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Antioxidant Activity of Essential Oil of *Baeckea Frutescens* L. (Ujung Atap) and GC-MS (Gas Chromatography-Mass Spectrometry) Analysis

Nurkhasanah Mahfudh*, Silvi Apriyani, Iin Narwanti

Faculty of Pharmacy, Universitas Ahmad Dahlan, Yogyakarta, Indonesia
*Corresponding author: Nurkhasanah Mahfudh | Email: nurkhasanah@pharm.uad.ac.id

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Abstract: *Baeckea frutescens* L. plant (Ujung atap) is a shrubby plant that resembles a pine tree and is usually found in tropical hills, mountains, and coastal environments. *B. frutescens* contains flavonoids and phenolic compounds which have known as potential antioxidants. *B. frutescens* also contains essential oils, which have been used in various treatments and aromatherapy. This study aims to measure the antioxidant activity of *B. frustescens* essential oils based on the IC_{50} value with the ABTS method and identify the chemical compound components of *B. frustescens* essential oils with the GC-MS. The stages of this research method consist of making *B. frustescens* essential oil using the steam-water distillation method, measuring the antioxidant activity of *B. frustescens* leaf essential oil using the ABTS method (2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonate)) and analysis of the chemical compound of *B. frustescens* essential oil using the GC-MS (Gas Chromatography and Mass Spectroscopy). Based on the results of this research, it is known that the antioxidant activity of the *B. frustescens* is included in the very weak category with an IC_{50} of 481.525 ± 5.455 µl/L or equivalent to 435.154 ± 4.930 ug/mL. Meanwhile, the antioxidant activity of quercetin has an IC_{50} value of 2.846 ± 0.156 µg/mL, which is included in the very strong category. The analysis of the essential oil compound content of *B. frustescens* found six main components of *B. frustescens* that had a concentration of more than 5%, namely, 1-beta- Pinene (21.62%), 1,8-Cineole (17.73%), alpha-pinene (14.93%), Gamma-Terpinene (6.84%), 3- Cyclohexene-1-methanol,α,α,4-trimethyl-(CAS) (5.78%), and Bicyclo[3.1.1]hept-2- ene,3,6,6 trimethyl-(CAS) (5.02%).

Keywords: *Baeckea frutescens*, ujung atap, antioxidant, essential oil, ABTS.

1. INTRODUCTION

Normal biochemical processes, increased environmental exposure, and high levels of xenobiotics in the diet can trigger the formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS). These ROS and RNS play a role in the occurrence of oxidative stress in various pathological conditions. In a situation of oxidative stress, the body's cellular components undergo changes, which can eventually trigger various types of diseases. However, this oxidative stress can be effectively controlled by strengthening the cellular defense system through the presence of antioxidants [1].

Antioxidants are chemical compounds naturally found in the body that provide hydrogen atoms to free radicals. Thus, antioxidants break the chain reaction and convert free radicals into a more stable form. Antioxidants play an important role in health by helping to prevent atherosclerosis,

reduce inflammation, fight tumors, prevent blood clots, and osteoporosis. Based on their origin, antioxidants are divided into two types, namely, natural and synthesized antioxidants [2].

Antioxidant capacity test using ABTS (2,2-azinobis(3-ethyl- benzothiazoline-6-sulfonate)) method, is a technique used to determine the amount of free radicals that can be neutralized by antioxidants. The basic principle of this method is to measure the ability of antioxidants to inhibit ABTS free radicals [3].

Baeckea frustescens L. (Ujung atap) is a shrub that generally grows in tropical environments along hills, mountains, and beaches. This plant grows in a number of places, including Java, Sumatra, Kalimantan, and Sulawesi [4]. This plant belongs to the *Myrtaceae* family and has a wide distribution. *B. frustescens* is known by various regional names such as sapu-sapu or jungrahab [5]. *B. frustescens* has various benefits such as its leaves can be used as a medicine for colds, fever, irregular menstruation and pain. While in Southeast Asia, *B. frustescens* leaves and flowers are useful as one of the raw materials for tea and its infusion becomes a refreshing drink. In addition, the essential oil of *B. frustescens* leaves can be used as massage oil for rheumatism [6].

