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Antioxidant and antibacterial activity of *Curcuma* spp. rhizome essential oil

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Abstract *Curcuma xanthorrhiza* Roxb. (temulawak) rhizome essential oil showed the highest antioxidant activity (IC_{50}) 380.95±18.49 ppm, with total phenolic 63.95±2.53 mg GAE/g, and total flavonoid 26.21±0.86 mg QE/g. *Curcuma xanthorrhiza* Roxb. rhizome essential oil showed the highest antibacterial activity with inhibition zone 11.93±0.43 mm towards *Eschericia coli*, inhibition zone 12.63±0.31 mm towards *Staphylococcus aureus*, MIC value 1.17% and MBC value 4.68% towards *Eschericia coli*, MIC value 1.13% and MBC value 4.52% towards *Staphylococcus aureus*. The main components of *Curcuma xanthorrhiza* Roxb. rhizome essential oil were p-cymene, ar-curcumene, 3,7-cyclodecadiene-1-one, and xanthorrhizol. The main components of *Curcuma longa* (turmeric) and *Curcuma purpurascens* (temu tis) rhizome essential oil were turmerone and curlone.

Keywords: Antibacterial, Antioxidant, *Curcuma* spp., Essential oil, Hydrodistillation

Introduction

Curcuma xanthorrhiza (temulawak) is one of *Zingiberaceae* with main components consisting of curcuminoid and essential oil. The main components of essential oil are xanthorrhizol, α-curcumin, β-curcumin, turmerone, cineol, and ar-turmerone. According to Jantan *et al*. (2012), *Curcuma xanthorrhiza* (temulawak) essential oil consists of curcuminoid, bisdemethoxycurcumin, and demethoxycurcumin which can act as an antioxidant, while xanthorrhizol is a compound that has capacity as an antibacterial agent.

Curcuma longa (turmeric) is one of the rhizomes that is usually used as a coloring and flavoring agent in food and beverage products. *Curcuma longa* (turmeric) main components consist of curcuminoid, bisdemethoxycurcumin, demethoxycurcumin, fat, protein, and essential oil. *Curcuma longa* (turmeric) essential oil has some bioactive compounds such as curcuminoid, α-turmerone, α- and β-tumeron, bisdemethoxycurcumin, demethoxycurcumin, tumerol, α-atlanton, β-caryophyllene, linalol and 1,8 cineol (Shan and Iskandar, 2018).

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Based on Suparmajid *et al*. (2016), *Curcuma longa* (turmeric) exhibited antioxidant activity caused by its active compounds such as ascorbic acid, β-carotene, caffeic acid, curcumin, eugenol, and p-coumaric acid. Not only is a coloring agent, but curcumin also can act as an antibacterial agent (Nadifah *et al*., 2017).

Curcuma purpurascens (temu tis) is one of the plants that can be found in Solo and Yogyakarta, Indonesia. The main bioactive compounds of *Curcuma purpurascens* are ar-turmerone, turmerone, and curlone (Rouhollahi *et al*., 2015). *Curcuma purpurascens* essential oil exhibited antioxidant activity due to its bioactive compounds such as ar-turmerone and turmerone (Stanojević *et al*., 2015).

The essential oil contains various volatile compounds including terpenes, alcohol, esters, aldehydes, ketones, lactones, coumarins, and ethers. Generally, essential oils can easily be degraded by oxygen, light, and heat (Preedy, 2016). Essential oils can be extracted using the hydrodistillation method. This method has the advantage of being able to process large amounts of solvent. A higher yield of essential oil can be obtained from herbs and spices using the hydrodistillation process (Hashemi *et al*., 2018). In the hydrodistillation method, water is used as a solvent, which can reduce the loss of volatile compounds that are bound with organic solvents (Preedy, 2016).

The aim of this research was to investigate the antioxidant and antibacterial capacity of *Curcuma* spp. rhizome essential oil. *Curcuma* spp. rhizomes such as *Curcuma xanthorrhiza* Roxb. *(*temulawak*), Curcuma longa* (turmeric), and *Curcuma purpurascens (*temu tis*)* were extracted by hydrodistillation method, then their antioxidant and antibacterial activity were assayed using DPPH and disc diffusion method. The chemical composition of the obtained essential oil was analyzed using the GC-MS method.

Materials and methods

The main material used in this research were *Curcuma xanthorrhiza* (*temulawak*), *Curcuma longa* (turmeric), and *Curcuma purpurascens* (*temu tis*) rhizomes.

