

Isolation and Analysis of Chemical Components of Essential Oil of Baru Cina (*Artemisia vulgaris* L) Leaves and Antioxidant Activities Test

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Abstract. Essential oil of Baru Cina leaves (*Artemisia vulgaris* L.) has been successfully isolated by hydrodistillation method using the Stahl apparatus. Baru Cina leaves were hydrodistillation for \pm 6 hours to produce the essential oil amount of 0.39 % (v/b). The essential oil obtained from Baru Cina leaves was characterized by using GC-MS spectroscopy. The GC-MS analysis results showed that there were 29 compounds and the main compounds identified as many as 14 compounds, such as Alpha-Pinene (0.33%), 1,8-Cineol (1.96%), Filifoline (1.41 %), 1-Octen-3-Ol (2.99 %), 2,4-Cycloheptadien-1-One (Eucarvone) (30.61 %), 2-Cyclohexen-1-Ol (Cis) (2.86 %), Bicyclo-3,3,1-Hepta-3-One (1.62 %), Trans-Caryophyllene (4.82 %), 3-Cyclohexen-1-Ol (1, 71 %), 2-Cyclohexen-1-Ol(Trans) (2.75 %), 2-Cyclohexen-1-One (Piperitone) (30.92 %), Trans Carveol (0.31%), Ar-Dimethylphenethyl Alcohol (0.88%), Eugenol (2.42%). This study also showed antioxidant activity from the essential oil of Baru Cina leaves with the IC₅₀ value was 95.76 mg/L.

Keywords: Essential Oil, Baru Cina Leaves, Antioxidant

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1 Introduction

In Indonesia, there have been many developments in food, beverage, pharmaceutical, aromatherapy, cosmetics, and other industries that require essential oils as raw materials or additives. Essential oil is known as etheric oils, essential oils, or flying oils. Essential oils are obtained from various parts of aromatic plants such as leaves, flowers, roots, stems, fruits, and seeds. The number of essential oil contents in each plant is different. The essential oils' properties that stand out include being volatile at room temperature, having a pungent taste, and smelling good according to the smell of the original plant (Yuliani and Satuhu, 2012).

Baru Cina (*Artemisia vulgaris* L.) plant in the Karo community is known as the Binara plant is a wild plant that is considered weeds that thrive and can inhibit the growth of producing crops in

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agricultural fields. This plant is a green leaf plant with a height of 50-150 cm, flowers, and thrives in the open field. According to the Karo people, this plant is useful for treating menstrual pain, strong medicine, cough medicine, seizure medicine, heartburn medicine, and increased appetite (Anonymous, 2012).

An antioxidant is a compound that donates electrons (electron donor) or reductant. The antioxidant compound has an important role in the body's defense against the bad effects caused by free radicals. Free radicals are known to induce cancer, arteriosclerosis, and aging, caused by tissue damage due to oxidation. The use of natural compounds as antioxidants has been around for a very long time. Natural antioxidants are found in almost all microorganisms, fungi, and even in animal and plant tissues. Most of these are phenolic compounds and some of the natural antioxidant groups are flavonoids, phenolic acids, and essential oils (Pokornya, 2001).

Research on *Artemisia vulgaris* L. has been carried out, among others, by analyzing the composition of the essential oil of *Artemisia vulgaris* L. which was obtained from Deli Serdang areas. The study results showed that based on the GC-MS analysis, as many as 28 compounds were found in *Artemisia vulgaris* L (Sembiring, 2011).

Some essential oil-producing plants show antioxidant activity, for example, Eucalyptus leaf which is useful as an antiseptic with its main content of 1,8-cineol and terpin acetate, Menta Peperita leaf has stimulant properties with the main ingredients of menthol, mentone, and menthyl acetate.

This research was expected to provide information about the chemical components of the essential oil of Cina Baru leaves (*Artemisia vulgaris* L.) as well as provide information about its antioxidant properties.

2 Materials and Methods

2.1 Equipments

In this study, the equipments used were: Stahl apparatus, gas chromatography-mass spectroscopy (GC-MS), UV-Visible (UV-Vis) spectroscopy, graduated cylinder, vial, bunsen, test tube, analytical balance, cuvette, and spatula.

2.2 Materials

The main materials used were Cina Baru leaves, anhydrous sodium sulfate, ethanol, aquadest, and 2,2-diphenyl-1-picryl-hydrazil (DPPH).