B. frustescens contains essential oils and causes this plant to smell aromatic. According to Mat Saad *et al*. [7] the essential oil of *B. frustescens* leaves obtained from Klang Gates Quartz Ridge, Malaysia has the main compounds, namely, p-Cymene (31.06%), a-Pinene (24.53%), 1,8- Cineol (11.49%), and g-Terpinene (8.31%). Then in the antioxidant test of *B. frustescens* essential oil with the DDPH method, the EC_{50} value> 50 mg/mL was obtained, indicating that the *B. frustescens* leaf essential oil was less active in capturing DPPH radicals.

In the research conducted Supandi *et al*. [8], the results from the extraction of essential oil from the *B. frustescens* leaves obtained from Sungai Nanjung Village, West Kalimantan used The steam distillation method obtained the main chemical compound components in the *B. frustescens* leaves essential oil, namely α-Pinene by 26.95%, β-Pinene by 21.55%, and 1,8 Cineol by 18.04%.

Research on the potential of *B. frustescens* essential oil still very limited. Variations in antioxidant test methods will also provide additional data and evidence of its potential. This study aims to provide evidence of the andioxidant activity of *B. frustescens* essential oil, using ABTS method and identifying the constituent components of its essential oil.

2. MATERIALS AND METHODS

Baeckea frustescens L. (Ujung atap) samples obtained from Sukamara, Central Kalimantan were authenticated at the Biology Laboratory of Gadjah Mada University with the certificate number 4807/UN1/FA.2/BF/PT.01.06/2024 to determine their authenticity.

2.1. Sample preparation

B. frustescens in the form of twigs and leaves are first cut into smaller shapes and then dried using an oven at 30° C for 3 days. After the sample is dry, extraction is carried out using the steam-water distillation method to isolate essential oil from the B. frustescens plant. The weight of the sample used is 1.2 kg with 6 liters of water.

2.2. Essential oil quality test

2.2.1. Organoleptical Test

Organoleptical tests were conducted to see the uniformity of essential oil quality. Organoleptical observations in this study include smell and color.

2.2.2. Yield Calculation

The results of essential oils that have been obtained are then calculated using the formula:

$$\%\text{Yield} = \frac{Oil\ weight\ (mL)}{Weight\ simplisia\ of\ B.frustescens\ \ (g)}\ x\ 100\%$$

2.2.3. Specific gravıty

The specific gravity of *B. frustescens* essential oil is determined by weighing 1 mL of *B. frustescens* essential oil at room temperature of 20-25° C and compared to the weight of 1 mL of water measured at the same temperature.

2.2.4. pH test

Measurement of the pH of the essential oil of the *B. frustescens* was carried out using a pH meter. The calibrated pH meter is then inserted into the essential oil of the *B. frustescens*. The pH is read when the number has stabilized, and replicated 3 times.

2.3. Antioxidant Activity Test

The ABTS methode was used for measuring antioxidant activity for the sensitivity of the method. A total of 36 mg of ABTS (7mM) was dissolved in distilled water as much as 10 ml in a volumetric flask [9]. Potassium persulfate ($K_2S_2O_8$, BM: 270.322): weighed as much as 5.4 mg and then dissolved with distilled water in a 10 ml volumetric flask [9] . Making ABTS radical solution by means of 5 mL ABTS added with 5 mL $K_2S_2O_8$, then the mixture was incubated for 12-16 hours at 22-24 ° C in a dark place until a dark blue color was obtained [9]. The blank solution was made by adding 5 mL of potassium persulfate to 5 mL of distilled water and incubated in a dark room at 22-24°C for 12-16 hours before use [10].

