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Screening of Aroma Compounds in Commercial Tea (*Camellia sinensis*) from Indonesia and Testing Their Activity as Antioxidants

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Abstract

Tea from tea leaves of the *Camellia sinensis* species is a superior industrial plant that promises superior bioactivity. For a more in-depth study of the essential oils contained in commercial teas on the market, their significant characteristic from their constituent compounds were determined. The essential oil yields of the three commercial samples were obtained by distillation using a Liken-Nickerson vessel and analyzed by gas chromatography-mass spectrometry (GC-MS), then tested for antioxidant properties by the DPPH method. The yield obtained ranged from 0.08-0.13%. The main major components (more than 0.5% identified) contained in the three samples identified were benzyl acetate, between 4,56-26.27%, and linalool compounds from tea leaves, as much as 20.59%, and 3.42% in samples C and B which were not found in sample A. As shown by our results, antioxidant activity significantly correlated with benzyl acetate and linalool composition. Sample A showed the best antioxidant profile, with highest inhibition (IC_{50} 20.19 mg/mL).

Keywords: tea, essential oil, isolation, chemical composition, antioxidant

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1 Introduction

Tea is one of the leading agricultural commodities in Indonesia and plays a role in contributing to the economy in the plantation sub-sector [1]. Tea is the oldest traditional drink, is in great demand, contains non-alcoholic caffeine, and is most widely consumed by people worldwide after water, so tea is an item exported to the international market [2]. Indonesia plays a vital role in the tea export market and occupies the fifth position, with an average export volume of 90 thousand tons annually [3]. The uniqueness of the aroma and taste, supported by tea's bioactivity, makes it a popular and valuable source of ingredients in various sectors of the food and beverage industry, medicine, and cosmetics [4].

Based on the type of processing, the teas commonly consumed are divided into four types, namely black, green, oolong, and white teas, with the highest consumption composition being black tea at 69%, while the rest are other types of tea [5]. The popularity of black tea consumption by Indonesian people is due to easy processing and abundant availability. The process of making black tea is carried out by enzymatic oxidation. The polyphenol oxidase enzyme catalyzes the catechin compounds in tea to produce theaflavin and thearubigin compounds [6].

Tea essential oil is generally obtained from the distillation process of the leaves of the Camellia sinensis tea plant [7]. The quality of Tea essential oil is influenced by various things, such as the method of picking, the part of the tea that is picked, and the tea leaves collecting and processing method [8]. The aroma compound in tea is the key to the quality of tea because it is an aspect of the judgment that determines acceptance or rejection before the tea is tasted. Aspects of tea assessment, such as color, aroma, and taste of tea, are influenced by the composition and concentration of the chemical compounds contained therein [9]. Tea leaf extract is rich in monoterpenes, terpene alcohol, sesquiterpenes, and phenolic compounds, which contribute to the characteristic aroma of tea [7].

Along with the development of the use of natural ingredients as antioxidants in several decades, tea considered to be excellent because of the various components of chemical compounds in it [4]. As previously reported, tea leaf extract (volatile and non-volatile) has excellent antioxidant activity (less than 52 µg/ml) [10]. Meanwhile, essential oils (volatile compounds) are known for their broad bioactivity, such as antioxidants, anti-inflammatories, anticancer, and others. In addition, the massive commodity and role of Indonesian tea commercial products globally make it fascinating to analyze aroma components that focus on the essential oils found in commercial teas and test their activity as antioxidants.

2 Experimental section

2.1 Plant materials and reagents

Samples of commercial tea leaves are three types of tea leaves sold in markets in the Malang area. As a control, healthy and pest-free jasmine tea plants are taken from tea plantations in Wonosari Lawang, East Java, in Indonesia. Wet tea leaves obtained are chopped and then oxidized (temperature 85-100 ºC) until dry tea leaves are obtained. Other ingredients are 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and solvent Dichloromethane (DCM, ethanol was purchased from Analytical Grade and purchased from Sigma Aldrich)

