Physicochemical Properties and Antioxidant Activity of Essential Oil from Fresh, Wilted, and Dried Leaves of Holy Basil (*Ocimum tenuiflorum* L.) Planted in Yogyakarta

Hetty Sri Mulyati, Rini Yanti*, Supriyadi Supriyadi

Department of Food Technology and Agricultural Products, Faculty of Agricultural Technology, Universitas Gadjah Mada, Flora Street No. 1, Bulaksumur, Yogyakarta 55281, Indonesia
*Corresponding authors: Rini Yanti, Email: riniyanti@ugm.ac.id

Submitted: January 26, 2022; Revised: August 31, 2022; Accepted: September 23, 2022

ABSTRACT

This study aimed to determine the effect of drying and distillation time on the physicochemical properties and antioxidant activity of essential oil from holy basil (*Ocimum tenuiflorum*) leaves. The leaves were subjected to different drying treatments to obtain fresh, wilted, and dried samples. The microstructure tissue of the various treatment samples was observed using a Scanning Electron Microscope (SEM). Subsequently, essential oil was extracted using the water and steam distillation method for 3, 4, 5, and 6 hours. The distilled essential oil was tested for its physicochemical properties (specific gravity, refractive index, acid number, and solubility in alcohol), and the compound composition evaluation was carried out using Gas Chromatography-Mass Spectrometry (GC-MS). The antioxidant activity was then determined using the DPPH (*1,1-diphenyl- 2-picrylhydrazyl*) method. The results showed that the fresh leaves distillation for 5 hours produced the highest oil yield of 0.62%. The physicochemical properties were 0.986 g/ml specific gravity, 1.339 refractive index, 1.8 mg KOH/g acid value, and 1:1 solubility in alcohol. The major component of the product was methyl isoeugenol (20.50%), bete-element (15.07%), eugenol (13.59%), germacrene D (14.2%), and ylangene (7.46). The results of the antioxidant activity test showed that the essential oil of holy basil could be included in the powerful natural antioxidant category, with an IC_{50} value of 7,895 µg/mL.

Keywords: Holy basil leaves; essential oil; methyl isoeugenol; microstructure of leaves; antioxidant activity

INTRODUCTION

The natural resources in Indonesia are known to exhibit remarkable diversity, but their full and optimal utilization remains a challenge. One such resource is the plethora of essential oil plants, with holy basil *(Ocimum tenuiflorum L.*) being one of the most common due to its distinct properties. Furthermore, this herbaceous plant is native to various regions of India and Southeast Asia and has also taken root in Indonesia, particularly abundant in the West Sumatra province. The Minangkabau community, residing in West Sumatra, often use its leaves as a culinary seasoning (Gardjito et al., 2018) and alternative medicine (Upadhyay et al., 2015; Rohini et al., 2019).

Holy basil leaves exhibit similarities to *Ocimum basilicum* and *Ocimum americanum*, but the aroma is more fragrant (Rinaldi, 2012). The genus *Ocimum* encompasses approximately 150 distinct species, which are globally distributed (Pandey et al., 2014). The aroma

DOI: http://doi.org/10.22146/agritech.72545
ISSN 0216-0455 (Print), ISSN 2527-3825 (Online)

emitted by holy basil leaves can be attributed to their essential oil content. This essential oil is composed of natural compounds derived from secondary metabolites with numerous benefits. Furthermore, aromatic plants are considered the primary source of flavor and aroma constituents in the food and pharmaceutical industries (Abed and Kurji, 2018).

The post-harvest handling of plant materials plays a crucial role in determining the quality of essential oil components. The optimization of the yield of these components can be achieved through adept pre-treatment of samples. Nugraheni et al. (2016) reported an increased essential oil yield from cinnamon wood through air drying. Muhtadin et al. (2013) also showed the effectiveness of pre-treatment involving the use of oven drying on citrus peels. A study on essential oil from *Thymus vulgaris* and *Melissa officinalis* leaves (Khalili et al., 2018) indicated that fresh samples provided higher levels of oxygenated compounds. Similar results were obtained by Sukardi et al (2014), where essential oil from kaffir lime leaves was obtained through water and steam distillation under fresh conditions.

According to previous studies, distillation time also plays a crucial role in determining the effectiveness of the extraction of these components. For example, in the study by Mayasari et al (2013), kaffir lime leaves were subjected to steam distillation for 4 hours. Khasanah (2015) reported the use of steam-water distillation for 3 hours on citrus leaves. Furthermore, Nurnasari and Prabowo (2020) reported a 6-hour water-steam distillation of tobacco leaves, and Putri et al. (2021) investigated steam distillation of basil leaves for 2 hours.

Essential oil derived from *Ocimum* species contains bioactive compounds, such as eugenol, linalool, eucalyptol, and bornyl acetate (Kartika, 2016). Various studies showed the potential of the holy basil plant as an antifungal (Piras et al., 2018), antidiabetic (Mousavi et al., 2018), anticancer agent (Boonyanugomol et al., 2021) and insecticidal (Bhavya et al., 2018). The plant is also recognized for its phenolic content, which exhibits antioxidant properties (Sharma and Kumar, 2011). Ahmed et al (2019) reported significant phenolic content in *Ocimum basilicum,* contributing to its free radical scavenging properties. Based on this background and scientific review, further study is needed to explore essential oil content and antioxidant activity of holy basil *(Ocimum tenuiflorum L.)*.

METHODS

Materials

The materials used in this study included holy basil leaves obtained from farmers in the Godean region, Yogyakarta Special Province, Indonesia. Furthermore, the plant was identified in the Plant Systematics Laboratory of the Biology Faculty at Gadjah Mada University, with the scientific name *Ocimum tenuiflorum L.* Water was used as the solvent for distillation, and the chemicals employed included Na_2SO_4, Methanol (PA), Toluene (PA), $AgNO_3$, NaCl, HNO_3, KOH from Merck (Germany), and DPPH (Sigma, USA).

The equipment used in this study included a set of water and steam distillation apparatus, with a 2 kg capacity. For analysis, an Abbe refractometer, pycnometer, UV-visible spectrophotometer, Bidwell sterling, Scanning Electron Microscope (JOEL JSM-6510, USA), and a set of GC-MS equipment (Shimadzu QP 20110S, Japan) were utilized. Furthermore, the column specifications included HP-5MS; length: 30 meters; ID: 0.25 mm; carrier gas: Helium; ionization.