Quercetin was used as a positive control, 10 mg of quercetin standard was weighed and dissolved in methanol p.a until the volume reached 10 mL, thus obtaining a concentration of 1000 μg/mL. 1 mL of stock solution was diluted in a volumetric flask with methanol p.a until the volume reached 10 mL. Then quercetin standard solution was made in a concentration series of 1, 1.5, 2, 2.5, and 3 μg/mL and dissolved with methanol p.a in a 5 mL volumetric flask. Making the main solution of essential oil samples, as much as 0.1 mL of *B. frustescens* essential oil was dissolved with methanol p.a in a 10 mL volumetric flask until a concentration of 10 (μl/mL) was obtained. A total of 1 mL of main solution was then diluted with methanol p.a in a 10 mL volumetric flask to obtain a concentration solution of 1 (μl/mL) or 1000 (μl/L). Essential oil sample solutions were made with concentration series of 200, 300, 400, 500, and 600 (μl/L) and dissolved with methanol p.a in a 5 mL volumetric flask.

2.3.1. Antioxidant Activity Measurement

Each concentration series of quercetin standard solution and *B. frustescens* essential oil was pipetted as much as 1 mL and added 1 mL of ABTS radical solution then incubated. After incubating during the operating time, measurements were taken at a wavelength of 734 nm to determine the absorbance. Then calculate the percent inhibition and IC_{50} value. Antioxidant activity of essential oil and quercetin was determined based on IC_{50} value.

$$\% \text{ Inhibition} = \frac{(Control\ absorbance - Sample\ absorbance)}{Control\ absorbance}\ x\ 100\%$$

2.4. Component Analysis of Compounds

Essential oil samples were injected 0.1-0.2 µL using a syringe into the GC-MS (Shimadzu QP2010 SE). The injector temperature was set at 150°C. Next, the column temperature was set with an initial temperature of 70°C for 5 minutes, then increased to. At 225°C, it was waited for 14 minutes. It was then increased to 300°C, and then waited for 7 minutes. 5% methyl or phenyl polysiloxane is used as the stationary phase and helium gas (He) serves as the carrier gas. Then the injected sample will move through the column to the detector and the separation results will form a chromatogram. Qualitative identification of essential oil components can be done by comparing mass spectra and retention periods obtained with reference data available in the literature.

3. RESULTS AND DISCUSSION

The sample used in the study was authenticated and confirmed that plant name is *Baeckea frutescens* L., which is included in the *Myrtaceae* family.

3.1. Essential Oil Quality Test

3.1.1. Organoleptical Test

Organoleptical observations in this study include smell and color. Based on the results of observations, the essential oil of B. frustescens has a clear yellow color and a distinctive and pungent aroma. In research Mugao, [11] the essential oil of B. frustescens leaves has a sharp aroma and is pale yellow in color. According to Elicia et al. [12] the essential oil of B. frustescens leaves has a clear yellow color and smells typical of B. frustescens.

3.1.2. Yield Calculation

The yield of essential oil that has been obtained is presented in Table 1. The current study found the yield of essential oil of *B. frustescens* is 0.63%. Other study conducted by Elicia *et al*. [12] which used the *B. frutescens* from Bangka Belitung found the yield of essential oil of *B. frustescens* of 0.45%. However, the research conducted by Supandi *et al*. [8] which used the *B.frutescens* from West Kalimantan found the yield of *B. frustescens* leaves essential was 0.999%. Oil production is influenced by various factors, namely: genetic factors, climate, extraction methods, land altitude, soil fertility, plant age, distillation methods, location, and pest and disease attacks. The higher the yield produced, the lower the quality obtained [13].

Table 1. Yield of *B. frustescens* Essential Oil

Sample weight (gram)	Essential oil (mL)	Yield (%) v/w
1.203	7.6	0.63%

3.1.3. Specific gravity

The specific gravity of the essential oil of *B. frustescens* obtained a value of 0.9037 <1 g/mL. In previous research by Supandi *et al*. [8] obtained a specific gravity of essential oil from the leaves of ujung atap is 0.878 g/mL. The specific gravity of essential oils usually ranges from 0.800 g/mL to 1.180 g/mL. This value is influenced by the content of chemical components in it. The higher the content of heavy fractions in essential oils, the greater the specific gravity produced [8].