2.2 Essential oil isolation

Samples of commercial and control tea leaves (500 g) were distilled in a Liken-Nickerson vessel (distillation-extraction) for 4 hours, respectively. DCM solvent was used to obtain tea essential oil extract, then separated from water in the distillation results using a

separatory funnel. The essential oil was concentrated using a rotary evaporator, and the remaining solvent was evaporated with a stream of N_2 gas. The isolated samples were then analyzed using a Gas Chromatography-Mass Spectrometer (GC-MS) on a gas chromatography system (GC, Agilent 7890B) and a mass spectrometer (MS, Agilent 5977B)

2.3 DPPH antioxidant activity test

Testing the free radical scavenging potential of 1,1-diphenyl-2-picrylhydrazyl (DPPH) from tea leaf essential oil was carried out by adopting the method from [11] with several modifications. A certain amount of DPPH solution (0.1 mmol) was dissolved from 3.94 mg of solid DPPH in 100 mL of ethanol. 2 mL of DPPH solution was mixed with essential oil solutions with concentration series of 32.0, 16.0, 8.0, 4.0, 2.0, 1.0 and 0.5 mg/mL then incubated for 30 minutes in the gel at room temperature. Absorbance readings for each concentration were taken at a wavelength of 517 nm using a UV-Vis spectrophotometer. Ascorbic acid was used as a positive control, and ethanol was measured as a negative control. All spectrophotometric data were performed in 3 replicates and analyzed. Antioxidant activity is expressed by inhibiting the essential oil concentration required to inhibit 50% DPPH radical solution or IC_{50}. The formula calculated the inhibition value: inhibition (%) = $(A_0 - A_1)/A_0 \times 100\%$, where A_0 is the absorbance of the DPPH solution blank, and A_1 is the absorbance of the sample. IC_{50} was estimated from inhibition versus concentration plots using a non-linear regression algorithm. The best activity of Tea essential oil against DPPH radicals was obtained from the lowest IC50 value.

3 Results and Discussion

3.1 Yield of Tea Essential Oil

Three commercial tea leaves were used, which were stated as samples A, B, and C. The tea leaves used had color characteristics. Sample A was brownish-black, while samples B and C were brownish green. The aroma produced from tea leaves before being processed in sample A tends to smell of flowers and wood, while samples B and C give off a sweet and jasmine aroma. In addition, the leaves of sample A tend to be brittle, while the leaves of samples B and C tend to be quite strong. To control the wet tea leaves used before being oxidized are green in color. After being oxidized at a temperature of 85-100 oC, the tea leaves are brownish green. After distillation with a Liken-Nickerson vessel, the distillate mixed with DCM was obtained. After concentration, the essential oil obtained had a physical appearance in the form of a yellow to brownish-yellow liquid.

Table 1 Essential Oil Yield

Sample	%Yield
A	0.131
B	0.078
C	0.078
Control	0.006

The essential oil from commercial tea leaves was weighed, and the yield percent obtained is shown in Table 1, with a range between 0.078% and 0.131%. The control sample produces the lowest % yield of only 0.006%. The highest yield obtained was in sample A at 0.0131%, more than in samples B and C due to the fragile physical characteristics of the leaves. Separated tea leaves provide a larger surface area so that the essential oil of sample A tea leaves is more easily separated during the distillation process. In addition, the distillation-extraction method using a Liken-Nickerson vessel in this study gave relevant results where the yield of tea leaf essential oil isolates obtained was 0.09% to 0.63% (v/w) [12]. This shows that the distillation-extraction method using a modified Likens Nickerson vessel can increase the essential oil isolates of tea leaves by extracting the essential oil distillate directly during the reaction.

3.2 Chemical Components of Essential Oils

After integrating the chromatograms and identifying the components of the tea essential oil compounds from 3 commercial samples and one control sample, with concentrations above 0.5%, they were grouped into the essential oil compounds shown in Table 2. Based on the results obtained, the highest component from sample A, Benzyl acetate (26.27%), is also the main component in samples B and C. In sample B, the large number of compounds causes the

highest compound content not to exceed 5%, where the highest component in sample B is Linalool (3.42%), Citral (3.17%), and Methyl-anthranilate (3.35%). Linalool was also found as a component with the highest percentage in sample C of 20.59%. As a comparison, the control sample also found the highest component, namely linalool, at 31.94%. This indicates that linalool can be found in most test samples.

