



## ANTIOXIDANT ACTIVITY OF *BLUMEA BALSAMIFERA* L. LEAVES EXTRACTS

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### ABSTRACT.

*Blumea balsamifera* L leaves were isolated and tested for antioxidant activity using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. *B. balsamifera* leaves were macerated as much as 1300 gram using a methanol solvent then partitioned with petroleum ether, n-hexane, and extracted with ethyl acetate and acetone to obtain petroleum ether, n-hexane, ethyl acetate, acetone and methanol extracts by weight and% yield respectively, namely 6.34 g (5.43%), 4.97 g (4.25%), 7.34 g (6.28%), 14.14 g (12.11%) dan 5.66 (4.85%). Phytochemical tests of petroleum ether and n-hexane extracts showed the presence of steroid compounds, while ethyl acetate, acetone, and methanol extracts showed the presence of alkaloids, saponins, flavonoids, terpenoids, and phenolic compounds. The antioxidant activity test of petroleum ether, n-hexane, ethyl acetate, acetone, and methanol extracts obtained IC<sub>50</sub> values respectively 177.47 ppm, 192.66 ppm, 4.28 ppm, 7.33 ppm and 8.61 ppm. Acetone extract of *B. balsamifera* (BBA) is separated by chemical components using chromatography of gravity column so that the combined fraction of acetone *B. balsamifera* extract (BBA1-BBA11) is obtained. Antioxidant activity test of BBA1, BBA2, BBA3, BBA4, BBA5, BBA6, BBA7, BBA8, BBA9, BBA10 and BBA 11 fractions IC<sub>50</sub> values obtained were 147.17 ppm, 81.36 ppm, 10.31 ppm, 3.84 ppm, 1.18 ppm, 3.37 ppm, 6.76 ppm, 9.32 ppm, 19.26 ppm, 16.82 ppm, and 30.71 ppm. The BBA extract is characterized by its structure using Gas Chromatography-Mass Spectrometry (GC-MS).

**Keywords:** *Blumea balsamifera* L leaves, acetone extract, Antioxidant activity, 1-diphenyl-2-Pikrilhidrazil (DPPH)

### INTRODUCTION

Indonesia is one of the countries with biodiversity that has long been used by the community as medicine. Aside from being a medicinal ingredient, these natural materials are also used as food, flavor ingredients, fragrance ingredients or various other commercial industrial materials. People used to use natural ingredients as traditional medicines which are believed to cure certain diseases, but further research is needed to prove the efficacy of these natural ingredients. The natural material used as traditional medicine usually comes from plants. One of the plants that are widely used by the community as traditional medicine is the *Blumea balsamifera* plant [1].

*Blumea balsamifera* is a plant species that belongs to the genus *Blumea* and *Astereaceae* family. The results of the study reported that the leaves of the *B. balsamifera* plant contained essential oils and borneols [1]. The *B. balsamifera* plant also contains cineol, limonene, palmitin, and myristic acids, sesquiterpenes alcohols, dimethyl ether chloracetophenone, tannins, pyrocatechol, glycosides, saponins, flavonoids, landerols, camphor, flavanol, resin and resin [2]. In addition, the results of another study also stated that there are 42 types of secondary metabolite compounds present in the essential oil of *B. balsamifera* leaves have antitumor and antioxidant effects. [3].

*Blumea balsamifera* leaves contain many flavonoid compounds [4]. Phenolic and flavonoid contents in plants play an important role in their benefits as antioxidants, but not all phenolic compounds are useful as antioxidants. The results showed that the content of phenols and flavonoids in blackberries was directly proportional to antioxidant activity [5]. The antioxidant activity does not only depend on the total phenol content but is also influenced by other compounds, such as ursolic acid, betulinic acid, and oleic acid [6]. Research on the chemical content of *B. balsamifera* plants is needed to determine the nutritional value of *B. balsamifera* plants.