Isolation of Essential Oil by Water and Steam Distillation Method

A total of 500 g fresh holy basil leaves were weighed and subjected to three treatments, namely fresh and wilted samples dried at room temperature of 29-31 °C for 48 hours, and dried leaves subjected to drying using a cabinet dryer at 50 °C for 10 hours. Each material was placed on a sieve previously filled with 8 L of water and then heated to a temperature of 100 °C with varying distillation times of 3, 4, 5, and 6 hours. The timing started from the first drop of oil appearing, and the hot vapor produced was condensed and collected. The remaining water from essential oil was dried using anhydrous sodium sulfate and stored at 4 °C for further analysis. The calculation of the yield value is presented in equation 1.

$$\text{Yield (\% g/g)} = \frac{oil\ weight\ (g)}{material\ weight\ (g)}\ x\ 100\% \quad (1)$$

Physical characteristic tests, compound component identification, and antioxidant activity tests were conducted on essential oil with the highest yield in each leaf drying treatment.

Scanning Electron Microscope (SEM)

SEM testing was conducted at the Integrated Research and Testing Laboratory at Gadjah Mada University. Holy basil leaves samples were placed on an aluminum stub and sputter-coated with a thin layer of platinum. Subsequently, the leaves structure was conditioned in a standard vacuum at a speed of 5.0 KV for surface analysis and 10 KV for cross-section analysis, followed by detection using secondary electrons.

Physical Properties of Essential Oil

Physical properties of essential oil included specific gravity, refractive index, acid number, and solubility in alcohol. The testing was based on the Indonesian National Standard 06-2385-2006 (SNI, 2006).

Compound Component Identification

GC-MS testing was performed at the Integrated Research and Testing Laboratory at Gadjah Mada University. A 0.2 mL essential oil sample was dissolved in 1 mL MeOH in a GC vial and vortexed until homogenized. The parameters used included an injector temperature of 260 °C, 60 °C column temperature, Helium carrier gas, pressure flow control mode, 12 kPa pressure, 25 mL/min total flow, 0.51 mL/min column flow, 26 cm/s² linear acceleration, 3.0 mL/min cleaning flow, and 42 split ratio. Agilent DB-1 column was used with a length of 30 m and a diameter of 0.25 mm, and the EI (Electron Impact) ionization was operated at 70 eV.

DPPH *Radical Scavenging Assay*

The DPPH radical scavenging assay was carried out based on the method proposed by Memarzadeh et al. (2020). A total of 100 µL essential oil sample was mixed with DPPH methanol solution (40 ppm) in a concentration range of 25–500 ppm. Subsequently, the mixture was vortexed and kept at room temperature and in the dark for 30 minutes (Ahmed et al., 2019). The absorbance was measured at a wavelength of 515 nm using a UV-Vis spectrophotometer. Methanol was used as a blank, and BHT was used as a positive control. Each treatment was repeated three times, and the inhibition level of the sample was calculated using the equation 2:

$$\% \text{ Inhibition} = \frac{(A^o - A^1)}{A^o} \, x \, 100\% \qquad (2)$$

Description : A^o = DPPH absorbance; A^1 = sample absorbance.

Experimental Design and Data Analysis

This study used a Completely Randomized Factorial Design (CRDF), consisting of two factors, namely drying treatment (fresh, wilted, and dried) and distillation time (3, 4, 5, and 6 hours), and three replicates of each sample were included. Furthermore, data were analyzed using ANOVA with SPSS version 22. Differences between samples were determined using the least significant difference test at $p < 0.05$, and the results were presented as means ± SD.

RESULTS AND DISCUSSION

Microstructure of Holy Basil Leaves Tissue

The results of the microstructure observation of holy basil leaves tissue are offered in Figures 1 and 2.

According to Benmoussa et al. (2018), tissue damage in the material became a parameter of effectiveness during the extraction process. In Figure 1, the location of essential oil sacs was distributed on the adaxial surface of the leaves. Zotti et al., (2020) stated that aromatic plants contained oil stored within the glandular trichomes, primarily found on the abaxial surface of the leaves. Figure 1a showed the surface of fresh samples, which were still covered by a cuticle layer, making the location of the sacs invisible. However, on the surface of wilted (Figure 1b) and dried samples (Figure 1c), the cuticle layer appeared to be damaged, leading to the exposure of the sacs. Figure 2 showed the inner tissue structure. In fresh leaves (Figure 2a), the tissue was still intact and capable of maintaining its shape. This structural preservation led to the non-compaction of water components, thereby making the tissue look intact, and essential oil sacs remained undamaged.

In wilted leaves (Figure 2b), the tissue structure appeared to have reduced stiffness, but due to the relatively high water content, essential oil sacs remain undamaged. However, in dried leaves (Figure 2c), the

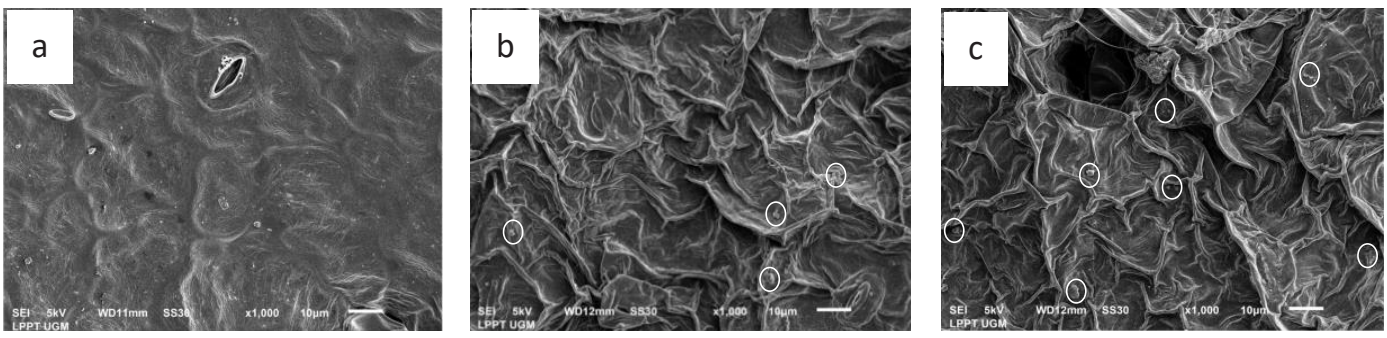

Figure 1. Microstructure of holy basil leaves surface (a) fresh, (b) wilted, and (c) dried