3.1.4. pH test

The pH measurement of the essential oil of the *B. frustescens* obtained a pH value of 3.76. This shows that the essential oil of the *B. frustescens* is acidic. Some essential oils have an acidic pH as in research Sohipah, [14] cinnamon essential oil (*Cinnamomum burmanii*) has a pH of 5.6. Then in research Changbunjong *et al*. [15] essential oil from bitter orange peel (*Citrus aurantium*) has a pH of 5. The physical properties of *B. frustescens* essential oil can be seen in Table 2.

Table 2. Chemical Properties of *B. frustescens* Essential Oil

Color	Smell	Specific gravity	pH
Clear yellow	A distinctive aroma	0.9037	3.76

3.2. Antioxidant Activity

Quercetin standard is used as a comparison because it has good antioxidant activity, this can be proven by the results of the antioxidant activity test of quercetin standard has an average IC_{50} value of 2.846± 0.156 µg/mL. Quercetin antioxidant activity test results are presented in Figure 1.

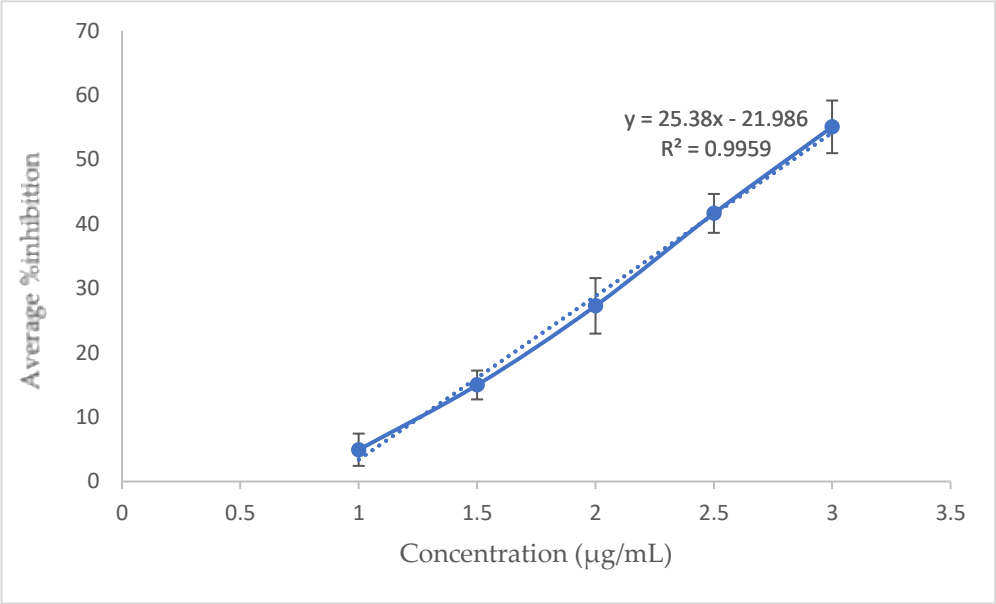


Figure 1. Graph of Quercetin Antioxidant Effectiveness with ABTS Method

The result of IC_{50} value of essential oil of *B. frustescens* is 481.525± 5.455 µl/L or equivalent to 435.154± 4.930 µg/mL or 0.435± 0.005 mg/ml. From these results, the essential oil of *B. frustescens* can be categorized as having very weak antioxidant activity because its antioxidant effectiveness is >50 µg/mL. Compounds are categorized as very strong antioxidants if they have IC_{50} values of less than 50 µg/mL, strong in the range of 50-100 µg/mL, moderate between 100-150 µg/mL, weak 150-200 µg/mL, and very weak if more than 200 µg/mL [16]. The antioxidant activity of *B. frustescens* essential oil against ABTS radicals can be seen in Figure 2.