Hydrodistillation essential oil

The rhizome was dried in the cabinet dryer at 50^{o}C and 24 hours. The dried rhizome was put into the blender until the texture changed into powder. In the next step, the powder was weighed and dissolved using aquadest with a ratio of 1:12 in the flat bottom flask that connected to the Dean-Stark tube. The heating

process was carried out at 100ºC and around 6 hours or more until the formation of two layers of essential oil and water. The mixture of essential oil and water was separated by using a separating funnel.

Experimental design

The experimental design used in this study was a three-factor factorial experiment in a completely randomized design and four replications. The variable was the type of *Curcuma* spp. rhizome. The different type of *Curcuma* spp. rhizome was *Curcuma xanthorrhiza* (temulawak)*, Curcuma longa* (turmeric) *and Curcuma purpurascens* (temu tis). All obtained data were analyzed by ANOVA using SPSS version 20.

Identification of plants

Family and species identification of *Curcuma xanthorrhiza* Roxb*., Curcuma longa* L. *and Curcuma purpurascens* Blume (Family Zingiberaceae) plant was determined by the Research Center of Biology, Indonesian Institute of Sciences (LIPI), Bogor, Indonesia.

Parameter of analysis

In this study, an analysis was carried out in the form of moisture content yield (AOAC, 2005), phytochemical screening (Sulaisyah and Aminin, 2018), total phenolic content (Tahir *et al*., 2017), total flavonoid content (Ahmad *et al*., 2015), antioxidant activity (Sandhiutami and Indrayani, 2012), antibacterial activity (Yunita *et al*., 2015), and Chemical composition using GC-MS method (Sukrasno *et al*., 2012).

Results

Phytochemical analysis of Curcuma spp. rhizome essential oil

The phytochemical analysis of *Curcuma* spp. rhizomes essential oil revealed the presence of some bioactive compounds such as alkaloid, saponin, polyphenols, flavonoid, triterpenoid and glicosides as shown in Table 1.

Table 1. Phytochemical screening results of *Curcuma* spp. essential oil

Chemical compound	*Curcuma xanthorrhiza (temulawak)*	*Curcuma longa* (turmeric)	*Curcuma purpurascens (temu tis)*
alkaloid	+	+	+
Saponin	+	+	+
Tanin	-	-	-
Polyphenols	+	+	+
Flavonoid	+	+	+
Triterpenoid	+	+	+
Steroid	-	-	-
Glycosides	+	+	+

Note: (+): detected, (-): not detected

Characteristics of Curcuma spp. rhizome essential oil

Characteristics of essential oils such as yield (%), color, and specific gravity indirectly indicate the quality of oils. The characteristics of *Curcuma* spp. rhizome essential oil can be observed in Table 2.

Table 2. Physicochemical characteristics of *Curcuma* spp. essential oil

Type of *Curcuma* spp. rhizomes.	yield (%)	essential oil color	specific gravity (g/mL)
Curcuma xanthorrhiza (temulawak)	2.02±0.0003	Clear yellow	0.9349
Curcuma longa (turmeric)	0.85±0.0001	Clear yellow	0.8855
Curcuma purpurascens (temu tis)	2.18±0.0004	Colorless	0.9098

Antioxidant activity of Curcuma spp. rhizomes essential oil

The highest total phenolic is in *Curcuma xanthorrhiza (*temulawak) essential oil (Table 3). The results of One-Way ANOVA statistical analysis showed that the type of the *Curcuma* spp. rhizome has a significant effect ($p < 0.05$) on the total phenolic of essential oils.

Table 3. Antioxidant activity of *Curcuma* spp. rhizomes essential oil

Type of *Curcuma* spp. rhizomes.	total phenolics (mg GAE/g)	total flavonoid (mg QE/g)	antioxidant activity-IC_{50} (ppm)
Curcuma xanthorrhiza (temulawak)	63.95 ± 2.53^{c}	26.21 ± 0.86^{c}	380.95 ± 18.49^{a}
Curcuma longa (turmeric)	30.65 ± 0.93^{b}	20.96 ± 0.87^{b}	542.97 ± 12.63^{b}
Curcuma purpurascens (temu tis)	26.43 ± 1.08^{a}	14.32 ± 0.53^{a}	901.94 ± 19.42^{c}

Notes: values with different letters within a column are significantly different ($p<0.05$)

Antibacterial activity of Curcuma spp. rhizomes essential oil

It can be seen that *Curcuma xanthorrhiza* (temulawak) essential oil showed the highest inhibition zone diameter both for gram-positive *Staphylococcus aureus* and gram-negative bacteria, *Eschericia coli* (Table 4). The results of the One-Way ANOVA statistical analysis showed that the higher concentration of essential oils was significantly affected ($p < 0.05$) on the diameter of the inhibitory zone of essential oils.