*Blumea balsamifera* plants in Aceh are usually used to treat several diseases such as fever, cough, bone pain, colds, heredity, and ambient. The use of *B. balsamifera* plant oil on wounded skin in mice has no acute toxicity. Rats fed *B. balsamifera* oil at a dose of 2000 mg/kg for 24 hours did not find allergic reactions and acute toxicity. Wounds in mice were found to be better healing than mice that were not given *B. balsamifera* oil. In addition to healing wounds, *B.*



*balsamifera* plants are also useful as antioxidants [7]. Previous studies reported that *B. balsamifera* leaf extract can increase the antioxidant enzymes glutathione (GSH) and catalase (CAT) in mice induced by streptozotocin. Streptozotocin can induce hyperglycemia which will create free radicals that cause DNA damage, protein degeneration, lipid peroxidase, and can damage various organs [8]. Previous research also showed that brewed *B. balsamifera* leaves had an antioxidant effect as big as  $0.10 \pm 0.00002$ ;  $0.03 \pm 0.00005$  mg GAE/g sample, while the leaves of *B. balsamifera* which were boiled had an antioxidant effect of  $0.18 \pm 0.0001$ ;  $5.55 \pm 0.01$  mg GAE / g of the sample [9], but the isolation and structural determination of the chemical compounds of *B. balsamifera* leaf extracts have not been done. Therefore, it is necessary to conduct research on the extraction and isolation of secondary metabolite compounds and the determination of antioxidant activity with DPPH from the active extract of *B. balsamifera* leaves. Determination of the chemical components of *B. balsamifera* leaf extracts was analyzed using GC-MS (*Gas Chromatography Mass Spectrometry*) to find out the components of their chemical compounds.

## METHODOLOGY

### Blumea balsamifera leaves extraction

*Blumea balsamifera* (*B. balsamifera*) leaves that have been cut into small pieces, dried at room temperature and crushed. The finely ground *B. balsamifera* leaves were weighed 1300 g, then macerated using methanol for 24 hours. Later on, the methanol extract was partitioned with petroleum ether to obtain petroleum ether extract and methanol extract. Furthermore, methanol extract was partitioned with *n*-hexane, *n*-hexane extract and residue were obtained. The residue is then concentrated and extracted with ethyl acetate to obtain ethyl acetate extract and residue (methanol extract). The methanol extract is then concentrated and extracted with acetone to obtain acetone extract and methanol extract. Each extract from various solvents obtained was concentrated using a rotary evaporator, then the concentrated extract obtained was weighed.

### Phytochemical Test of *Blumea balsamifera* Leaf Extract

Phytochemical tests which include alkaloids, flavonoids, saponins, phenolics, steroids, and terpenoids are carried out based on the method conducted by Harborne [10].

### Antioxidant activity test by DPPH method (1,1-diphenyl-2-picrylhydrazyl)

#### 1.1. Making a blank solution

Making a blank solution is done by piping 1 mL of 0.4 mM DPPH solution into a 5 mL measuring flask, then adding methanol (p.a) to the volume limit mark. The absorbance was then measured using a UV-Vis spectrophotometer at a maximum wavelength of 517 nm.

#### 1.2. Making Positive Control Solutions

Vitamin C as much as 3 mg was weighed and then put into a 50 mL measuring flask, afterward dissolved with methanol (p.a) to mark the volume limit (60 mg/mL or ppm mother liquor). Later on, made a series solution with concentrations of 3, 6, 9, 12 and 15  $\mu\text{g/mL}$  or ppm, 1 mL of DPPH solution of 0.4 mM and methanol (p.a) were added to a volume of 5 mL. Furthermore, it was incubated at  $37^\circ\text{C}$  for 30 minutes and the absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 517 nm.

#### 1.3. Preparation of Test Material Solution

Preparation of the test material 250 mg/mL was carried out by weighing 2 mg of *B. balsamifera* leaves extract and dissolved in 8 mL methanol, then shaken until homogeneous. Then 25, 50, and 100 ppm series solutions were made, then incubated at room temperature for 30 minutes and absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 517 nm. Measurement of antioxidant activity is calculated by the following formula:

$$\text{DPPH radical scavenging} = \frac{\text{Abs}_{\text{blank}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{blank}}} \times 100\% \quad [11]$$

### Isolation of *Blumea balsamifera* leaf acetone extract

Isolation of active extracts of *Blumea balsamifera* leaves was carried out using the gravity column chromatography method with  $\text{F}_{254}$  silica gel adsorbent dissolved in a solvent to be used in the tracing process. The fractions obtained were analyzed by thin-layer chromatography (TLC). TLC tests were carried out with an eluent



mixture system using *n*-hexane, ethyl acetate, acetone, chloroform, and methanol solvents. After elution on the TLC plate, spots/stains are then seen under UV light and the chromatogram is sprayed using vanillin sulfate solution to see the spots/spots of the TLC. Each fraction that produces a separation pattern with the same  $R_f$  (Retardation factor) on the chromatogram is combined and concentrated to obtain 11 combined fractions [12]. The combined fractions obtained were then tested for antioxidant activity by the DPPH method.