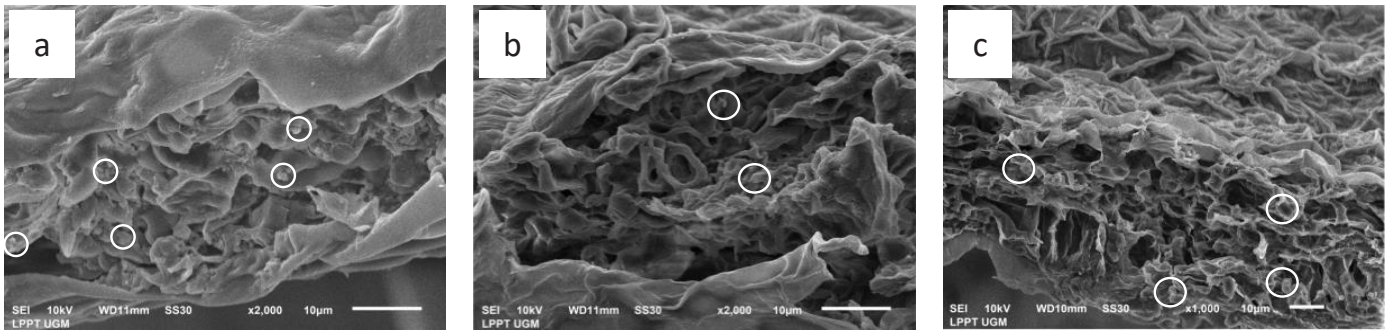

Figure 2. Microstructure of cross-sectional holy basil leaves (a) fresh, (b) wilted, and (c) dried

Table 1. The yield of holy basil essential oil

Drying Method	Distillation Time (hours)			
	3	4	5	6
Fresh Leaves	0.23±0.04^{b}	0.31±0.01^{d}	0.62±0.03^{g}	0.61±0.01^{g}
Wilted Leaves	0.22±0.01^{ab}	0.27±0.02^{c}	0.50±0.01^{f}	0.46±0.02^{e}
Dried Leaves	0.18±0.02^{a}	0.22±0.04^{ab}	0.32±0.01^{d}	0.31±0.01^{d}

Description: Different letters in the same row indicate significant differences (p>0.05)

structure had been compromised. This was due to the decreasing water content in the tissue, leading to changes in leaves shape. The changes were indicated by bending toward the upper surface of the leaves that was covered by the cuticle (wax). Variations in shape due to leaves contraction could damage tissue containing essential oil sacs, leading to the release of various aromas.

Essential Oil Yield

Table 1 presented the yield of *Ocimum tenuiflorum* essential oil. The measurement results showed that fresh leaves distilled for 5 hours had the highest yield at 0.62%. The results obtained by Hadipoentyanti and Wahyuni (2008) for various types of *Ocimum* plants included *O. gratissimum from* Bogor (0.780%), *O. gratissimum* from Serang (0.564%), *O. basillicum from* Bogor (0.999%), *O. canum* from Bogor (0.560%), *O. santum* from Bogor (0.509%), and *O. minimum* from Malang (0.401%). Furthermore, Fattahi et al (2019) examined O*. basilicum* from Iran and obtained a yield of 0.39%. The variation in the values recorded across different drying methods was due to the thin tissue structure of holy basil leaves, making the penetration of vapor during distillation easier. The results showed that wilted and dried leaves yield was 0.50% and 0.32%, respectively. Decreases in yield could be attributed to the heating process during sample preparation, as volatile compounds tended to evaporate when heated. The volatile nature of compounds with easy evaporation (Voo et al., 2012) and high-temperature drying could lead to the loss of essential oil from leaves tissue (Anh et al., 2019). The yield of holy basil essential oil increased along with the longer distillation time. The maximum distillation time was 5 hours, and there was a decrease in the amount of extract after 6 hours. This reduction could be attributed to the complete extraction or partial decomposition and evaporation of essential oil compounds due to prolonged heating (Nurnasari and Prabowo, 2020).

Physicochemical Properties of Essential Oil

The physicochemical properties analyzed in this study referred to the Essential Oil Association (EOA) standard for *Ocimum basilicum*. The EOA standard for *Ocimum basilicum* was used for comparison, as there was no standardized reference for *Ocimum tenuiflorum L.* essential oil. The analysis of physicochemical properties (Table 2) of the products was based on the yield value with a distillation time of 5 hours.

Specific Gravity

Specific gravity referred to the ratio of the weight of a sample to an equal volume of water. The typical value of this parameter for essential oil ranged from 0.696 to 1.188. The results showed that the specific

Table 2. Characteristics of physicochemical properties of *Ocimum tenuiflorum L*. essential oil

Parameter	Fresh leaves	Wilted leaves	Dried leaves	EOA Standard *Ocimum basillum*
Specific gravity (g/mL)	0.986±0.0009[b]	0.966±0.0070[a]	0.972±0.0046[a]	0.952-0.973
Refractive index	1.339±0.0125[a]	1.346±0.0064[a]	1.474±0.0557[b]	1.512-1.519
Acid number (mg KOH/g)	1.8 ±0.3054[a]	1.8 ±0.3241[a]	1.6 ±0.0127[a]	> 1
Solubility in alcohol	1:1	1:1	1:1	4:1

Description: Different letters in the same row indicate significant differences (p > 0.05).

gravity of holy basil essential oil for fresh, wilted, and dried leaves was 0.986, 0.966, and 0.972, respectively. When compared to the EOA standard, which ranged from 0.952 to 0.973, the values obtained in this study were higher. These higher values could be attributed to the high weight fraction contained in the extract, which was evident in the GC-MS results (Table 3). According to Slamet et al. (2019), the higher the weight fraction, the higher the specific gravity. Compounds, such as sesquiterpenes and eugenol benzoate, were part of the heavy fraction of essential oil (Arpima et al., 2020).

Refractive Index

The refractive index value typically ranged from 1.3 to 1.7. The results of this study showed values of 1.339, 1.346, and 1.474 for essential oil from fresh, wilted, and dried leaves, respectively. The low refractive index in fresh leaves was due to the presence of water. When compared to the EOA standard range of 1.510 to 1.516, the values obtained in this study were lower.

Acid Number

The acid number could also affect the quality of essential oil. The higher the level of free fatty acids in the oil, the higher the acid content (Qorriaina et al., 2015), thereby influencing the distinctive aroma. The results showed that the acid number for fresh, wilted, and dried leaves was 1.8, 1.8, and 1.6, respectively. These values were higher compared to the EOA standard of < 1, but were lower compared to those obtained by Hadipoentyanti and Wahyuni (2008), where the acid number was 1.9562 mg KOH/g for Ocimum basilicum oil. The high acid number could also be attributed to the oxidation of aldehyde compounds, forming carboxylic acid.

