidant activities. These findings suggest that both oils might be good natural antioxidant agents.

ACKNOWLEDGEMENT

We thank to the Research Institute and Development of Universitas Muhammadiyah Prof. DR. HAMKA, Jakarta, for Funding Research Grants Development of Science and Technology Batch I 2019.

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E-ISSN 2477-0612
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