#### Characterization of extracts using Gas Chromatography Mass Spectrometry (GC-MS)

BBA extracts were analyzed for their chemical structure using GC-MS (*Gas Chromatography Mass Spectrometry*) Shimadzu QP2000A. A sample of 1  $\mu$ L was injected into GC-MS which was operated using a glass column with a length of 25 m, a diameter of 0.25 mm, and a thickness of 0.25  $\mu$ m with a stationary phase CP-Sil 5CB with the oven temperature set between 70-270°C and rate of temperature rise of 10°C/minute, Helium carrier gas-pressurized 12 kPa, total rate of 30 mL/min, and a split ratio of 1: 50 [13].

## RESULTS AND DISCUSSION

### *Blumea balsamifera* leaves extraction

*Blumea balsamifera* leaves (1300 g) were extracted using methanol as a solvent for 24 hours to obtain 116.8 g (10.01 %). The methanol extract (116.8 g) of *B. balsamifera* leaves was partitioned using petroleum ether to remove chlorophyll content, so as to obtain as much as 5.43% petroleum ether extract and methanol extract. then the methanol extract was partitioned again using *n*-hexane to extract nonpolar compounds from the extract to obtain 4.25% *n*-hexane extract and methanol extract. The methanol extract was then concentrated and extracted using ethyl acetate to attract semipolar compounds to obtain 6.28% methanol extract and ethyl acetate extract. The methanol extract was then concentrated and extracted with acetone so that the acetone extract was obtained as much as 12.11% and the methanol extract.

### Phytochemical Test of *Blumea balsamifera* Leaf Extract

*B. balsamifera* Phytochemical test results of *Blumea balsamifera* leaves extract can be seen in Table 2.

Table 2. Phytochemical tests of *B. balsamifera* leaves extracts

No	Phytochemical test	Extract				
		BBM	BBA	BBE	BBH	BBPE
1	Alkaloids	+	+	+	-	-
2	Fenoliks	+	+	+	-	-
3	Flavonoid	+	+	+	-	-
4	Saponin	+	+	+	-	-
5	Steroids	-	-	-	+	+
6	Terpenoid	+	+	+	-	-

Information :  
 BBPE = *Blumea balsamifera* petroleum eter  
 BBH = *Blumea balsamifera n*-hexane  
 BBE = *Blumea balsamifera* ethyl acetate  
 BBA = *Blumea balsamifera* acetone  
 BBM = *Blumea balsamifera* methanol

Table 2. shows that the phytochemical tests for the five *B. balsamifera* leaves extracts were BBM, BBE, BBA, BBH, and BBPE. Alkaloid test on the four extracts showed that BBM, BBE, and BBA positive extracts contained alkaloids which were characterized by the formation of red sediment using Dragendorff reagents, brown sediment with Wagner reagents and white sediment using Mayer reagents. Phenolic test shows that BBM, BBE and BBA positive extracts contain phenolic compounds which are marked by the formation of a black or blue color change. The flavonoid test showed that BBM, BBE and BBA extracts were positive there were flavonoid compounds that were shown by the formation of a yellow color change. Saponin test showed that the positive saponin compound was found in the BBM, BBE and BBA extracts which were characterized by the formation of stable foam  $\pm$  30 seconds. BBH and BBPE



extracts showed positive results on steroid tests which were marked by the formation of green color when Liebermann Burchard's reagent was added. Whereas the BBM, BBE and BBA extracts showed negative results. BBM, BBE and BBA extracts showed positive results in the terpenoid test which was marked by the formation of a brownish-red color when the Liebermann Burchard reagent was added. Whereas the BBH and BBPE extracts showed negative results which showed that the BBH and BBPE extracts contained no terpenoid compounds.

**The antioxidant activity test of *B. balsamifera* leaves extract with DPPH method**

The antioxidant activity test was carried out on BBM, BBE, BBH and BBPE extracts using the DPPH method. DPPH test results on the four *B. balsamifera* leaves extracts can be seen in Table 3.