Solubility in Alcohol

The solubility of holy basil essential oil in alcohol was tested at a 1:1 ratio, leading to easy solubility in 96% alcohol and the yield of a clear solution. Furthermore, the solubility was attributed to the presence of terpenes with low oxygenation. Alcohol contained hydroxyl groups, which contributed to its ability to dissolve in essential oil. The physicochemical properties of holy basil essential oil in this study were only compared to *Ocimum basilicum*, as there was no EOA standard. The difference in oil quality was caused by geographical location, variety, pre-distillation processes (Hadipoentyanti and Wahyuni, 2008), weather, and climate (Zarlaha et al., 2014). The EOA standard used for *Ocimum basilicum* plants belonged to the methyl chavicol type, while holy basil oil was different. Consequently, the physicochemical property analysis results were different from the EOA standard.

Identification of Essential Oil Compounds

Chemical content analysis using Gas Chromatography-Mass Spectrometry (GC-MS) was conducted to identify the compounds present in holy basil leaves essential oil. The GC-MS test results indicated that the extract of fresh, wilted, and dried leaves each contained 56, 71, and 80 compounds, respectively. Furthermore, the dominant components were terpenoids, especially monoterpenes, and sesquiterpenes, which had carbon numbers of C10 and C15 (Table 3). Terpenoids had distinct boiling points that could influence their retention times. In gas chromatography systems, compounds with lower boiling points were eluted first due to their easier evaporation, leading to faster retention times (Harianingsih et al., 2017). The compound identification results (Table 3) were obtained based on the yield with a distillation time of 5 hours.

Terpenoids produced by fresh, wilted, and dried holy basil leaves oil in the form of monoterpenes and sesquiterpenes did not show significant differences in quantity. The dominant chemical components in the extract were Methyl isoeugenol (20.50%), Beta-elemene (15.07%), Eugenol (13.59%), Germacrene D (14.20%), and Ylangene (7.46%). Several studies stated that essential oil of *O. tenuiflorum* from India

Table 3. Identification results of holy basil essential oil compounds

No	Compound components	Fresh leaves		Wilted leaves		Dried leaves	
		RT (Min)	Area (%)	RT (Min)	Area (%)	RT (Min)	Area (%)
1	Hexahydro-1,3-benzodioxol-2-one	-	-	-	-	4.97	0.01
2	3-Hexen-1-ol, (Z)-	-	-	-	-	5.09	0.01
3	a-Pinene	-	-	-	-	6.92	0.06
4	Bicyclo[3.1.1]hept-2-ene, 3,6,6-trimethyl-	-	-	6.93	0.02	-	-
5	Camphene	-	-	7.32	0.03	7.32	0.08
6	Benzaldehyde	-	-	7.73	0.02	7.72	0.06
7	Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	-	-	8.03	0.01	8.03	0.03
8	ß-Pinene	-	-	8.11	0.04	8.10	0.09
9	1-Hepten-3-ol	-	-	-	-	8.29	0.01
10	ß-Myrcene	-	-	8.54	0.01	8.54	0.01
11	Octanal	-	-	-	-	8.89	0.01
12	D-Limonene	-	-	9.61	0.05	9.61	0.1
13	Eucalyptol	9.68	0.01	9.67	0.08	9.67	0.14
14	Benzeneacetaldehyde	-	-	10.13	0.04	10.12	0.14
15	ß-Ocimene	10.21	0.01	10.20	0.13	10.20	0.12
16	?-Terpinene					10.50	0.02
17	3-Carene	-	-	10.50	0.01	-	-
18	5-Isopropyl-2-methylbicyclo[3.1.0]hexan-2-ol #	10.80	0.02	10.80	0.05	10.79	0.06
19	2-Furanmethanol, 5-ethenyltetrahydro-a,a,5-trimethyl-, cis-	10.94	0.01	10.93	0.01	10.94	0.01
20	Ethanone, 2,2-dihydroxy-1-phenyl-	-	-	11.00	0.03	10.98	0.06
21	trans-Linalool oxide (furanoid)	11.39	0.06	11.39	0.1	11.38	011
22	Linalool	11.79	0.78	11.80	1.38	11.80	1.58
23	2,7-Octadiene-1,6-diol, 2,6-dimethyl-	11.93	0.04	11.93	0.07	11.93	0.08
24	cis-p-mentha-1(7),8-dien-2-ol	-	-	-	-	12.08	0.02
25	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-, (Z,E)-	-	-	12.20	0.01	-	-
26	Myrtenyl methyl ether	-	-	-	-	12.38	0.01
27	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S)-	13.01	0.02	13.01	0.05	13.00	0.05
28	endo-Borneol	13.71	2.51	13.72	3.45	13.72	3.79
29	Terpinen-4-ol	-	-	-	-	14.03	0.11
30	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)-	14.03	0.08	14.03	0.1	-	-
31	1,7,7-Trimethylbicyclo[2.2.1]heptan-2-ol	-	-	14.25	0.02	-	-
32	Terpineol	14.45	0.08	14.45	0.02	14.44	0.10
33	a-Terpineol	-	-	14.45	0.09	-	-
34	2-Isopropylidene-3-methylhexa-3,5-dienal	14.62	0.01	-	-	14.62	0.01
35	Estragole	-	-	14.62	0.01	-	-