Table 2 Tea essential oil composition

No	Compound	Retention time (min)	% Composition			
			A	B	C	Control
1	Linalool	6,931	-	3,42	20,59	31,94
2	5-Methylfurfural	7,316	-	1,45	-	-
3	Benzoic acid	7,724	-	1,67	-	-
4	α-terpineol	8,224	-	2,05	-	-
5	Benzyl acetate	8,522	26,27	2,05	24,04	-
6	trans-linalool oxide	8,573	-	-	-	12,51
7	Citronelol	8,673	-	2,15	-	5,25
8	Citral	8,96	-	3,17	-	-
9	2-phenylmethyl ester	9,173	-	2,51	-	-
10	Geraniol	9,286	-	3,1	-	7,41
11	α-ionene	9,428	-	0,88	-	-
12	Benzyl alcohol	9,61	-	1,73	-	-
13	Phenylethyl-alcohol	9,868	-	2,3	-	-
14	2-Cyclopenten-1-one, 3-methyl-2-(2Z)-2-pentenyl-	9,988	-	1,97	-	-
15	p-Cresol	11,016	-	1,37	-	-
16	*3,Hexen-1-ol, benzoat*	11,309	-	2,69	-	-
17	Isoeugenol acetate	11,552	-	1,84	10,86	-
18	2-methoxy naphtalene	11,795	-	1,37	-	-
19	α-Cadinol	11,959	-	0,91	-	-
20	Methyl-anthranilate	12,087	-	3,35	-	-
21	Tetracosane	12,724	5,84	-	-	-
22	2-Hexyldecanol	12,856	-	0,58	-	-
23	2-methyl pentacosane	13,85	-	0,85	-	-
24	Eicosane	13,849	5,36	-	-	-
25	Hexacosane	13,849	5,78	-	-	-
26	Hexadecanol	13,981	5,59	-	-	-
27	Fitol	13,985	-	2,9	3,05	-
28	Benzyl benzoate	14,33	-	1,69	-	-
29	2-methyl hexacosane	14,878	-	0,78	-	-
30	Octacosane	15,366	-	0,78	-	-
	Total non-essential oil compounds		12,39	5,15	0	1,59

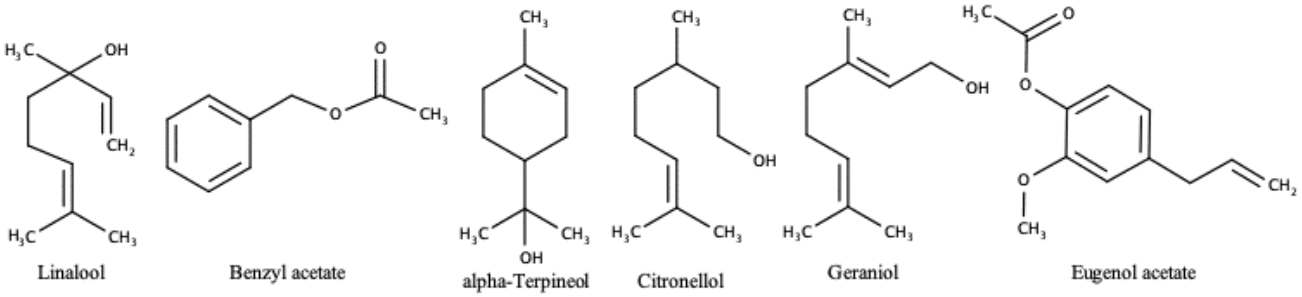

Figure 1 Chemical structure of the main components of tea essential oil

Other components contained in tea leaf essential oil from this study were: α-terpineol, citronellol, geraniol, phytol, and eugenol acetate. The types of tea leaf essential oil components detected in this study were also found in studies of jasmine tea leaf essential oil conducted by [13] and [14]. When compared research on essential oils from various tea leaves without mixing jasmine flowers by [15] and [16] showed different results where benzyl

acetate compounds were not found, while compounds such as linalool, citronellol, and phytol are found in high levels [15], [16]. Thus, it can be stated that the benzyl acetate compound detected in the essential oil of tea leaves in this study is thought to have come from mixed jasmine flowers. Figure 1 shows the structure of the main components of tea essential oil compounds.