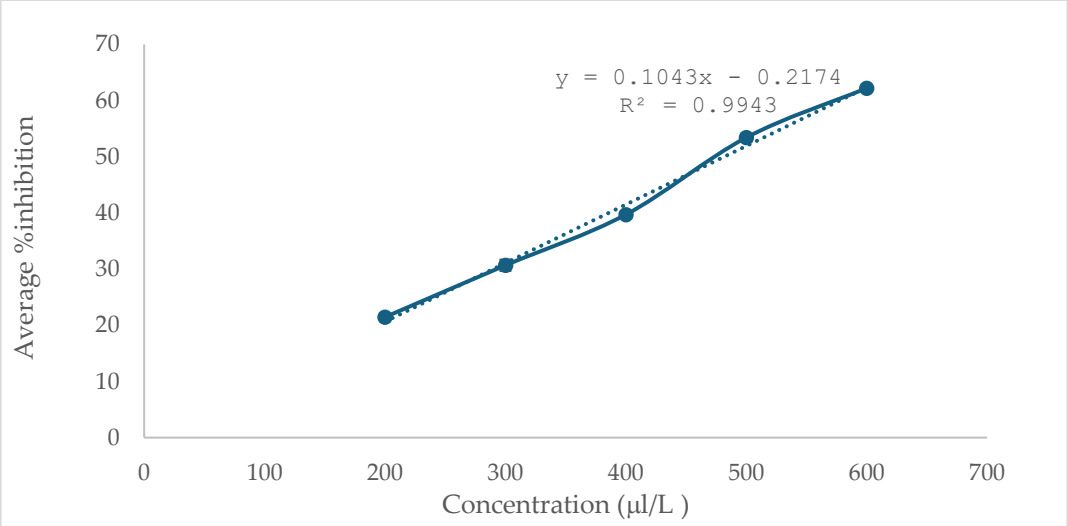


Figure 2. Graph of Antioxidant Effectiveness of *B. frustescens* Essential Oil with ABTS Method

However, the essential oil of *B. frustescens* used in this study has greater antioxidant activity than that reported Mat Saad *et al*. [7] in the essential oil leaf of *B. frustescens* obtained from Klang Gates Quartz Ridge, Malaysia has antioxidant activity EC_{50}>50 mg/mL using DPPH method.

Another study conducted by Nisa *et al.* [17] on the ethanol extract of *B. frustescens* found the strong antioxidant whic had a value of IC_{50} was 41.96±6.74 µg/mL while the water extract from the *B. frustescens* had a value of IC_{50} was 93.3±3.41 µg/mL. The present study recommended to develop further research using *B. frustescens* extract because it has high antioxidant activity.

Essential oils can show considerable differences in terms of their yield, chemical content, and effectiveness, even when derived from the same plant species. These differences are influenced by various internal plant factors such as genotype, age, plant population density, as well as the plant parts used for extraction [11].

Factors such as variations in environmental conditions, including altitude, temperature, illumination, rainfall, humidity, and soil characteristics at different growing sites, contribute to differences in the active compound content as well as antioxidant activity of medicinal plants [18].