No	Compound components	Fresh leaves		Wilted leaves		Dried leaves	
		RT (Min)	Area (%)	RT (Min)	Area (%)	RT (Min)	Area (%)
37	2-Nonen-4-one, 2-methyl-	15.06	0.01	-	-	-	-
38	2-Methyl-4-octenal	-	-	15.06	0.01	15.05	0.01
39	5-Hepten-1-ol, 2-ethenyl-6-methyl-	15.51	0.01	-	-	-	-
40	2-Cyclohexen-1-one, 3-methyl-6-(1-methylethyl)-	-	-	16.19	0.01	-	-
41	1b,5,5,6a-Tetramethyl-octahydro-1-oxa-cyclopropa[a]inden-6-one	-	-	-	-	16.24	0.01
42	Citral	16.63	0.01	-	-	-	-
43	Cinnamaldehyde, ß-methyl-	-	-		-	16.72	0.01
44	2,5,5,8a-Tetramethyl-3,4,4a,5,6,8a-hexahydro-2H-chromene	17.11	0.02	17.24	0.03	17.24	0.02
45	Falcarinol	-	-	-	-	17.57	0.01
46	a-Guaiene	17.91	0.3	17.91	0.01	17.91	0.01
47	2-Methoxy-4-vinylphenol	-	-	-	-	17.98	0.04
48	(1S,2E,6E,10R)-3,7,11,11-Tetramethylbicyclo[8.1.0]undeca-2,6-diene	18.41	0.02	-	-	-	-
49	a-Cubebene	18.75	0.36	18.74	0.4	18.74	0.44
50	Aciphyllene	18.83	0.12	18.83	0.13	18.82	0.14
51	Eugenol	19.09	13.33	19.10	13.59	19.09	12.53
52	Phenol, 2-methoxy-3-(2-propenyl)-	-	-	19.39	1.3	-	-
53	Ylangene	19.49	6.57	19.48	6.94	19.46	7.46
54	(-)-ß-Bourbonene	19.76	2.4	19.77	2.79	19.76	2.93
55	Isogermacrene D	19.91	3.22	19.87	3.36	19.86	2.76
56	Metil isoeugenol	20.39	18.23	20.37	19.24	20.38	20.5
57	Caryophyllene	20.97	4.63	20.98	3.23	20.99	1.81
58	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-,[1R-(1R*,4Z,9S*)]-	-	-	21.12	0.01	-	-
59	cis-a-Bergamotene	21.14	0.09	-	-	-	-
60	Bete-elemene	21.51	14.65	21.21	14.55	21.12	15.1
61	Humulene	21.67	2.98	21.66	2.73	21.66	2.73
62	Germacrene D	22.26	14.06	22.26	14.2	22.27	14.2
63	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4aa,7a,8aß)]-	-	-	-	-	22.52	0.57
64	Bicyclo[5.3.0]decane, 2-methylene-5-(1-methylvinyl)-8-methyl-	22.74	1.61	22.60	0.8	-	-
65	?-Elemene	22.68	1.47	22.68	1.28	22.66	1.7
66	(1S,4aR,8aS)-1-Isopropyl-7-methyl-4-methylene-1,2,3,4,4a,5,6,8a-octahydronaphthalene	23.06	0.12	23.06	0.07	23.06	0.09
67	(3S,3aR,3bR,4S,7R,7aR)-4-Isopropyl-3,7-dimethyloctahydro-1H-cyclopenta[1,3]cyclopropa[1,2]benzen-3-ol	23.14	0.99	23.13	0.7	23.13	0.7
68	1-Isopropyl-4,7-dimethyl-1,2,3,5,6,8a-hexahydronaphthalene	-	-	23.27	1.52	23.27	1.6
69	β-copaene	23.33	2.05	-	2.81	23.33	3.12

No	Compound components	Fresh leaves		Wilted leaves		Dried leaves	
		RT (Min)	Area (%)	RT (Min)	Area (%)	RT (Min)	Area (%)
71	a-Muurolene	23.58	0.04	23.58	0.03	23.58	0.04
72	Aromadendrene oxide-(1)	23.65	0.19	24.37	0.18	23.64	0.12
73	Cadala-1(10),3,8-triene	-	-	-	-	23.72	0.02
74	Cyclohexanemethanol, 4-ethenyl-a,a,4-trimethyl-3-(1-methylethenyl)-, [1R-(1a,3a,4ß)]-	23.92	1.6	23.90	0.95	23.90	0.9
75	Caryophyllene oxide	24.64	0.67	23.97	0.74	23.96	0.98
76	Cubenol	26.12	0.65	25.40	0.37	24.08	0.4
77	Nerolidyl acetate	-	-	-	-	24.15	0.02
78	Bicyclo[4.4.0]dec-2-ene-4-ol, 2-methyl-9-(prop-1-en-3-ol-2-yl)-	23.73	0.01	23.72	0.01	24.19	0.02
79	(1S,3aR,4R,8R,8aS)-1-Isopropyl-3a-methyl-7-methylenedecahydro-4,8-epithioazulene	24.30	0.08	24.07	0.06	24.29	0.09
80	(2E,4S,7E)-4-Isopropyl-1,7-dimethylcyclodeca-2,7-dienol	24.53	0.35	24.52	0.31	24.52	0.31
81	3,7-Cyclodecadiene-1-methanol, a,a,4,8-tetramethyl-, [s-(Z,Z)]	24.38	0.06	24.15	0.15	24.81	0.1
82	Isoaromadendrene epoxide	-	-	25.02	0.26	24.91	0.48
83	5-Azulenemethanol, 1,2,3,4,5,6,7,8-octahydro-a,a,3,8-tetramethyl-	25.80	0.13	25.79	0.1	25.79	0.13
84	10,10-Dimethyl-2,6-dimethylenebicyclo[7.2.0]undecan-5ß-ol	-	-	25.91	0.05	-	-
85	Caryophylla-4(12),8(13)-dien-5a-ol	25.93	0.04	-	-	-	-
86	Alloaromadendrene oxide-(1)	-	-	26.54	0.02	25.85	0.06
87	11,11-Dimethyl-4,8-dimethylenebicyclo[7.2.0]undecan-3-ol	-	-	-	-	25.92	0.04
88	.tau.-Cadinol	26.02	0.45	26.00	0.29	26.00	0.33
89	2-Naphthalenemethanol, decahydro-a,a,4a-trimethyl-8-methylene-, [2R-(2a,4aa,8aß)]-	26.24	0.06	26.23	0.04	26.24	0.22
90	Neointermedeol	-	-	26.33	0.53	26.32	0.59
91	Globulol	-	-	26.43	0.03	-	-
92	(-)-Globulol	26.44	0.07	-	-	26.43	0.04
93	(1R,7S,E)-7-Isopropyl-4,10-dimethylenecyclodec-5-enol	27.03	0.17	27.03	0.13	27.03	0.28
94	Longipinocarveol, trans-	-	-	-	-	27.81	0.05
95	Cedren-13-ol, 8-	27.50	0.04	-	-	-	-
96	2,5-Octadecadiynoic acid, methyl ester	28.86	0.01	-	-	-	-
97	Cholestan-3-ol, 2-methylene-, (3ß,5a)-	-	-	-	-	30.22	0.05
98	2-Acetoxy-1,1,10-trimethyl-6,9-epidioxydecalin	30.23	0.01	-	-	-	-
99	1-Oxaspiro[2.5]octane, 5,5-dimethyl-4-(3-methyl-1,3-butadienyl)-	-	-	30.23	0.01	-	-
100	Methyl 2-hydroxy-octadeca-9,12,15-trienoate	-	-	-	-	31.23	0.01
101	1-Heptatriacotanol	-	-	-	-	31.52	0.01
102	2-Methyl-4-(2,6,6-trimethylcyclohex-1-enyl)but-2-en-1-ol	-	-	31.69	0.01	31.66	0.03
103	1,2-15,16-Diepoxyhexadecane	-	-	32.28	0.01	-	-