Table 3. The antioxidant activity Test of *B. balsamifera* leaves extracts

Extract	Concentration (ppm)	Absorbance	% Inhibition	IC <sub>50</sub>
BBM	100	0.38	48.50	8.61
	50	0.08	89.08	
	25	0.03	95.70	
BBA	100	0.36	50.64	7.33
	50	0.09	87.65	
	25	0.02	97.13	
BBE	100	0.35	52.97	4.28
	50	0.09	87.24	
	25	0.01	97.85	
BBH	100	0.70	5.59	192.66
	50	0.69	7.24	
	25	0.57	23.22	
BBPE	100	0.70	5.59	177.47
	50	0.68	7.69	
	25	0.56	24.60	
Vitamin C	15	0.46	37.67	3.30
	12	0.23	67.91	
	9	0.08	88.59	
	6	0.05	92.43	
	3	0.04	94.49	

Information : Negative control absorbance (DPPH) = 0,74

Table 3 shows that the extract of BBM, BBA, and BBE had the greatest antioxidant activity with IC<sub>50</sub> values of 8.61 ppm, 7.33 ppm and 4.28 ppm, respectively, with the highest linearity that can be seen on the correlation curve between% inhibition and concentration extract BBM, BBA, BBE, BBH and BBPE (Figure 1). IC<sub>50</sub> values were

obtained from the linear equation of the correlation curve between % inhibition and concentration of extracts of BBM, BBA, BBE, BBH and BBPE, that is  $y = 0.5583x + 45.19$ ;  $y = 0.5584x + 45.895$ ;  $y = 0.5432x + 47.665$ ;  $y = 0.2471x - 2.4$  dan  $y = 0.2656x - 2.865$ . The y value is replaced by 50 so that the  $IC_{50}$  value is obtained (shown in Figure 1). A compound is expressed as a free antiradical category very strong when  $IC_{50}$  values  $<10 \mu\text{g/mL}$ , strong categories when  $IC_{50}$  values are  $10\text{-}50 \mu\text{g/mL}$ , moderate categories when  $IC_{50}$  values range from  $50\text{-}100 \mu\text{g/mL}$ , weak categories when  $IC_{50}$  values range between  $100\text{-}250 \mu\text{g/mL}$  and inactive when  $IC_{50}$  is above  $250 \mu\text{g/mL}$ [15].

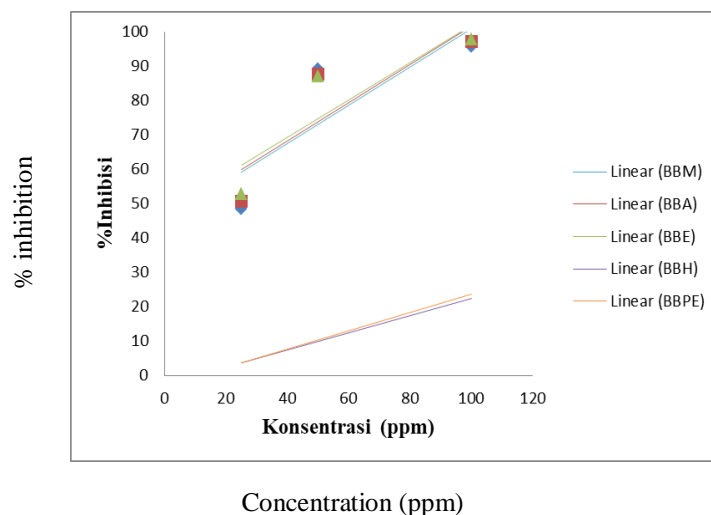


Figure 1. % inhibition correlation curve and concentration of *B. balsamifera* extract

#### Isolation of chemical components of acetone extract of *B. balsamifera* leaves

12.11% BBA extract was separated by chemical components using gravity column chromatography. The chromatography results of the BBA extract column showed that there were 11 combined fractions that were monitored with TLC shown in Figure 2.

