

ABTS ASSESSMENT AND CHEMICAL PROFILING OF Syzygium aromaticum and Cananga odorata ESSENTIAL OILS FOR ANTIOXIDANT POTENTIAL IN EAST KALIMANTAN

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ABSTRACT. This present study was to explore the antioxidant activity and total phenolic compound of two essential oils from East Kalimantan. Essential oils used in this research are *Syzygium aromaticum* leaves and *Cananga odorata* flowers. Chemical profiling of *S. aromaticum* leaves by GC-MS showed that eugenol (71.97%) and trans (Beta)-Caryophyllene (23.48%) are the major components. Whereas in *C. odorata* flowers, the major constituents are trans-caryophyllene (26.91%), Germacrene (18.15%), and alpha-bergamotene (12.41%). The Folin-Ciocalteu technique was used to determine the plant's total phenolic content. The results of a total phenolic compound of gallic acid equivalents (GAE)/mg 115.20 mg GAE/g and 9.67 mg GAE/g, respectively. The free radical ABTS was utilized to investigate antioxidant activity with IC₅₀ values of *S. aromaticum* leaves and *C. odorata* flowers should be ascorbic acid was 5.05 μ g/mL. The finding suggests that an antioxidant test using ABTS is suitable for essential oils and *S. aromaticum* leaves has the potency for natural antioxidants.

Keywords: Essential oils, Syzygium aromaticum, Cananga odorata, ABTS

INTRODUCTION

Essential oils have been widely utilized in the pharmaceutical, cosmetic, and food industries. Natural antioxidants derived from plants have recently surpassed synthetic antioxidants in popularity [1]. Essential oils can function as a source of antioxidants that come from nature [2], so that the use of essential oils is increasing throughout the world. Awareness of the health risks posed by synthetic chemicals used in food has strengthened the search for natural sources of antimicrobials and antioxidants. The increased incidence of undesirable aspects such as carcinogenicity, teratogenicity and slow degradation is largely related to the use of chemicals to control food spoilage such as synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) [3]. Their replacement with relatively less harmful compounds has taken research in the food industry to a new level. Essential oils or compounds obtained from plants have been accepted by the industry as having little or no danger to human health. So, it can be used as a substitute for synthetic antimicrobials and a potential antioxidant.

Essential oils that have potential in the pharmaceutical and food industries include *Cananga odorata* and *Syzygium aromaticum*. *Cananga odorata* includes family the Annonaceae not only is it a vital raw resource in the perfume industry, but it is also thought to be a potentially valuable plant in agriculture and medicine [4]. There are several uses of *C. odorata* in several countries, for example in India, people use *C*.

odorata leaves for treat dandruff and topical direct application for ease the itching. In Indonesia, the cananga oils use as boost euphoria feel during sex and also lower the sexual tension [5]. *Syzygium aromaticum* is a valuable spice that has been used for generations as a food preservative and for a variety of therapeutic applications. Clove is indigenous to Indonesia, but it is now grown in many parts of the world, including the Brazilian state of Bahia. This plant holds significant promise for uses in medicine, cosmetics, food, and agriculture because to its high concentration of phenolic chemicals such eugenol, eugenol acetate, and gallic acid. The most important studies on the biological activities of clove and eugenol are covered in this overview. Clove deserves special attention because it contains more potent antioxidant and antibacterial properties than a lot of other fruits, vegetables, and spices [6].

There are various ways to measure the antioxidant activity of substances, and one of those methods is the ABTS test. This test evaluates the ability of antioxidants to neutralize the 2,2'-azinobis (3- ethylbenzthiazolin-6-sulfonic acid) ABTS++ stable radical cation, which appears as a blue-green chromophore [7]. The extent of the blue-green color fading, which is measured by a sudden decrease in absorbance at 734 nm, is dependent on the reaction time, intrinsic antioxidant activity, and sample concentration. Potassium persulfate is the most commonly used oxidant for generating ABTS++ [8]. The ABTS test is a mixed-mode test that typically involves hydrogen atom transfer, electron transfer, and proton-coupled electron transfer (PCET) mechanisms that may perform different functions in varying proportions, depending on the reaction conditions, such as the pH and solvent [9].