No	Compound components	Fresh leaves		Wilted leaves		Dried leaves	
		RT (Min)	Area (%)	RT (Min)	Area (%)	RT (Min)	Area (%)
105	Phytol	35.41	0.11	35.41	0.03	35.41	0.20
106	Phytol, acetate	-	-	37.26	0.13	-	-
107	Ethyl iso-allocholate	43.15	0.03	-	-	-	-
	Hydrocarbon compounds		54.70		55.17		55.22
	Oxygenated compounds		42.57		44.82		44.70
	Sesquiterpene hydrocarbons		54.69		54.87		54.71
	Monoterpene hydrocarbons		0.01		0.30		0.51
	Oxygenated monoterpenes		3.65		5.47		6.06
	Oxygenated sesquiterpenes		5.57		4.97		5.81
	Others		33.35		34.38		32.83
	Total identification (%)		97.27		99.99		99.92

contained a significant amount of compounds, such as methyleugenol (Rou, 2011) and eugenol (Wang, 2015). Methyl isoeugenol was a derivative of eugenol that was widely used in flavor and aroma formulations (Riyanto et al., 2015). Several studies also reported the use of Eugenol as a strong aroma agent (Wongpraneekul et al., 2022).

Antioxidant Activity

Antioxidant activity measurement was run to determine the potential of holy basil leaves essential oil as a free radical scavenger. Furthermore, the IC50 value was used to determine the effective concentration of the oil in inhibiting DPPH radicals, as shown in Table 4. According to Sayuti and Yenrina (2015), antioxidant compounds reacted with DPPH through a hydrogen atom donation mechanism, leading to a color change from purple to yellow. The antioxidant activity results (Table 4) were obtained from the yield value with a distillation time of 5 hours.

Table 4. IC_{50} Values of Holy Basil Essential Oil

Sample	IC_{50} Value (µg/mL)	Antioxidant Activity
BHT	7.002±0.368^{a}	Very Strong
Fresh Leaves	11.1851±0.509^{c}	Very Strong
Wilted Leaves	8.603±0.843^{b}	Very Strong
Dried Leaves	7.895±0.472^{ab}	Very Strong

Description: Different letters in the same row indicate significant differences (p>0.05).

Table 4 showed that holy basil leaves essential oil had a strong potential as an antioxidant. According to (Molyneux P, 2004), the IC50 values for antioxidants were categorized as very strong (less than 50 µg/mL), strong (50-100 µg/mL), moderate (101-150 µg/mL), and weak (above 150 µg/mL). Furthermore, the lower the IC50 value in the sample, the higher its activity (Badarinath et al., 2010).

The activity in holy basil oil was attributed to the presence of compounds, such as eugenol and methyl isoeugenol. Eugenol was a compound that contributed to the scavenging of free radicals (Devi & Ganjewala, 2011), and one of the major phenolic compounds found in clove oil (Nurjannah et al., 2013). It also exhibited very strong antioxidant properties in essential oil of *Ocimum gratissimum* Linn (Mahapatra and Roy, 2014). According to Tucker and Adams (2014), the use of plant-based antioxidants, such as eugenol and its derivatives, in food products was a safe alternative for health. BHT was used as a positive control in this study, and the results showed the extract produced had comparable antioxidant capabilities with BHT. This indicated that it could be used as an excellent natural antioxidant agent.

CONCLUSION

In conclusion, essential oil from fresh leaves with a distillation time of 5 hours had the highest yield of 0.62%. However, the drying method and distillation time did not significantly affect its physicochemical properties. The dominant compounds in holy basil essential oil were Methyl isoeugenol (20.50%), Beta-

elemene (15.07%), Eugenol (13.59%), Germacrene D (14.2%), and Ylangene (7.46%). The antioxidant activity test results showed that the product could be categorized as a very strong natural antioxidant, with an IC_{50} value of 7.895 µg/mL. These dominant compounds could contribute to other potential benefits of holy basil (*Ocimum tenuiflorum*) essential oil.

CONFLICT OF INTEREST

All authors declare no conflict of interest regarding the submitted manuscript entitled: "Physicochemical Properties and Antioxidant Activity of Essential Oil from Fresh, Wilted, and Dried Leaves of Holy Basil (*Ocimum tenuiflorum* L.) Planted in Yogyakarta at Various Distillation Times " for consideration in the Journal of Agritech.

REFERENCES

Abed, K. M., & Kurji, B. M. (2018). *Extraction of Ocimum basillicum Oil by Solvents Methods*. (April), 3–6. https://doi.org/10.14233/ajchem.2018.21032

Ahmed, A. F., Attia, F. A. K., Liu, Z., Li, C., Wei, J., & Kang, W. (2019). Antioxidant activity and total phenolic content of essential oils and extracts of sweet basil (*Ocimum basilicum* L.) plants. *Food Science and Human Wellness*, *8*(3), 299–305. https://doi.org/10.1016/j.fshw.2019.07.004

Anh, T. T., Duyen, L. T., Hang, L. M., Lam, T. D., Bach, L. G., Nguyen, D. C., & Toan, T. Q. (2019). Effect of drying temperature and storage time on *Ocimum gratissimum* Linn. leaf essential oil from Central Highlands, Vietnam. *Materials Today: Proceedings*, *18*, 4648–4658. https://doi.org/10.1016/j.matpr.2019.07.449

Badarinath, A. V, Rao, K. M., Chetty, C. M. S., Ramkanth, S., Rajan, T. V. S., & Gnanaprakash, K. (2010). *A Review on In-vitro Antioxidant Methods : Comparisions , Correlations and Considerations*. *2*(2), 1276–1285.