2.3 Isolation of Essential Oil Using Stahl Apparatus

As much as 600 g of Cina Baru leaves were put into a 2000 mL of bottom flask, and added with enough water. Then, isolated using a Stahl apparatus for \pm 6 hours. The distillate obtained was a mixture of essential oils and water and then was separated from water by adding anhydrous sodium sulfate. The yield obtained was measured and then analyzed by GC-MS and antibacterial activity test.

2.4 Antioxidant Test of Essential Oil of Cina Baru Leaves (*Artemisia vulgaris L.*) with DPPH Method

2.4.1 Preparation of DPPH 0.3 M Solution

As much as 11.85 g DPPH was dissolved with 100 mL of ethanol, then homogenized.

2.5 China's New Leaf Essential Oil Variation Manufacturing

Essential oil of Cina Baru leaves was prepared as a mother liquor of 1000 ppm. As much as 0.025 g of essential oil was dissolved with ethanol. Then, mother liquor of 1000 ppm was diluted to 100 ppm, and from 100 ppm was diluted again to variations in the concentration of solutions of 5, 10, 15, and 20 ppm to test its antioxidant activity.

2.6 Antioxidant Test

2.6.1 Blank Solution

As much as 1 mL of DPPH 0.3M solution was added with 2.5 mL of ethanol, homogenized in a test tube, and left for 30 minutes in a dark room. After that, the absorbance was measured with a maximum wavelength of 515 nm.

2.6.2 Antioxidant Activity Test of Essential Oil of Cina Baru Leaves

As much as 1 mL DPPH 0.3M solution was added with 2.5 mL of essential oil 5 ppm, homogenized in a test tube, and left for 30 minutes in a dark room. After that, the absorbance was measured with a maximum wavelength of 515 nm. The same procedure was performed for essential oils of 10, 15, and 20 ppm.

3 RESULT AND DISCUSSION

The GC-MS chromatogram obtained was as many as 29 chromatogram peaks indicated in figure 1 and only 14 chromatogram peaks could be adjusted to the spectrum available in the standard library presented in Table 1.