3.3 Antioxidant Activity of Essential Oils

The primary analysis of the radical scavenging activity of essential oils was carried out on DPPH from three commercial tea samples and one control sample. The IC_{50} values are shown in Table 3, and ascorbic acid (Vit C) was used as a standard comparison. The principle of measuring the antioxidant activity of this method is based on the absorbance value after DPPH is added to the essential oil solution. Essential oil solutions inhibit free radicals in DPPH, so they change the color of DPPH to violet. The color change indicated an inhibition reaction of tea leaf essential oil with some DPPH solution. The IC_{50} value of tea leaf essential oil was obtained by calculating the linear regression equation of the curve between ln concentration and % inhibition. The level of antioxidant activity of the samples can be expressed into very weak categories (IC_{50} > 200 µg/mL), weak (IC_{50} 150-200 µg/mL), moderate (IC_{50} 100-150 µg/mL), strong (IC_{50} 50-100 µg/mL). mL), and very strong (IC_{50} <50).

Table 3 DPPH Radical Scavenging Activity

Sample	IC_{50} (µg/mL)	Activity category
A	20.19	Very strong
B	44.33	Very strong
C	49.70	Very strong
Control	356.68	Weak
Ascorbic acid	1.30	Very strong

The results in Table 3 show that the IC_{50} value of tea leaf sample A has the highest antioxidant properties (IC_{50} 20.19 µg/mL), which is indicated by the lowest IC_{50} value. The IC_{50} value is still higher than the IC_{50} of ascorbic acid, but sample A is still classified as having very strong antioxidant properties and can inhibit DPPH free radicals. Meanwhile, samples B and C had lower antioxidant activity than sample A, and both were still relatively strong in inhibiting DPPH free radical compounds. The antioxidant activity of tea leaf essential oil is influenced by its constituent compounds. The linalool compound in sample C is thought to reduce the antioxidant properties of commercial tea leaf essential oil compared to sample A. Besides that, the antioxidant properties of sample B can be affected by the low levels of the benzyl acetate compound so that the DPPH free radical inhibition process decreases. The same results were shown in the control sample, where no measurable benzyl acetate content accompanied a high linalool value of 31.94%. Based on the GC-MS results, benzyl acetate is a compound contained in three types of tea leaves. It has an area % of more than 24% in samples A and C, so it is suspected that the benzyl acetate compound can inhibit free radicals from DPPH solutions.

Benzyl acetate is an ester compound with the chemical formula $C_9H_{10}O_2$. This compound is colorless and has a sweet and fragrant aroma [17]. The content of benzyl acetate compounds can be found in jasmine, water hyacinth, and gardenia flowers. Benzyl acetate has also been widely used in the fragrance industry [16]. Benzyl acetate compound is the main compound of jasmine tea leaf essential oil. Research on the essential oil of jasmine tea previously mentioned that the main components include linalool, benzyl acetate, alpha-farnesene, benzyl alcohol, and methyl acetate [14]. Other research states that the components of tea leaf essential oil mixed with jasmine contain linalool compounds of approximately 17-19%, 24% benzyl acetate compounds, followed by other compounds such as benzyl alcohol, methyl anthranilate, and indole below 10% [1]. 13]. This result shows that apart from aroma being influenced by chemical components, antioxidant activity is also influenced by the compounds that make up the essential oil.

4 Conclusions

In this research, we studied the oil yield, essential oil composition, and antioxidant activities of three commercial teas from Indonesia compared to local tea leaves from the Malang region, East Java. Different sources produced a comparative analysis to explore the relationship between antioxidant activity and the main constituents of essential oils. The main

component of essential oils from the three commercial samples is benzyl acetate, with a concentration of around 3.42 - 26.27%. Commercial tea sample variety A had the highest benzyl acetate content of the other three samples. It significantly affected its antioxidant activity, so the IC_{50} value of 20.19 µg/mL was obtained in the DPPH test. The three commercial essential oil samples provided very strong antioxidant activity, showing a positive correlation between the constituents of benzyl acetate and linalool essential oils in tea.

5 Declarations

5.1 Acknowledgements

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5.2 Author contribution

Warsito: Conceptualization, methodology, writing draft. M. Fadel Alief: Carried out experiment essential oil isolation. Vina Octavia Azzahra: Carried out experiment DPPH antioxidant activity test. M.Farid Rahman: Reviewing and editing. Rurini Retnowati: supervised the project.

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5.4 Conflict of Interest

The authors declare there is no conflict of interest in this study.

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