Benmoussa, H., Elfalleh, W., He, S., Romdhane, M., Benhamou, A., & Chawech, R. (2018). Microwave hydrodiffusion and gravity for rapid extraction of essential oil from Tunisian cumin (*Cuminum cyminum* L.) seeds: Optimization by response surface methodology. *Industrial Crops and Products*, *124*(August), 633–642. https://doi.org/10.1016/j.indcrop.2018.08.036

Bhavya, M. L., Chandu, A. G. S., & Devi, S. S. (2018). *Ocimum tenuiflorum* oil, a potential insecticide against rice weevil with anti-acetylcholinesterase activity. *Industrial Crops and Products*, *126*(October), 434–439. https://doi.org/10.1016/j.indcrop.2018.10.043

Boonyanugomol, W., Rukseree, K., Prapatpong, P., Reamtong, O., Baik, S. C., Jung, M., Shin, M. K., Kang, H. L., Lee, W. K. (2021). An in vitro anti-cancer activity of ocimum tenuiflorum essential oil by inducing apoptosis in human gastric cancer cell line. *Medicina (Lithuania)*, 57. https://doi.org/10.3390/medicina57080784

Devi, S. A., & Ganjewala, D. (2011). Antioxidant activities of methanolic extracts of sweet-flag (*Acorus calamus*) leaves and rhizomes. *Journal of Herbs, Spices and Medicinal Plants*, *17*(1), 1–11. https://doi.org/10.1080/10496475.2010.509659

Fattahi, B., Arzani, K., Souri, M. K., & Barzegar, M. (2019). Effects of cadmium and lead on seed germination, morphological traits, and essential oil composition of sweet basil (*Ocimum basilicum* L.). *Industrial Crops and Products*, *138* (June). https://doi.org/10.1016/j.indcrop.2019.111584

Gardjito, M., Putri, G, R., & Dewi, S. (2018). *Profil Struktur, Bumbu dan Bahan dalam Kuliner Indonesia*. Yogyakarta: Gadjah Mada University.

Hadipoentyanti, E dan Wahyuni, S. (2008). Keragaman selasih (Ocimum Sp) Berdasarkan karakter morfologi produksi dan mutu Herba. *Jurnal Littri*, *14*(4), 141–149.

Harianingsih, Wulandari, R., Harliyanto, C., & Andiani, C. N. (2017). Identifikasi GC-MS ekstrak minyak atsiri dari sereh wangi (*Cymbopogon winterianus*) menggunakan pelarut metanol. *Techno*, *18*(1), 23–27.

Katika, J. G., Suketi, K., & Mayasari, H. (2016). Produksi biomassa dan minyak atsiri kemangi (Ocimum basilicum L.) pada Berbagai dosis pupuk nitrogen dan pupuk cair hayati. *J. Hort. Indonesia*. *7*(April), 56–63. https://doi.org/10.29244/jhi.7.1.56-63

Khalili, G., Mazloomifar, A., Larijani, K., Tehrani, M. S., & Azar, P. A. (2018). Solvent-free microwave extraction of essential oils from Thymus vulgaris L. and Melissa officinalis L. *Industrial Crops and Products*, *119*(September 2017), 214–217. https://doi.org/10.1016/j.indcrop.2018.04.021

Khasanah, L. U. (2015). Pengaruh perlakuan pendahuluan terhadap karakteristik mutu minyak atsiri daun jeruk purut (*Citrus hystrix* DC). *Jurnal Aplikasi Teknologi Pangan*, *04*(02), 48–55. https://doi.org/10.17728/jatp.2015.10

Mahapatra, S. K. & Roy, S. (2014). Phytopharmacological approach of free radical scavenging and anti-oxidative potential of eugenol and *Ocimum gratissimum* Linn. *Asian Pacific Journal of Tropical Medicine*, *7*(S1), S391–S397. https://doi.org/10.1016/S1995-7645(14)60264-9

Mayasari, D., Jayuska, A., & Wibowo, M. A. (2013). Pengaruh variasi waktu dan ukuran sampel terhadap komponen minyak atsiri dari daun jeurk purut (*Citrus hystrix* DC.). *Jurnal Kimia Khatulistiwa*, *2*(2), 74–77.

Memarzadeh, S. M., Gholami, A., Pirbalouti, A. G., & Masoum, S. (2020). Bakhtiari savory (*Satureja bachtiarica*

Bunge.) essential oil and its chemical profile, antioxidant activities, and leaf micromorphology under green and conventional extraction techniques. *Industrial Crops and Products*, *154*(January), 112719. https://doi.org/10.1016/j.indcrop.2020.112719

Muhtadin, A. F., Wijaya, R., & Prihatini, P. (2013). Pengambilan minyak atsiri dari kulit jeruk. *Jurnal Teknik Pomits*, *2*(1), 98–101.

Molyneux P. (2004). The use of the stable free radical diphenylpicryl-hydrazyl (DPPH) for estimating anti-oxidant activity. *Songklanakarin Journal of Science and Technology*, *26*(May), 211–219.

Mousavi, L., Salleh, R. M., & Murugaiyah, V. (2018). Phytochemical and bioactive compounds identification of *Ocimum tenuiflorum* leaves of methanol extract and its fraction with an antidiabetic potential. *International Journal of Food Properties*, *21*(1), 2390–2399. https://doi.org/10.1080/10942912.2018.1508161

Nugraheni, K. S., Khasanah, L. U., Utami, R., & Ananditho, B. K. (2016). Pengaruh perlakuan pendahuluan dan variasi metode destilasi terhadap karakteristik mutu minyak atsiri daun kayu manis (*C. burmanii*). *Jurnal Teknologi Hasil Pertanian*, *IX*(2), 51–64.

Nurjannah, D. A., Retnowati, R., & Juswono, U. P. (2013). Aktivitas antioksidan dari minyak bunga cengkeh (*Syzygium aromaticum*) kering berdasarkan aktivitas antiradikal yang ditentukan menggunakan Electron Spin Resonance. *Jurnal Ilmu Kimia Universitas Brawijaya*, *1*(2), 283–288.