Considerable research has examined the chemical composition and antioxidant properties of essential oils derived from *S. aromaticum* and *C. odorata*. However, the ABTS test for essential oils extracted from *S. aromaticum* leaves and *C. odorata* flowers in East Kalimantan has yet to be conducted. This study aims to address this knowledge gap and provide a comprehensive analysis.

MATERIALS AND METHODS

Material and chemicals

Samples of aromatic plants were gathered from several locations in East Kalimantan. The leaves of *S. aromaticum* from Samarinda, and *C. odorata* flowers were collected from Tenggarong, Kutai Kartanegara, East Kalimantan province. Gallic acid, quercetin, Ascorbic acid, methanol, sodium carbonate (Na2CO3), Folin-Ciocalteu reagent was purchased from Merck (Darmstadt, Germany). The ABTS reagent (2,2'-azinobis-(3-ethylbenzothiaziline-6-sulfonate), Potassium Persulfate (K2S2O8), MgSO4.

Distillation method

Water and steam distillation were used to gather the essential oils. The water steam distillation method was adopted from [10] with slight modifications. Water-steam distillation was performed by running steam through a distillation stainless kettle containing dried plant material with different weight of samples and distilled water for 3 hours, afterward collecting the condensate (water and oil) in at Erlenmeyer flask. To eliminate moisture, magnesium sulphate was applied to the flask. Weighed and maintained at 4°C in a hermetically sealed glass flask for further analysis, the generated essential oil was measured. In Table 1, the amounts of essential oils were determined based on the dry weight of the plant material and expressed as a percentage (w/w).

Determination of total phenolics

The Folin-Ciocalteu test was used to determine the total phenolic content (TPC) [11]. About 0,1 mL of sample was entered in a test tube, then followed by 0.25 mL of Folin-Ciocalteu reagent. After standing for 5 minutes in the dark, 1.25 mL of sodium carbonate was added into the solution. The test tube was wrapped with parafilm and stood in the dark place for 1 hour. The samples were measured for absorbance using UV-Vis Spectrophotometer at wavelength 765 nm, while the comparison was gallic acid standard curve. The results were given in milligrams of gallic acid per gram of material. Each assay was carried out in triplicate.

Determination of antioxidant activity using ABTS free radical scavenging method

The ABTS radical scavenging method was used to assess the antioxidant activity of essential oils [11] with slight modification. The decolorization of ABTS was the reaction of a mechanism involves the radicals to donate electrons. ABTS was initially dissolved to a concentration of 7 mM in deionized water. The solution K2S2O8 solution was then produced at a concentration of 2.45 mM. After mixing the two solutions at a 1:1 ratio, they were kept in the dark for 24 to 48 hours. After that, the ABTS solution was diluted 1:25 with aqueous methanol. Aqueous methanolic plant extract in 20 L (diluted 1:10) was combined with 2 mL of ABTS+ solution and held at a constant temperature of 30°C. At 0, 5, and 10 minutes after initial mixing, absorbance was measured at 734 nm. On the day of preparation, all solutions were utilized, and all measurements were taken in triplicate. The results are given in milligrams of ascorbic acid per gram of dry material. The percentage inhibition of ABTS+ was estimated using the procedure below:

$$I \% = \frac{At=0-At}{At=0} X 100$$
Eqn. 1

where I denote ABTS inhibition (%), At=0 denotes the absorbance of the control sample (time = 0 hours), and At denotes the absorbance of the tested sample in 5 or 10 minutes.

Chemical profilling

Analysis of the components of *S. aromaticum* leaves and *C. odorata* flowers essential oil resulting from steam distillation for 3 hours carried out using a gas chromatographymass spectrometer GC-MS QP2010S SHIMADZU. A total of 0.05 μ L of *S. aromaticum* leaves and *C. odorata* flowers essential oil was injected into the GC-MS instrument using a syringe. The chromatography column used is a Restek capillary column Rtx-5 MS with a length of 30 m and a stationary phase of 5% diphenyl / 95% dimethyl polysiloxane. Helium gas was used as the mobile phase with a gas flow rate of 84.2 mL/minute (Split ratio 158.4). The temperature in the column is set at 60-215oC (10°C/minute) meanwhile the injector temperature is set at 225°C. The mass spectrometer uses the EI ionization type with an energy of 70 eV. The results of the analysis of *S. aromaticum* leaves and *C. odorata* flowers essential oil components are chromatogram (TIC) consisting of component peaks with % area and time retention of each, as well as the mass spectrum of the component compared with the spectrum mass in the Wiley7.lib library contained in the instrument.