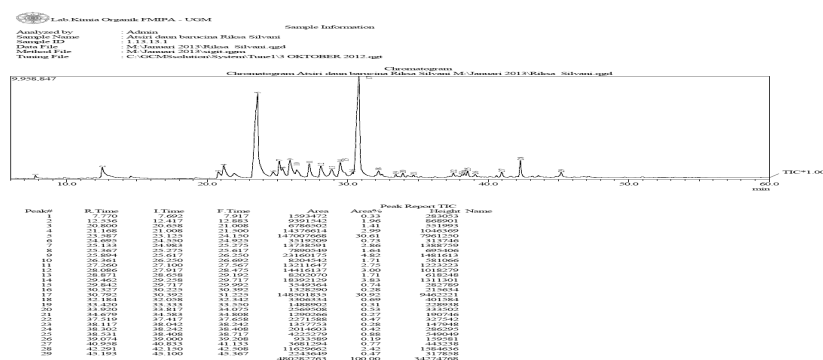


Figure 1. GC-MS chromatogram of essential oil of Cina Baru leaves

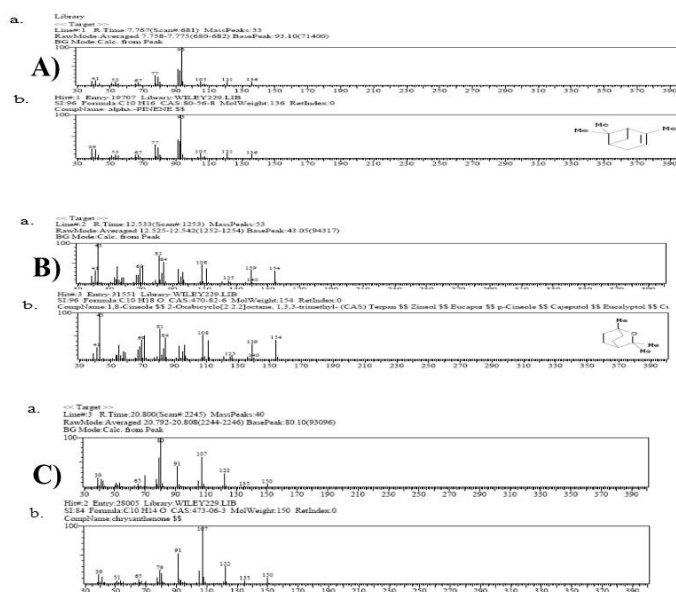
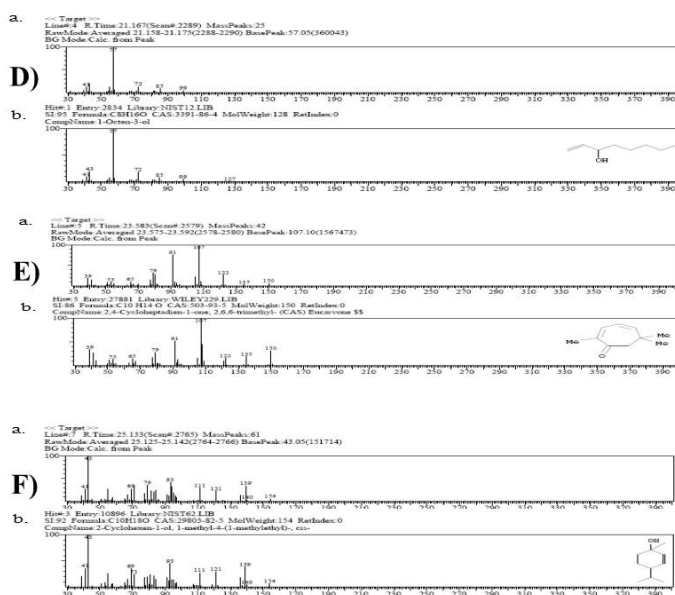
Tabel 1. Compounds obtained from the essential oil of Cina Baru leaves

No	Formula Molecule	Content (%)	Retention Time (Minute)	Fragmentation Peak	Suspected Compound
1	C ₁₀ H ₁₆	0.33	7.767	136, 121, 105, 93, 77, 67, 53, 39	Alpha-Pinene
2	C ₁₀ H ₁₈ O	1.96	12.533	154, 140, 139, 125, 108, 84, 81, 69, 43, 41	1,8-Cineole (Eucalyptol)
3	C ₁₀ H ₁₄ O	1.41	20.792	150, 135, 122, 107, 91, 80, 65, 39	Filifoline
4	C ₈ H ₁₆ O	2.99	21.167	128, 99, 85, 72, 57, 41	1-Octen-3-ol
5	C ₁₀ H ₁₄ O	30.61	23.583	150, 135, 122, 107, 91, 79, 65, 53, 39	2,4-Cycloheptadien-1-one (Eucarvone)
6	C ₁₀ H ₁₈ O	2.86	25.133	154, 140, 139, 121, 111, 93, 79, 69, 43, 41	2-Cyclohexen-1-ol (Cis)
7	C ₁₀ H ₁₄ O	1.62	25.367	150, 135, 122, 108, 93, 81, 69, 53, 41	Bicyclo-3,3,1-Hepta-3-one
8	C ₁₅ H ₂₄	4.82	25.892	204, 189, 175, 161, 147, 133, 120, 105, 93, 79, 69, 55, 41	Trans-Caryophyllene
9	C ₁₀ H ₁₈ O	1.71	26.358	154, 136, 121, 111, 93, 71, 69, 43, 41	3-Cyclohexen-1-ol
10	C ₁₀ H ₁₈ O	2.75	27.250	154, 140, 139, 121, 111, 93, 79, 69, 43, 41	2-Cyclohexen-1-ol (Trans)
11	C ₁₀ H ₁₆ O	30.92	30.792	154, 152, 137, 124, 110, 95, 82, 67, 54, 41	2-Cyclohexen-1-one (Piperitone)
12	C ₁₀ H ₁₆ O	0.31	33.417	152, 137, 119, 109, 84, 83, 55, 39	Trans-Carveol
13	C ₁₀ H ₁₄ O	0.88	38.533	150, 131, 119, 105, 91, 77, 65, 51, 41	Ar-imethylphenethyl Alcohol