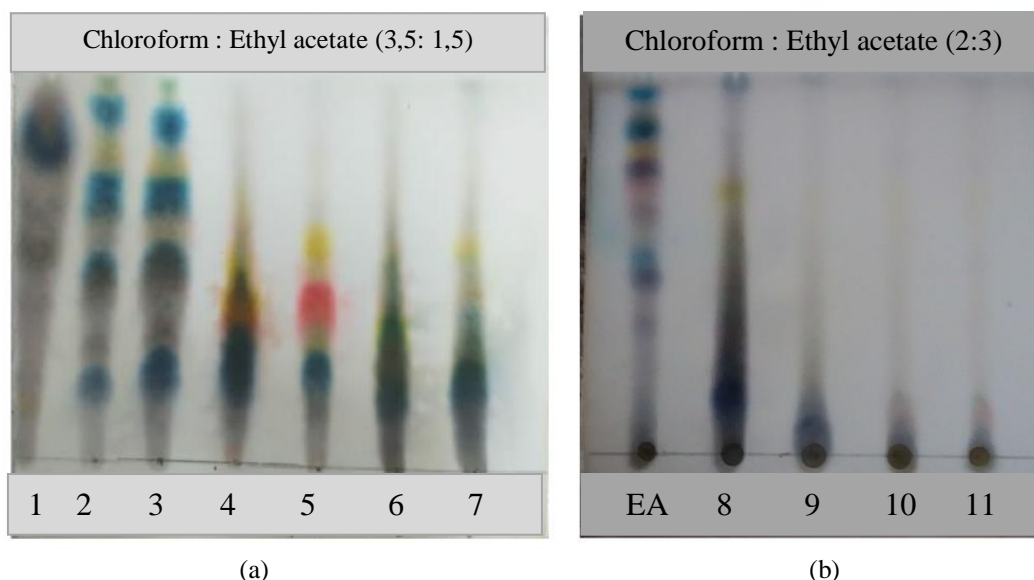


Figure 2. (a) TLC BBA1 to BBA7 with vanillin sulfate reagent  
(b) TLC BBA8-BBA11 with vanillin sulfate reagent

Figure 2 (a) shows the TLC results from BB1 to BB7 using the chloroform-ethyl acetate eluent (3.5: 1.5). The BB5 fraction shows bright red and yellow stains. The bright red stain when tested with  $\text{FeCl}_3$  solution shows a blackish color which is a characteristic of a group of phenolic compounds. Figure 2 (b) shows the TLC results from BBA8 to



BBA11 using the chloroform-ethyl acetate eluent (2: 3). This is because the compounds in BBA8 to BBA11 are more polar so that the eluent used is increased in polarity.

**Test the combined antioxidant activity of the BBA extract fraction by the DPPH method**

The combined fraction of BBA extracts, namely BBA1 to BBA11, was carried out by the DPPH test to determine its antioxidant activity. This antioxidant activity test was carried out with DPPH as a radical compound and vitamin C as a positive control. DPPH test results from all combined fractions of BBA extract can be seen in Table 5.

Table 5. Test the combined antioxidant activity of the BBA extract fraction by the DPPH method

Extract	Concentration	Absorbance	% Inhibition	IC <sub>50</sub>
BBA1	100	0.61	36.40	147.17
	50	0.71	25.92	
	25	0.81	15.23	
BBA2	100	0.41	56.91	81.36
	50	0.58	39.60	
	25	0.73	24.40	
BBA3	100	0.08	91.41	10.31
	50	0.24	74.28	
	25	0.44	53.60	
BBA4	100	0.06	93.00	3.84
	50	0.24	74.49	
	25	0.41	57.49	
BBA5	100	0.06	93.62	1.18
	50	0.24	74.66	
	25	0.39	58.94	
BBA6	100	0.06	93.31	3.73
	50	0.21	77.69	
	25	0.42	56.04	
BBA7	100	0.06	93.34	6.76
	50	0.22	77.00	
	25	0.43	54.87	
BBA8	100	0.06	93.38	9.32
	50	0.22	76.42	
	25	0.44	53.80	
BBA9	100	0.06	93.31	19.26
	50	0.23	75.35	
	25	0.50	48.01	
BBA10	100	0.07	92.31	16.82
	50	0.24	75.07	
	25	0.48	49.56	
BBA11	100	0.07	92.41	30.71
	50	0.31	67.66	
	25	0.55	42.77	
Vitamin C	15	0.05	94.41	3.44
	12	0.05	94.17	
	9	0.07	89.2	
	6	0.23	75.28	
	3	0.66	31.29	