RESULTS AND DISCUSSION

Yield of essential oils

Based on the results of sample distillation (Table 1), the yield of essential oils from S. aromaticum leaves showed the highest yield oil by 97 g from 5000 g weight of sample, while the *C. odorata* flowers yield of essential oil by 5.25 g from 700 g weight of sample. The yield percentation of S. aromaticum leaves and C. odorata flowers essential oils was 1.94% and 0.76%, respectively.

Tuble 1. The yield of essential offs								
No	Plants	Yield (%)	Total phenolic content (GAE mg / g)					
1	Syzygium aromaticum leaves	1.94	115.20 ± 0.02					
2	Cananga odorata flowers	0.76	9.67 ± 002					

Table 1 The wield of assertial oils

Distillation is a method for extracting essential oils. This study employed both steam and water distillation techniques. These processes involved isolating the oils and separating a water-insoluble chemical [12].

The distillation of S. aromaticum leaves yielded 1.94% oil, as shown in Table 1. This yield was lower than the 2.7% obtained in study [13] but higher than the range of 0.7-0.92% reported in study [14]. For C. odorata flowers, the distillation yielded 0.76% oil, which was higher than the 0.075% yield found in study [15].

Differences in yield are influenced by differences in growing places, varieties, soil conditions and climate. In addition, drying conditions and extraction methods also affect the oil yields. The several variables that affect yield variety, including the location and timing of sample collection, the type and portion of plant utilized, and the method of extraction [13].

Total phenolic content of essential oils

The total phenolic content of the essential oils, as shown in Table 1, was 115.20 mg GAE/g for S. aromaticum leaves and 9.67 mg GAE/g for C. odorata flowers. These results indicate that the phenolic content in the essential oils from S. aromaticum leaves was higher than that in the essential oils from C. odorata flowers.

The Folin-Ciocalteu (FC) test was used to assess the total phenolic content of essential oils, with gallic acid serving as a reference. Folin-Ciocalteu and gallic acid can be stable blue complexes. By combined to form reducing heteropoly acid (phosphomolybdate-phosphotungstate), spectrophotometers can detect the presence of a blue molybdenum-tungsten complex. This reaction occurs when the hydroxy group in gallic acid phenolics ion combines with the Folin-Ciocalteu reagent. The hydroxy group found in phenolic ion compounds and oxidized by the molybdenum while the ion reduces from Mo6+ to Mo5+. The sodium carbonate was added to the samples, it is known that Folin-Ciocalteau reagents sustained the combination of phenolics in alkaline and caused the protons in phenolic compounds was disintegrate into ions. The blue color was the reaction of the process. The development of the blue hue will lead to an increase in the production of phenolic ions; as more phenolic ions are formed, the amount of heteropoly acid is reduced, increasing the concentration of the blue hue [16].

Antioxidant IC₅₀ of essential oils

The antioxidant activity analysis used ABTS to assess free radical scavenging. The results, presented in Fig 1, show IC50 values of 9.45 μ g/mL for the essential oils from S. aromaticum leaves and 21.89 μ g/mL for those from C. odorata flowers. In comparison, the positive control, ascorbic acid, had an IC50 value of 5.05 μ g/mL.