Table 4. Antibacterial activity of *Curcuma* spp. essential oil

Type of *Curcuma* spp. rhizomes	inhibition zone diameter to *Eschericia coli* (mm)	inhibition zone diameter to *Staphylococcus aureus* (mm)
Curcuma xanthorrhiza (temulawak)	11.93 ± 0.43^{c}	12.63 ± 0.31^{c}
Curcuma longa (turmeric)	9.92 ± 0.30^{a}	10.65 ± 0.35^{a}
Curcuma purpurascens (temu tis)	10.42 ± 0.50^{b}	12.36 ± 0.33^{b}

Notes: values with different letters within a column are significantly different ($p<0.05$)

It can be seen that the best antibacterial activity is in *Curcuma xanthorrhiza (temulawak)* essential oil because it has the largest inhibitory zone diameter for both Gram-positive *Staphylococcus aureus* bacteria and Gram-negative *Escherichia coli* bacteria (Table 5).

Table 5. MIC and MBC values of *Curcuma* spp. essential oil

Bacteria	essential Oil	MIC (%)	MBC (%)
Eschericia coli	*Curcuma xanthorrhiza (temulawak)*	1.17	4.68
	Curcuma longa (turmeric)	2.38	9.51
	Curcuma purpurascens (temu tis)	1.39	5.56
Staphylococcus aureus	*Curcuma xanthorrhiza (temulawak)*	1.13	4.52
	Curcuma longa (turmeric)	1.41	5.64
	Curcuma purpurascens (temu tis)	1.17	4.67

Chemical composition of Curcuma spp. essential oil

The results of the GC-MS analysis can be seen in Tables 6,7 and 8. It showed the chemical components of essential oil. Temulawak essential oil was found to contain the main active compounds p-cymene, ar-curcumene, 3,7-cyclodecadiene-1-one, and xanthorrhizol.

Table 6. Chemical components in *Curcuma xanthorrhiza (temulawak)* essential oil

peak	retention time (minute)	% area	compounds	quality (%)
1	4,738	1,68	*Camphor*	95
4	8,226	0,53	*1-Methyl-2,4-bis (1-Methyehtylidene)-1-vinylcyclohexane)*	95
5	8,431	0,94	*β-Farnesene*	96
6	8,747	0,36	*γ-Curcumene*	94
7	8,850	36,15	*Ar-Curcumene*	97
8	9,055	4,29	*Benzofuran*	98
9	9,192	36,93	*p-cymene*	80
13	11,397	5,88	*3,7-Cyclodecadien-1-one*	86
14	11,919	5,42	*Xanthorrhizol*	70

The main compounds identified in *Curcuma longa* (turmeric) essential oil are turmerone and curlone compounds (Table 7), where these two compounds showed a larger peak area than the other compounds, namely 77.09% and 13.03%.

Table 7. Organic compounds in *Curcuma longa* (turmeric) essential oil

peak	retention time (minute)	% area	compounds	quality (%)
1	3,216	0,38	*α-Phellandrene*	90
2	3,481	0,85	*Eucalyptol*	97
4	8,799	0,79	*Ar-Curcumene*	81
5	8,944	2,08	*α-Zingiberene*	89
6	9,089	0,15	*β-Bisabolene*	89
7	9,294	1,45	*β-Farnesene*	89
8	9,987	0,26	*1,2-Methyl-2-isopropylbenzene*	90
11	11,107	77,09	*Turmerone*	81
12	11,423	13,03	*Curlone*	95

Curcuma purpurascens (temu tis) essential oils found a component of the main compounds identified as turmerone and curlone (Table 8), which had a peak area of 43.49% and 15.88% respectively.

Table 8. Organic compounds in *Curcuma purpurascens (temu tis)* essential oil

peak	retention time (minute)	% area	compounds	*quality* (%)
1	3,481	4,02	*Terpan*	95
2	4,046	3,99	*α-Terpinolene*	96
3	4,738	3,56	*Camphor*	96
4	7,183	0,89	*1-Ethoxy-4-ethylbenzene*	91
5	7,705	0,97	*α-Myrcene*	81
6	8,089	1,70	*Caryophyllene*	95
7	8,217	1,14	*γ-Elemene*	95
8	8,799	2,22	*Ar-curcumene*	93
9	8,935	3,54	*α-Zingiberene*	90
10	9,038	4,46	*Benzofuran*	98
11	9,286	3,38	*Cyclohexene*	91
17	11,047	43,49	*Turmerone*	91
19	11,380	15,88	*Curlone*	94

Discussion

Phytochemical analysis was carried out to determine whether there was any bioactive compound present in the plant. Result of phytochemical analysis of *Curcuma* spp. rhizome essential oil showed the presence of some bioactive compounds such as alkaloids, saponins, polyphenols, flavonoids, triterpenoids, and glycosides. Alkaloids, saponins, polyphenols, and flavonoids have been shown to exhibit antioxidant, and antimicrobial activities (Anjusha and Gangaprasad, 2014). The phytochemical content of essential oil could be influenced by soil type, planting season, and mineral content of the soil used for growing rhizomes (Koomson *et al.*, 2018).