14	$C_{10}H_{12}O_2$	2.42	42.292	164, 149, 131, 121, 103, 91, 77, 65, 55, 39	Eugenol
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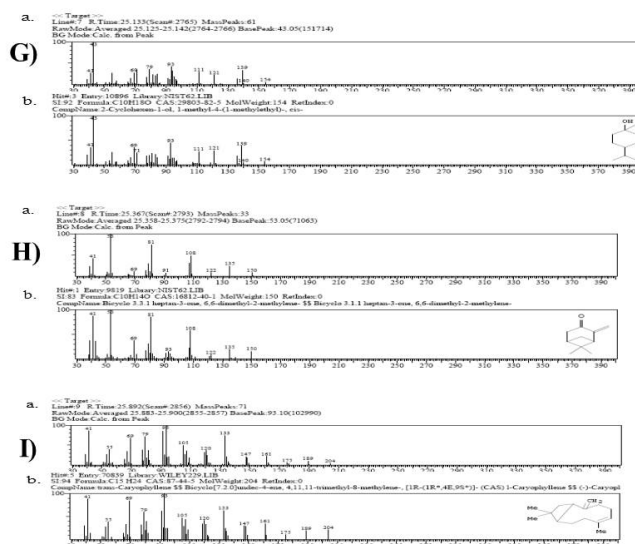
The peak with retention time (RT) of 7.767 minutes was 0.33%, which MS results showed molecular ion peaks at $m/e = 136$ followed by fragmentation peaks at m/e 121, 105, 93, 77, 67, 53, 39. Based on the standard library, the compound has the molecular formula $C_{10}H_{16}$ which was the skeletal isomer of a monoterpene compound with a spectrum as shown in figure 2. A. The peak with RT of 12.533 minutes was 1.96%, and MS results indicated molecular ion peaks at $m/e = 154$ followed by fragmentation peaks m/e 140, 139, 125, 108, 84, 81, 69, 43, 41. Based on the standard library, the compound has the molecular formula $C_{10}H_{18}O$, which was a skeletal isomer of one oxygenated monoterpene compound with a spectrum as shown in figure 2. B. The peak with RT of 20.792 minutes was 1.41%, and MS results demonstrated molecular ion peaks at $m/e = 150$ followed by fragmentation peaks at m/e 135, 122, 107, 91, 80, 65, and 39. Based on the standard library, the compound has the molecular formula $C_{10}H_{14}O$, which was a skeletal isomer of one oxygenated monoterpene compound with a spectrum as shown in figure 2. C.

The peak with RT of 21.167 minutes was 2.99%, and MS results assigned molecular ion peaks at $m/e = 128$ followed by fragmentation peaks at m/e 99, 85, 72, 57, and 41. Based on the standard library, the compound has the molecular formula $C_8H_{16}O$, which was a skeletal isomer of a monoterpene compound that has been decarboxylated with a spectrum as shown in Figure 3.D. The peak with RT of 23.583 minutes was 30.61%, and MS results showed molecular ion peaks at $m/e = 150$ followed by fragmentation peaks at m/e 135, 122, 107, 91, 79, 65, 53, 39. Based on the standard library, the compound has the molecular formula $C_{10}H_{14}O$, which was an isomer framework of an oxygenated monoterpene compound with a spectrum as shown in figure 3. E. The peak with RT of 25.133 minutes was 2.86%, and MS results indicated molecular ion peaks at $m/e = 154$ followed by fragmentation peaks at m/e 140, 139, 121, 111, 93, 79, 69, 43, 41. Based on the standard library, the compound has the molecular formula $C_{10}H_{18}O$, which was a skeletal isomer of an oxygenated monoterpene compound with a spectrum as shown in figure 3. F.

Figure 2. Fragmentation peaks of A). $C_{10}H_{16}$, B). $C_{10}H_{18}O$, C). $C_{10}H_{14}O$ Figure 3. Fragmentation peaks of D). $C_8H_{16}O$, E). $C_{10}H_{14}O$, F). $C_{10}H_{18}O$

The peak retention time of 25,367 minutes was 1.64%. MS results gave molecular ion peaks at $m/e = 150$ followed by fragmentation peaks at m/e 135, 122, 108, 93, 81, 69, 53, 41. Based on the standard library, the compound has the molecular formula $C_{10}H_{14}O$, which was an isomer framework of an oxygenated monoterpene compound with a spectrum as shown in figure 4.G. The peak with RT of 25,892 minutes was 4.82%, which MS results showed molecular ion peaks at $m/e = 204$ followed by fragmentation peaks m/e 189, 175, 161, 147, 133, 120, 105, 93, 79, 69, 55, 41. has the molecular formula $C_{15}H_{24}$, which was a skeletal isomer of a sesquiterpene compound with a spectrum as shown in figure 4.H. The peak with RT of 26.358 minutes was

1.71%, which MS results assigned molecular ion peaks at $m/e = 154$ followed by fragmentation peaks at m/e 136, 121, 111, 93, 71, 69, 43, 41. Based on the standard library, the compound has the molecular formula $C_{10}H_{18}O$, which was a skeletal isomer of an oxygenated monoterpene



with a spectrum as shown in figure 4. I.