Fig 1. Antioxidant IC₅₀ of essential oils

The overall antioxidant potency of single components and complex mixtures of various plants are commonly evaluated using the ABTS assay. The ABTS methodology is commonly employed to assess the efficacy of hydrophilic and lipophilic antioxidants, pure compounds, and extracts. This method is used to determine the ability of the tested materials to scavenge free radicals and neutralize their effects [17]. In both organic and aqueous solutions, absorption at 734 nm can be used to measure the antioxidant activity of essential oils [18]. By removing the ABTS cation's color, the ABTS approach measures the antioxidant capacity that directly interacts with the ABTS cation radicals. Antioxidants can diminish the blue-green color of the ABTS radical, which has a nitrogen core, turning it into a colorless form [19].

The antioxidant test using the ABTS method shows that the essential oil *S*. *aromaticum* leaves has very strong activity with an IC_{50} value close to that positive control of ascorbic acid as a comparison. This result is stronger than the results of research by Teles et al (2021) which obtained an IC_{50} of 78.98 µg/mL [20]. According to a previous report, clove essential oil has been found to exhibit superior antioxidant capacity when compared to other herbs and spices such as cinnamon, nutmeg, basil, oregano, and thyme. This suggests that clove essential oil could potentially be a more effective natural antioxidant compared to the other herbs and spices listed [21].

The major phenolic component of *S. aromaticum* leaves, eugenol, plays an important role in *S. aromaticum* antioxidant activity. Gülçin (2011) reported that eugenol is the most powerful antioxidant and eugenol is believed to have an aromatic ring [22]. This phenolic group stabilized a radical formed on a-carbon with conjugation in the eugenol molecule. It has various biological antioxidant mechanisms [23]. Eugenol demonstrates remarkable ferric ion (Fe3+) reducing ability and serves as an electron donor, effectively counteracting the damaging effects of free radicals by forming stable end products [22]. Apart from eugenol, Caryophyllene in the form of beta-caryophyllene in cloves also has antioxidant effects. Antioxidant properties of Caryophyllene were evaluated by DPPH and FRAP scavenging assays, also obtained positive results and the IC₅₀ value was lower than the positive control of ascorbic acid [24].

The major compounds of *C. odorata* flowers was Trans (Beta)-Caryophyllene from the sesquiterpene group. Beta caryophyllene is volatile, difficult to dissolve in water and has pharmaceutical potential as an analgesic, antioxidant, antimicrobial activity [25]. The high percentage of antioxidants in *C. odorata* is probably caused by the presence of the sesquiterpene hydrocarbon germacrene-D. Germacrene D, known as part of sesquiterpene is a powerful antioxidant because its chemical structure has extra cyclic methylene [26].

Chemical profiling

The chemical composition of *S. aromaticum* leaves (clove) and *C. odorata* flowers (cananga) essential oils were shown in Table 2 and 3.

Area 0.05 71.97
0.05 71.97
71.97
71.97
0.14
23.48
2.38
0.26
1.13
0.53
0.07
0
71.97

Table 2. Chemical composition of essential oils extracted from S. aromaticum leaves by water and steam distillation

Nine compounds were detected in the essential oil of *S. aromaticum*. The primary constituents were eugenol (71.97%) and trans-(β)-Caryophyllene (23.48%). Among these, two compounds were identified as phenylpropanoids, contributing to 73.1% of the total, with eugenol being the dominant component. Additionally, five compounds were classified as hydrocarbon sesquiterpenes, making up 26.31% of the oil, with trans-(β)-Caryophyllene being the major component. Furthermore, two compounds were characterized as oxygenated sesquiterpenes, comprising 0.6% of the composition (Fig. 2).