Nurnasari, E. & Prabowo, H. (2020). Pengaruh ukuran sampel dan lama waktu destilasi terhadap rendemen minyak atsiri tembakau lokal Indonesia. *Buletin Tanaman Tembakau, Serat & Minyak Industri*, *11*(2), 47. https://doi.org/10.21082/btsm.v11n2.2019.47-57

Pandey, A. K., Singh, P., & Tripathi, N. N. (2014). Chemistry and bioactivities of essential oils of some Ocimum species: An overview. *Asian Pacific Journal of Tropical Biomedicine*, *4*(9), 682–694. https://doi.org/10.12980/APJTB.4.2014C77

Piras, A., Gonçalves, M. J., Alves, J., Falconieri, D., Porcedda, S., Maxia, A., & Salgueiro, L. (2018). *Ocimum tenuiflorum* L. and *Ocimum basilicum* L., two spices of Lamiaceae family with bioactive essential oils. *Industrial Crops and Products*, *113* (December 2017), 89–97. https://doi.org/10.1016/j.indcrop.2018.01.024

Putri, I. A., Fatimura, M., Husnah, H., & Bakrie, M. (2021). Pembuatan minyak atsiri kemangi (*Ocimum basilicum* L.) dengan menggunakan metode distilasi uap langsung. *Jurnal Redoks*, *6*(2), 149–156. https://doi.org/10.31851/redoks.v6i2.5202

Qorriaina, R., Hawa, L. C., & Yulianingsih, R. (2015). Aplikasi pra-perlakuan microwave assisted extraction (MAE) Pada ekstrak daun kemangi (*Ocimum sanctum*) Menggunakan rotary evaporator (studi pada variasi suhu dan waktu ekstraksi). *Jurnal Bioproses Komoditas Tropis*, *3*(1), 32–38.

Rao, B. R. R., Kothari, S. K., Rajput, D. K., Patel, R. P., & Darokar, M. P. (2011). Chemical and biological diversity in fourteen selections of four ocimum species. *Natural Product Communications* 6(11):1705-1710 https://doi.org/10.1177/1934578X1100601134

Rinaldi, M. (2012). *Tekenang Daun Ruku-ruku*. Retrieved March 13, 2020, from Wordpress.com website: https://rinaldimunir.wordpress.com/2012/03/01/terkenang-daun-ruku-ruku/

Riyanto, A., Yunilawati, R., Rahmi, D., Aidha, N. N., & Ratnawati, E. (2015). Isomerisasi eugenol menjadi isoeugenol dengan metode sonikasi. *Jurnal Kimia dan Kemasan*, *37*(1), 37. https://doi.org/10.24817/jkk.v37i1.1810

Rohini, J., Mansoureh, N. V., Fouad Saleih, R. A. S., Rabeta, M. S., & Shah, A. M. (2019). Preliminary screening on wound healing potential of *Ocimum tenuiflorum* L. using in vitro assays. *Food Research*, *3* (3), 258–264. https://doi.org/10.26656/fr.2017.3(3).010

Sayuti, K. & Yenrina, R. (2015). *Antioksidan Alami dan Sintetik*. Padang: Andalas University.

Sharma, R., & Kumar, B. S. V. (2011). Isolation characterization and antioxidant potential of endophytic fungi of *Ocimum sanctum* Linn. (Lamiaceae). *Indian Journal of Applied Research*, *3*(7), 5–10. https://doi.org/10.15373/2249555x/july2013/23

Slamet, S., Ulyarti, U., & Rahmi, S. (2019). Pengaruh lama fermentasi terhadap rendemen dan mutu fisik minyak nilam *Pogostemon cablin* Benth). *Jurnal Teknologi dan Industri Pertanian Indonesia*, *11*(1), 19–25. https://doi.org/10.17969/jtipi.v11i1.11671

Standar Nasional Indonesia (2006). *Standarisasi Mutu Minyak Atsiri*. Jakarta: Badan Standarisasi Nasional.

Sukardi., Rivitac, A., Pulungan, M. H., Mulyadi, A. F. (2014). Penerapan PEF (*Pulsed Electric Field*) pada ekstraksi minyak atsiri daun jeruk purut (*Citrus hystrix D.C*) dengan metode destilasi air dan uap. Universitas Brawijaya. Malang.

Tucker, R. P. & Adams, J. C. (2014). Adhesion networks of Cnidarians: A postgenomic view, *11*(2), 323–377. https://doi.org/10.1016/b978-0-12-800097-7.00008-7

Upadhyay, A. K., Chacko, A. R., Gandhimathi, A., Ghosh, P., Harini, K., Joseph, A. P., Joshi, A. G., Karpe, S. D., Kaushik, S., Kuravadi, N., Lingu, C. S., Mahita, J., Malarini, R., Malhotra, S., Malini, M., Mathew, O. K., Mutt, E., Naika, M., Nitish, S., Pasha, S. N., Raghavender, U. S., Rajamani, A., Shilpa, S., Shingate, P. N., Singh, H. R.,

Sukhwal, A., Sunitha, M. S., Sumathi, M., Ramaswamy, S., Gowda, M., Sowdhamini, R. (2015). Genome sequencing of herb Tulsi (*Ocimum tenuiflorum*) unravels key genes behind its strong medicinal properties. *BMC Plant Biology*, *15*(1), 1–20. https://doi.org/10.1186/s12870-015-0562-x

Voo, S. S., Grimes, H. D., & Lange, M. B. (2012). Assessing the biosynthetic capabilities of secretory glands in citrus peel. *Plant Physiology*, *159* (1), 81–94. https://doi.org/10.1104/pp.112.194233

Wang, Z. (2015). Antagonistic activity of Ocimum sanctum L . essential oil on growth and zearalenone production by Fusarium graminearum in maize grains. *6* (September), 1–11. https://doi.org/10.3389/fmicb.2015.00892

Wongpraneekul, A., Havananda, T., & Luengwilai, K. (2022). Variation in aroma level of holy basil (*Ocimum tenuiflorum* L.) leaves is related to volatile composition, but not trichome characteristics. *Journal of Applied Research on Medicinal and Aromatic Plants*, *27*(May 2021). https://doi.org/10.1016/j.jarmap.2021.100347

Zarlaha, A., Kourkoumelis, N., Stanojkovic, T. P., & Kovala-Demertzi, D. (2014). Cytotoxic activity of essential oil and extracts of *Ocimum basilicum* against human carcinoma cells. Molecular docking study of isoeugenol as a potent cox and lox inhibitor. *Digest Journal of Nanomaterials and Biostructures*, *9*(3), 907–917.

Zotti-Sperotto, N. C., Melo, E. de C., de Souza, M. I. L., Fonseca, M. C. M., Gonzaga, D. A., de Ávila, M. B. R., Demuner, A. J., Ventrella, M. C., Lelis, A. C. V. (2020). Effect of drying with ultrasonic pretreatment on the yield and quality of the essential oil of Varronia curassavica Jacq. and Ocimum gratissimum Linn. *Industrial Crops and Products*, *147*(January), 112211. https://doi.org/10.1016/j.indcrop.2020.112211