Figure 4. Fragmentation peaks of G). $C_{10}H_{14}O$, H). $C_{15}H_{24}$, I). $C_{10}H_{18}O$

The peak with RT of 27.250 minutes was 2.75%, and MS results indicated molecular ion peaks at $m/e = 154$ followed by fragmentation peaks at m/e 140, 139, 121, 111, 93, 79, 69, 43, 41. Based on the standard library, the compound has the molecular formula $C_{10}H_{18}O$, which is a skeletal isomer of an oxygenated monoterpene compound with a spectrum as shown in figure 5.

J. The peak with RT of 30.792 minutes was 30.92%, which MS results assigned molecular ion peaks at $m/e = 152$ followed by fragmentation peaks at m/e 152, 137, 124, 110, 95, 82, 67, 54, 41. Based on the standard library, the compound has the molecular formula $C_{10}H_{16}O$, which was a skeletal isomer of an oxygenated monoterpene compound with a spectrum as shown in figure 5.

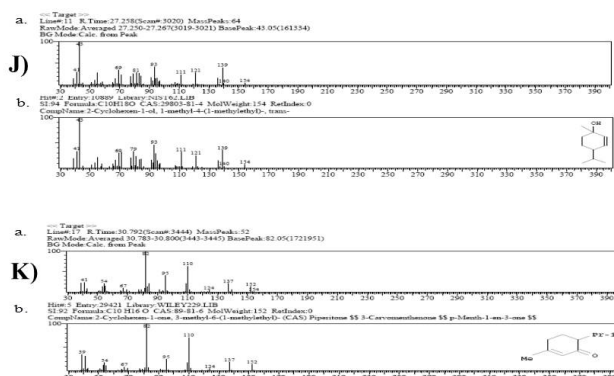
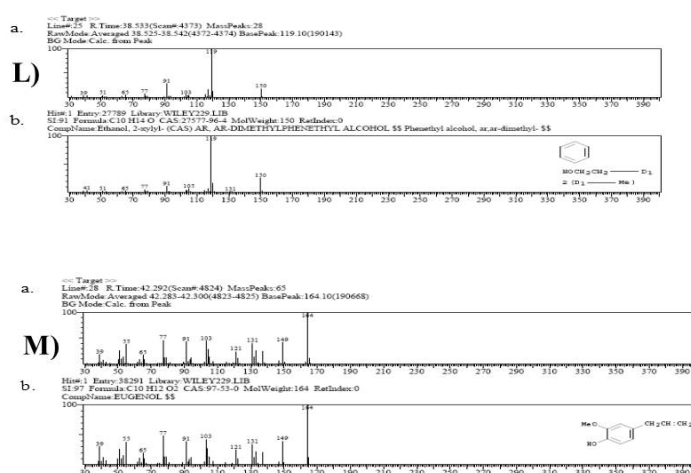


Figure 5. Fragmentation peaks of J). $C_{10}H_{18}O$, K). $C_{10}H_{16}O$

The peak with RT of 33,417 minutes was 0.31%, which MS results showed molecular ion peaks at $m/e = 152$ followed by fragmentation peaks at m/e 137, 119, 109, 84, 83, 55, 39. Based on the standard library, the compound has the molecular formula $C_{10}H_{16}O$, which was the skeletal isomer of an oxygenated monoterpene compound with a spectrum as shown in figure 6. L

The peak with RT of 38,533 minutes was 0.88%, which MS results presented molecular ion peaks at $m/e = 150$ followed by fragmentation peaks at m/e 131, 119, 105, 91, 77, 65, 51, 41. Based on the standard library, the compound has the molecular formula $C_{10}H_{14}O$, which was an isomer framework of an oxygenated monoterpene compound with a spectrum as shown in figure 6.M.

Figure 6. Fragmentation peaks of L). $C_{10}H_{16}O$, M). $C_{10}H_{14}O$

Antioxidant activity testing of essential oil of Cina Baru leaves was carried out using the DPPH method and showed that there was a reduction of DPPH free radicals which was indicated by a decrease in the absorbance of DPPH free radicals after the addition of essential oils, with the Least square equation, the IC_{50} value was 95.76 mg/L.at a maximum wavelength of 515 nm. According to Armala (2009), the level of the antioxidant power of the compounds tested using the DPPH method can be classified according to the IC_{50} value.