PeakRTCompoundsMolecularMolecularMolecular1 3.925 l-Phellandrene $C_{10}H_{16}$ 136 Hydrocarbon 0	% Area
1 3.925 l-Phellandrene $C_{10}H_{16}$ 136 Hvdrocarbon 0	
)37
Monoterpene	
2 4.034 Alphapinene $C_{10}H_{16}$ 136 Hydrocarbon 0).39
Monoterpene	
3 4.499 Sabinene $C_{10}H_{16}$ 136 Hydrocarbon 0).53
Monoterpene	
4 4.653 betaMyrcene $C_{10}H_{16}$ 136 Hydrocarbon 1	.20
Monoterpene	
5 5.050 alpha-Terpinene $C_{10}H_{16}$ 136 Hydrocarbon 0).46
Monoterpene	
6 5.127 Methyl para cresol $C_8 H_{10} O$ 122 Others 0).87
7 5.269 1.3.6-Octatriene, 3.7- C_{10} H ₁₆ 136 Hydrocarbon 2	2.66
dimethyl (Z)- Monoterpene	
(CAS) cis-3.7-	
Dimethyl-1.3.6-	
octatriene	
8 5.422 1.3.6-Octatriene, 3.7- C ₁₀ H ₁₆ 136 Hydrocarbon 3	3.17
dimethyl (E)- Monoterpene	
(CAS) .Beta.	
ocimene Y	
9 6.057 Alpha-terpinolene C_{10} H ₁₆ 136 Hydrocarbon 0).33
Monoterpene	
10 6.199 Linalol C_{10} H ₁₈ O 154 Oxygenated 1	1.01
Monoterpene	
11 8.237 2.3- $C_9 H_{12} O_2$ 152 Others 0).82
Dimethoxytoluene	
12 10.21 Nervl Acetate $C_{12} H_{20} O2$ 196 Others 0	0.40
13 10.274 alphaCopaene $C_{15}H_{24}$ 204 Hydrocarbon 0	0.42
Sesquiterpene	
14 10.459 Alloaromadendrene $C_{15}H_{24}$ 204 Hydrocarbon 0).34
Sesquiterpene	
15 10.589 Methyl Eugenol C_{11} H ₁₄ O ₂ 178 Hydrocarbon 0).77
Sesquiterpene	
16 10.958 trans- $C_{15} H_{24}$ 204 Hydrocarbon 2	26.91
Carvophyllene Sesquiterpene	
17 11.220 betaFarnesene $C_{15}H_{24}$ 204 Hydrocarbon 0).44
Sesquiterpene	
18 11.400 alphaHumulene $C_{15}H_{24}$ 204 Hydrocarbon 6	5.45
Sesquiterpene	
19 11.767 Germacrene D C_{15} H ₂₄ 204 Hydrocarbon 1	8.15
Sesquiterpene	
20 11.909 Farnesene $C_{15} H_{24}$ 204 Hydrocarbon 7	.29
Sesauiternene	-
21 11.977 alpha $C_{15} H_{24}$ 204 Hydrocarbon 1	2.41
Bergamotene Sescuiterpene	. =
22 12.234 Naphthalene $C_{15}H_{24}$ 204 Hydrocarbon 0).53
Sesquiterpene	

Table 3. Chemical composition of essential oils extracted from C. odorata flower by water and steam distillation

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23	13.113	(-)-Caryophyllene	$C_{15} H_{24} O$	220	Oxygenated	0.65
		oxide			Sesquiterpene	
24	15.374	Benzyl benzoate	$C_{14}H_{12}O_2$	212	Others	2.89
25	16.189	Farnecyl acetate	$C_{17} H_{28} O_2$	264	Others	0.49

Twenty-five compounds were identified in the essential oil of *C. odorata* flowers, making up 99.95% of its composition. The majority, 71.71%, were hydrocarbon sesquiterpenes, with trans-caryophyllene (26.91%), Germacrene (18.15%), and alpha-Bergamotene (12.41%) as the primary components. Eight compounds were monoterpene hydrocarbons, representing 9.11%, with beta ocimene as the main component at 3.17%. Linalool, an oxygenated monoterpene, constituted 11.01% of the essential oil. Additionally, (-)-Caryophyllene oxide, an oxygenated sesquiterpene, accounted for 0.64%. Five other compounds collectively made up 5.47% of the essential oil (Fig. 3).



Fig 2. Distribution of the main identified classes of compounds in the essential oil of S. aromaticum leaves



Fig 3. Distribution of the main identified classes of compounds in the essential oil of C. odorata flowers

Despite the vast array of chemical compositions found in essential oils, they can be broadly divided into two categories based on their hydrocarbon structure: terpenoids and phenylpropanoids. Terpenoids are synthesized from 2 (monoterpene), 3 (sesquiterpene),

or 4 (diterpene) isoprene units, while phenylpropanoids comprise a distinct class of compounds. Both terpenoids and phenylpropanoids are often noted as the primary components of many essential oils [27].