Information : Negative control absorbance (DPPH) = 0,96



Table 5. shows the level of antioxidant activity of the combined fraction of BBA extract. Based on the results of  $IC_{50}$  values obtained in antioxidant tests on 11 BBA extract fractions, it can be said that BBA3 fractions up to BBA10 have strong antioxidant activity with  $IC_{50}$  values respectively 1.18 to 19.26 ppm. The BBA5 fraction has a very strong antioxidant activity with the lowest  $IC_{50}$  value of 1.18 ppm compared to the positive control of 3.44 ppm. This can be seen from the results of the BBA5 fraction TLC showing yellow stains. Yellow stains indicate the presence of flavonoid compounds.  $IC_{50}$  value is obtained from the linear equation of the correlation curve between % inhibition and concentration of BBA1 extract up to BBA11 in a row namely  $y = 0.2719x + 9.99$ ;  $y = 0.421x + 15.745$ ;  $y = 0.4811x + 45.035$ ;  $y = 0.4587x + 48.235$ ;  $y = 0.4505x + 49.46$ ;  $y = 0.4706x + 48.23$ ;  $y = 0.4863x + 46.7$ ;  $y = 0.5008x + 45.32$ ;  $y = 0.569x + 39.03$ ;  $y = 0.5378x + 40.94$  dan  $y = 0.638x + 30.395$ . The y value is replaced by 50 so that the  $IC_{50}$  value is obtained (can be seen in Figure 4).