3.3. Compound Analysis Using GC-MS

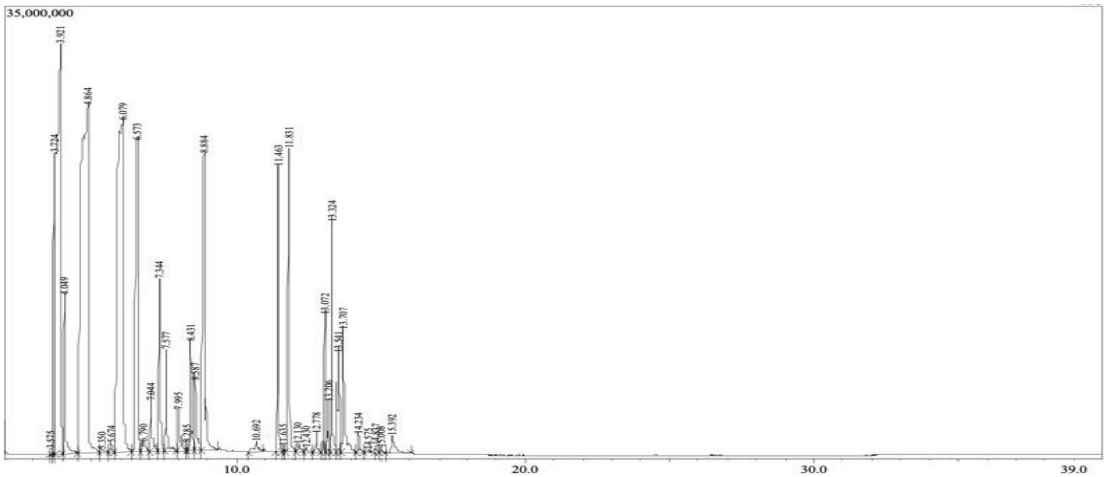


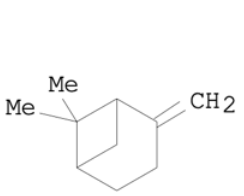
Figure 3. Results of Essential Oil Chromatogram of *B. frustescens*

Based on the chromatogram results in Figure 3, the results of GC-MS of B. frustescens essential oil contained 35 compounds (35 peaks).

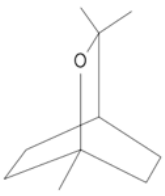
The essential oil B. frustescens contains 35 constituent compound components with the main compound content, namely, 1-beta-Pinene (21.62%), 1,8-Cineole (17.73%), alpha- pinene (14.93%), Gamma-Terpinene (6.84%), 3-Cyclohexene-1-methanol,.alpha.,alpha.,4-trimethyl-(CAS) (5.78%), and Bicyclo[3.1.1]hept-2-ene,3,6,6-trimethyl-(CAS) (5.02%). The chemical compound components of B. frustescens essential oil are presented in Table 3.

Table 3. Chemical Components of *B. frustescens* Essential Oil

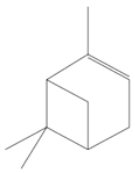
No.	Retention time	% Area	SI	BM	RM	Approximate Compound	Compound Group
1	4.864	21.62	95	136	$C_{10}H_{16}$	1-beta-Pinene	Monoterpen
2	6.075	17.73	95	154	$C_{10}H_{18}O$	1,8-Cineole	Monoterpen
3	3.921	14.93	96	136	$C_{10}H_{16}$	Alpha-pinene,(-)-	Monoterpen
4	6.573	6.84	95	136	$C_{10}H_{16}$	Gamma-Terpinene	Monoterpen
5	8.884	5.78	96	154	$C_{10}H_{18}O$	3-Cyclohexene-1-methanol, .alpha.,.alpha.,4-trimethyl-(CAS)	Monoterpen
6	3.724	5.02	93	136	$C_{10}H_{16}$	Bicyclo[3.1.1]hept-2-ene,3,6,6-trimethyl-(CAS)	Monoterpen
7	11.831	4.64	96	204	$C_{15}H_{24}$	alpha-Humulene	Sesquiterpen
8	11.463	3.20	97	204	$C_{15}H_{24}$	trans-Caryophyllene	Sesquiterpen
9	13.707	2.20	91	222	$C_{15}H_{26}O$	.beta.-Eudesmol	Sesquiterpen
10	13.324	2.14	87	220	$C_{15}H_{24}O$	Humulene oxide	Sesquiterpen
11	4.049	2.11	96	136	$C_{10}H_{16}$	Camphene	Monoterpen
12	13.541	2.07	84	220	$C_{15}H_{24}O$	(-)-Caryophyllene oxide	Sesquiterpen