Eugenol is a naturally occurring aromatic molecule found in *S. aromaticum* that belongs to multiple chemical classifications [22]. Its core identity lies within the phenylpropanoids, a family of organic compounds derived from amino acids and characterized by a three-carbon side chain branching off a benzene ring [28]. Within this family, eugenol further qualifies as a phenol, owing to the hydroxyl group gracing its aromatic ring. Simultaneously, the double bond within its side chain grants it membership among the alkenes, a vibrant community known for its reactive nature. Eugenol proudly wears the mantle of an essential oil component, lending its spicy, clove-like aroma and potential antimicrobial properties to fragrant blends [29,30].

Trans (Beta)-caryophyllene is classified as a sesquiterpene due to its 15-carbon structure formed by three isoprene units, it belongs to the extensive group of aromatic hydrocarbons prevalent in various plants and essential oils [31]. The distinctive configuration, featuring two fused rings, further establishes its status among bicyclic hydrocarbons. The presence of a double bond within its carbon chain classifies it as an alkene, influencing its reactivity and chemical behaviours [32]. Trans (Beta)-Caryophyllene plays a significant role in the composition of essential oils such as clove and hops, contributing to their characteristic scents and potential therapeutic attributes. Its spicy and peppery aroma has also found applications in the food and beverage industry as a flavoring agent, imparting a distinctive essence to culinary creations [33,34].

Trans-Caryophyllene is a natural sesquiterpene that is present in the essential oils of various spices, foods, and medicinal plants. It has been the subject of several studies that have explored its potential health benefits, including its ability to act as an antimicrobial [35], anti-fungal [36], anti-Alzheimer [37], analgesic [38], and anti-inflammatory agent [39]. Moreover, Trans-Caryophyllene has been identified as a specific agonist of the type 2 cannabinoid receptor (CB2R), which enables it to promote fatty acid oxidation in the C_2C_{12} mouse myoblast cell line [40] and reduce cerebral ischemic injury in rodents [41].

Germacrene D, a sesquiterpene, possesses a structure that includes three double bonds. The germacrene family comprises five distinct types, namely A, B, C, D, and E. Recent research has highlighted the antioxidant potential of germacrene [42,43].

Bergamotene is a sesquiterpene that possesses a chemical formula of $C_{15}H_{24}$ and exists in four isomeric forms, namely α -cis, β -cis, α -trans, and β -trans. This molecule is commonly found in various types of citrus such as *Citrus bergamia* [44]. One of the intriguing properties of bergamotene is its ability to function as a pheromone [45,46].

It's interesting to note that the chemical composition of *C. odorata* essential oil can vary depending on the country of origin. In the Philippines, farnesene (18.0%) and benzyl acetate (12.6%) are the major components, while in China, Beta-caryophyllene (33.0%) and Gamma-muurolene (19.8%) take the lead (Buccellato, 1982; Cu, 1988). In Thailand, p-methyl anisole (20.27%) and geranyl acetate (15.07%) are the primary constituents. A report from another researcher in Indonesia suggests that Trans-caryophyllene is the major component of *C. odorata*, which is consistent with the present study [47]. It is pertinent to acknowledge that the chemical composition of essential oils can exhibit variation even among the same species. This variation may be attributed to several factors, such as abiotic and biotic factors, post-harvest treatment, extraction methods, and conservation conditions. It is therefore imperative to consider these variables while analyzing the chemical composition of essential oils [48].

CONCLUSION

Researchers have found that two types of essential oils, from *S. aromaticum* leaves and *C. odorata* flowers, sourced from East Kalimantan, may possess promising natural antioxidant properties. The essential oil from *S. aromaticum* leaves contains a higher total phenolic content and exhibits greater antioxidant activity compared to the essential oil from *C. odorata* flowers, primarily due to its eugenol content. The ABTS method has proven to be a reliable way to assess the antioxidant activity of essential oils. Additionally, this study found that the yield of essential oil from *C. odorata* flowers is significantly higher than previous reports, suggesting the potential for increased production of this essential oil.

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