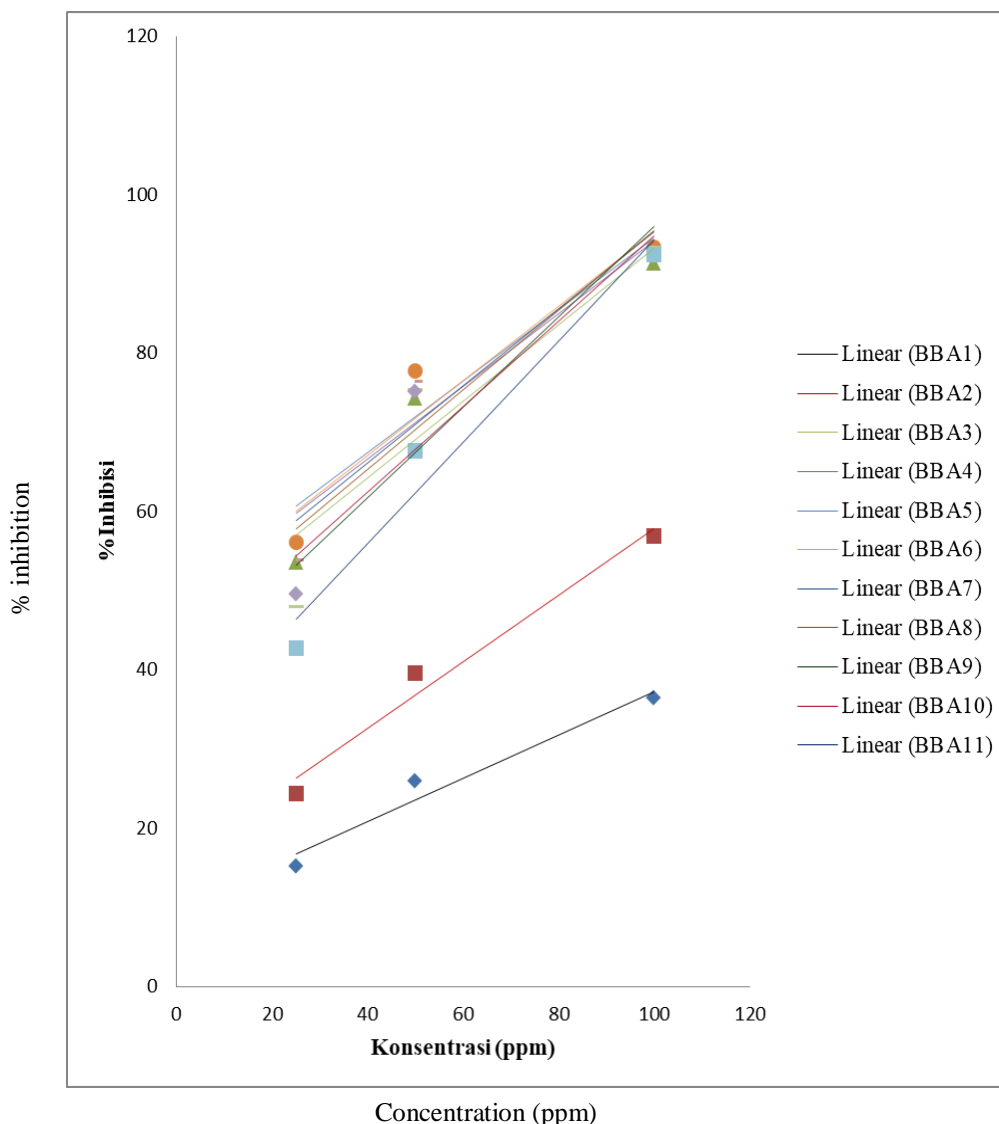


Figure 4. % inhibition correlation curve and concentration of BBA1-BBA11 extract

The BBA5 extract had the greatest antioxidant activity compared to the combined fraction of other BBA extracts with  $IC_{50}$  values of 1.18 ppm. This can be seen from the results of TLC BBA5 fraction which showed the presence of phenolic compounds in the extract. Antioxidant activity can be caused by the presence of phenolic compounds. The ability to capture free radical DPPH is strongly influenced by the OH groups contained in phenolic compounds [16]. The difference in phenolic antioxidant activity is determined by the chemical structure, number, and



position of the hydroxy and methyl groups in the ring. The more hydroxyl groups substituted in the molecule, the stronger the ability to capture free radicals because more hydrogen atoms are donated [17].

## 2. Characterization of BBA extract

BBA extracts were analyzed using GC-MS, chromatograms can be seen in Figure 5.

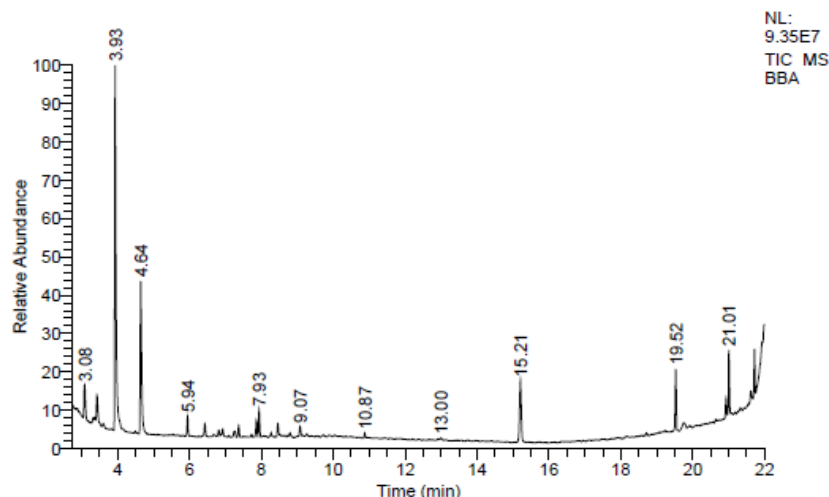


Figure 5. GC-MS chromatogram of BBA extract

Figure 5 shows that BBA extract has 2 dominant compounds with retention times of 3.93 and 4.64 minutes. The compounds detected were adjusted to the existing database so that 14 compounds were obtained for both extracts. The types of compounds analyzed can be seen in Table 6.

Table 6. Results of GC-MS BBA extract

No	Isolate	Retention time	% area	Compound
1	BBA	3.08	4.45	8-Phenyloctanoic_acid
		3.42	5.80	3-Hexen-2-one
		3.93	35.76	Tyranton
		4.64	17.26	Cyclohexanone
		5.94	2.03	Hemellitol
		6.42	1.57	2-(1-Phenyl-ethylamino)-2-thioxo-acetamide
		7.37	1.24	p-Cymene
		7.86	1.73	P-Cimene
		7.93	2.65	p-Cymol
		9.07	1.88	Naphthalene
		15.21	9.97	Dodecanoid_acid,_methyl_ester
		19.52	5.75	Methyl_tetradecanoate
		21.01	6.33	Cryptomeridiol
		21.72	3.57	Pentadecanoid_acid,_13-methyl-,_methyl_ester

Table 6. showed that the main compound contained in the BBA extract contained the main secondary metabolite compound namely *Tyranton* with % area in BBA extract of 35.76% and BBA4 extract of 25.56%.





## CONCLUSION

BBA extract showed a very strong antioxidant activity with  $IC_{50}$  7.33 ppm. The combined fraction of BBA extract which showed the highest antioxidant activity was BBA5 with  $IC_{50}$  1.18 ppm.

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