The highest yield of essential oils was 2.18% with a colorless color and a specific gravity of 0.9098 g/mL. According to Setyawan (2003), *Curcuma xanthorrhiza* (temulawak) has an essential oil content of 4-6%, *Curcuma longa* (turmeric) has an essential oil content of 1.5-2.5% and *Curcuma purpurascens* (temu tis) has an essential oil content of 2-3%. Differences in essential oil content could be influenced by several things including harvest age, plant parts that are being used, harvest season and climate, varieties or species of plants, isolation methods, and other environmental factors (Setyawan, 2003).

The total phenolic content of *Curcuma longa* (turmeric) essential oil is lower than the *Curcuma xanthorrhiza's* (temulawak) because the content of phenolic compounds in *Curcuma longa* (turmeric) essential oil only consists of bisdemethoxycurcumin (Setyowati and Suryani, 2013). While *Curcuma purpurascens* (temu tis) essential oils have a phenolic content in the form of curcumin and bisacuron (Vitasari *et al.*, 2016). The highest total flavonoid is in

Curcuma xanthorrhiza (temulawak) essential oil. The best antioxidant activity (IC_{50}) is found in *Curcuma xanthorrhiza* (temulawak*)* essential oil, followed by *Curcuma longa* (turmeric) essential oil and *Curcuma purpurascens* (temu tis) essential oil.

Curcuma xanthorrhiza (temulawak) essential oil was found to be the highest inhibition zone diameter for either gram-positive, *Staphylococcus aureus* or gram-negative bacteria *Escherichia coli*. Components in temulawak essential oils that play an important role as antibacterial are terpenoids and phenols. The antibacterial activity of terpenoids involves the breakdown of membranes by lipophilic compounds. Whereas phenolic component is able to act as an antibacterial compound through enzyme inhibition by reaction with sulfhydryl groups or through interactions with protein microorganisms. The best MIC and MBC were shown by *Curcuma xanthorrhiza* (temulawak) essential oil. According to Diastuti (2014), *Curcuma xanthorrhiza* (temulawak) essential oil contains xanthorrhizol and curcumene which play a role in inhibiting the growth of gram-positive and gram-negative bacteria. Essential oils are more active against gram-positive bacteria than gram-negative bacteria because gram-negative bacteria have an outer membrane that prevents the diffusion of hydrophobic compounds across the bacterial lipopolysaccharide layer (Nugraheni *et al.,* 2017).

Curcuma xanthorrhiza (temulawak) essential oil yielded 2.02% with a clear yellow color and a specific gravity of 0.9349 g/mL. *Curcuma longa* (turmeric) essential oil yielded 0.85% with a clear yellow color and the specific gravity was 0.8855 g/mL. *Curcuma purpurascens (temu tis)* essential oil yielded 2.18% with colorless color and a specific gravity of 0.9098 g/mL.

The best antioxidant activity found in *Curcuma xanthorrhiza* (temulawak) essential oil with a total phenolic content of 63.95 ± 2.53 mg GAE/g, total flavonoids 26.21 ± 0.86 mg QE/g, and antioxidant activity (IC_{50}) 380.95 ± 18, 49 ppm. The best antibacterial activity was found in *Curcuma xanthorrhiza* (temulawak) essential oil with diameter of inhibition zone 10.13 ± 0.22 to 14.18 ± 0.69 mm at different concentrations towards *Eschericia coli*, diameter of inhibition zone 10.56 ± 0.33 to 14, 61 ± 0.14 mm at different concentrations towards *Staphylococcus aureus*, MIC value 1.17% and MBC value 4.68% for *Eschericia coli*, and MIC value 1.13% and MBC value 4.52% for *Staphylococcus aureus*.

The main chemical compounds in the *Curcuma xanthorrhiza* (temulawak) essential oils are found to be p-cymene, ar-curcumene, 3,7-cyclodecadiene-1-one, and xanthorrhizol. *Curcuma longa* (turmeric) and *Curcuma purpurascens* (temu tis) essential oil contain turmerone and curlone

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