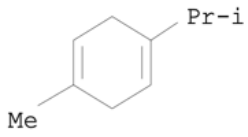
1-beta-Pinene



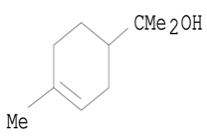
1,8-Cineole



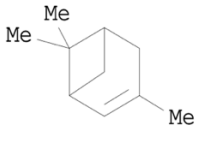
Alpha-pinene



Gamma-Terpinene



3-Cyclohexene-1-methanol, α,α,4-trimethyl-(CAS)



Bicyclo[3.1.1]hept-2-ene,3,6,6-trimethyl- (CAS)

Figure 4. Structure of the main compounds that make up the essential oil of *B. frustescens*

Based on the results of GC-MS analysis of *B. frustescens* essential oil in this study, there are some similarities in compound components with previous studies. Comparison of compound components in this study and previous studies is presented in Table 4.

Table 4. Comparison of Compound Content of *B. frustescens* Essential Oil in This Study and Previous Studies

No.	Compound Name	(Supandi et al., 2019)	(Wahyuni et al.,2022)	(Mat Saad et al., 2021)	(Dai et al., 2015)
1.	1-beta-Pinene	√	-	-	-
2.	1,8-Cineole	√	√	√	-
3.	Alpha-pinene,(-)-	√	-	√	-
4.	Gamma-Terpinene	-	-	√	-
5.	3-Cyclohexene-1-methanol, .alpha.,.alpha.,4-trimethyl-(CAS)	-	√	-	-
6.	Bicyclo[3.1.1]hept-2-ene,3,6,6-trimethyl- (CAS)	-	-	-	-
7.	alpha-Humulene	-	√	-	√

In Table 4, these compounds generally have strong antioxidant effectiveness but one of the factors causing very weak antioxidant activity can occur because essential oils are composed of heterogeneous compounds and are also influenced by other compounds contained in the essential oil. The heterogeneous level allows the presence of compounds that do not have antioxidant activity or inhibit antioxidant reactions [19].

Beta-pinene is a major compound of B.frutescens essential oil was reported to have weak antioxidant activity with IC_{50} values for DPPH and ABTS were 3116.3 μg/mL and 2245.0 μg/mL respectively and FRAP value was 6.5 μM Fe/mg pinene [20]. Alpha-pinene was also reported to have a weak antioxidant activity with IC_{50} values evaluated by DPPH test and FRAP test were 310±10μg/mL and 238±18.92μg/mL respectively [21]. However, beta-pinene and alpha-pinene were reported to have a good activities as cytogenetic, gastroprotective, anxiolytic, cytoprotective, anticonvulsant, and neuroprotective effects.

Eucalyptol (1,8-cineole) was monoterpene which found as a major compound in the essential oil of Eucalyptus showed a better antioxidant power than alpha and beta-pinene. 1,8-cineole which isolated from myrtle (Eucalyptus family) showed IC_{50} value using FRAP methode was 85.32 ± 1.43 μg/mL [22].

The present study found the weak antioxidant activity of *B. frutescent* essential oil could be caused as the two major compound of essential oil alpha-pinene and beta-pinene are very weak antioxidant, while one another (1,8-cineole) is strong antioxidant. The number of compound with very weak antioxidant were dominant than that of strong antioxidant.

4. CONCLUSION

B. frustescens essential oil has very weak antioxidant activity after testing using the ABTS method with an IC_{50} value of 481.525± 5.455 μl/L or equivalent to 435.154± 4.930 μg/mL. *B. frustescens* essential oil has 35 components of chemical compounds composed in it, and the main components of *B. frustescens* essential oil are 1-beta-Pinene (21.62%), 1,8-Cineole (17.73%), alpha-pinene (14.93%),

Gamma- Terpinene (6.84%), 3-Cyclohexene 1methanol,α, ,α,4-trimethyl (CAS) (5.78%), and Bicyclo[3.1.1]hept-2-ene,3,6,6-trimethyl-(CAS) (5.02%).

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Conflicts of interest: The authors declare no conflict of interest.

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