Table 2. Absorbance measurement results of essential oil of Cina Baru leaves

No	Sample	Absorbance
1	Blank	0.743
2	5 ppm	0.726
3	10 ppm	0.716
4	15 ppm	0.705

5

20 ppm

0.677

The DPPH radical scavenging was followed by monitoring the decrease in absorbance at the maximum wavelength that occurs due to the reduction by the antioxidant AH or the reaction with the apex radical (R.) data, which was often reported as IC_{50} , was the concentration of antioxidant required for 50% of DPPH radical scavenging in a given period of time. (15 to 30 minutes) (Pokornya, 2001). The absorbance measurement results of essential oil of Cina Baru leaves with variations of the concentration are presented in Table 2. DPPH is a free radical molecule that is stabilized by the resonance form as shown in figure 7. (Ionita, 2005).

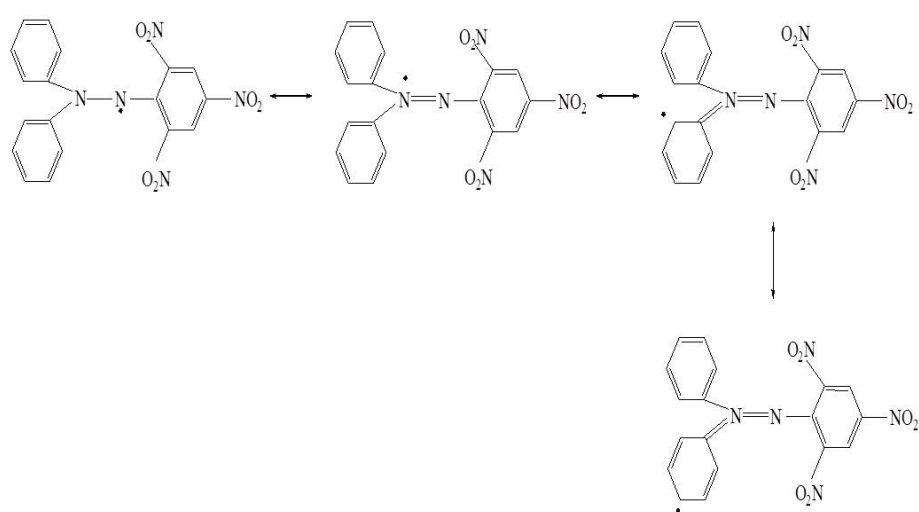


Figure 7. DPPH free radical stability

Table 3. Antioxidant power levels with the DPPH method

No	Intensity	IC_{50} value
1	Very Strong	< 50 mg/L
2	Strong	50-100 mg/L
3	Currently	101-150 mg/L
4	Weak	>150 mg/L

Antioxidant levels indicated in Table 3 show that the essential oil of Cina Baru leaves has the ability as a strong antioxidant. This is probably due to the compounds that play a role in the antioxidant activity being more semipolar so they will be more soluble. Hussain (2003) explained that antioxidant activity will also be influenced by the solubility of these compounds in a solvent. The potential antioxidant activity of essential oils has been demonstrated by the presence of attenuation percent of DPPH free radicals. This antioxidant activity was influenced by the content of oxygenated monoterpenes and sesquiterpenes of the essential oil (Sharififar et al, 2007).

4 Conclusion

Based on the results of the study, it can be concluded that the compounds contained in the essential oil of the Cina Baru leave by GC-MS analysis are 29 compounds, of which only 14 chromatogram peaks can be adjusted to the spectrum available in the standard library, namely Alpha-Pinene (0.33%), 1,8-Cineol (1.96%), Filifoline (1.41 %), 1-Octen-3-Ol (2.99 %), 2,4-Cycloheptadien-1-One (Eucarvone) (30.61 %), 2-Cyclohexen-1-Ol (Cis) (2.86 %), Bicyclo-3,3,1-Hepta-3-One (1.62 %), Trans-Caryophyllene (4.82 %), 3-Cyclohexen-1-Ol (1.71 %), 2-Cyclohexen-1-Ol(Trans) (2.75 %), 2-Cyclohexen-1-One (Piperitone) (30.92 %), Trans Carveol (0.31%), Ar-Dimethylphenethyl Alcohol (0.88%), Eugenol (2.42%) and the antioxidant activity of essential oil of Cina Baru leaves were obtained through DPPH method, which included a strong antioxidant group with an IC₅₀ value was 95.76 mg/L.

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