https://doi.org/10.31925/farmacia.2024.3.15 **ORIGINAL ARTICLE**

PHYTOCHEMICAL PROFILING BY LC-HRMS AND ANTIOXIDANT ACTIVITY OF *BLUMEA BALSAMIFERA* LEAVES EXTRACT BASED ON DIFFERENCES IN THE GROWING LOCATION AND EXTRACTING SOLVENT

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Abstract

This study aims to determine the total phenolic and flavonoid content, antioxidant activity and phytochemical profiling of *B. balsamifera* leaves using UHPLC-Q-Orbitrap HRMS from two different growth locations and solvent composition extraction. The samples were obtained from Bogor and Sukabumi district, West Java, Indonesia and macerated using water and 70% ethanol. The highest yield (17.52%) was found in 70% ethanol extract of *B. balsamifera* leaves from Bogor, which also had the highest total phenolic (62.68 mg GAE/g dry powder) and flavonoid (19.06 mg QE/g dry powder) contents. The activities were determined by using DPPH (2,2-diphenyl-1-picrylhydrazyl) (antioxidant activity), CUPRAC (cupric ion reducing anti-oxidant capacity) and FRAP (ferric-reducing antioxidant power). We found the highest antioxidant activity in 70% ethanol extract of *B. balsamifera* leaves from Bogor with antioxidant capacity values of about 60.35, 1815.25 and 309.91 μmol Trolox/g dry powder. A total of 47 compounds were identified in the *B. balsamifera* extracts. These compounds were identified as monoterpenes, flavonoids, phenolic acids, coumarin, carboxylic acids, lignans, furans, phthalic acid, sesquiterpene lactone and chalcone.

Rezumat

Acest studiu își propune să determine conținutul total de fenoli și flavonoide, activitatea antioxidantă și profilul fitochimic al frunzelor de *B. balsamifera* folosind UHPLC-Q-Orbitrap HRMS din două zone geografice diferite și extrase în solvenți diferiți. Probele au fost obținute din districtele Bogor și Sukabumi, Java de Vest, Indonezia, și au fost macerate folosind apă și etanol 70%. Cel mai mare randament (17,52%) a fost găsit în extractul etanolic 70% din frunzele de *B. balsamifera* din Bogor, care a avut, de asemenea, cel mai mare conținut total de fenoli (62,68 mg GAE/g pulbere uscată) și flavonoide (19,06 mg QE/g pulbere uscată). Proprietățile biologice au fost determinate prin utilizarea DPPH (2,2-difenil-1-picrilhidrazil) (activitate antioxidantă), CUPRAC (capacitate antioxidantă de reducere a ionilor de cupru) și FRAP (putere antioxidantă de reducere a ionilor de fier). Astfel, s-a evidențiat cea mai importantă activitate antioxidantă în extractul etanolic 70% din frunzele de *B. balsamifera* din Bogor, cu valori ale capacității antioxidante de aproximativ 60,35, 1815,25 și 309,91 μmol Trolox/g pulbere uscată. Un total de 47 de compuși au fost identificați în extractele de *B. balsamifera*. Acești compuși au fost: monoterpene, flavonoide, acizi fenolici, cumarină, acizi carboxilici, lignani, furani, acid ftalic, lactonă sesquiterpenică și chalcone.

Keywords: antioxidant, *Blumea balsamifera*, metabolite profiling, UHPLC-Q-Orbitrap HRMS

Introduction

Blumea balsamifera is a medicinal plant from the *Asteraceae* family found in India, South China and Southeast Asia [1, 2]. The characteristics of this plant are soft woody stems and fine hair. They can reach four meters in height and have flowers. Traditionally, this plant has been widely used to treat cough, urinary tract infection, gastric ulcers, headache, fever, eczema, dermatitis, rheumatism and as an insecticide [3, 4]. The leaves are reported to have antioxidant, antifungal, antibacterial, anti-gastralgic, anti-diarrhoea, anti-tumour and anti obesity properties [5, 6].

Plants contain complex primary and secondary metabolites with unique characteristics, varying structures and different functions. The metabolite composition varies and can be affected by several factors, such as environmental growth conditions (temperature, humidity, light and wind), soil fertility, harvest, post-harvest processing and extraction type and concentration solvents [7]. *B. balsamifera* is rich in metabolites such as flavonoids, monoterpenes, sesquiterpene, terpenes, alcohols, dihydroflavones, sterols and organic acids [5]. These compounds are widely used for disease treatment. They are developed as drug candidates, one of which is an antioxidant.

The antioxidant activity of the *B. balsamifera* plant has been widely studied and linked to its total phenolic and flavonoid content. Nine flavonoid compounds were isolated from *B. balsamifera*, and the compound with the most significant antioxidant activity was luteolin, with an IC_{50} value of 2.38 ± 0.01 µM [8]. The total content of flavonoids in the ethanol extract was 23.34 ± 0.67 mg catechins/g and the antioxidant activity was obtained with an IC_{50} value of 0.21 mg/mL [9]. The varying composition and concentration of plant metabolites will affect their bioactivity capabilities. Therefore, in this study, we reported for the first time the content of total phenolics and flavonoids, phytochemical profiling and antioxidant activity of *B. balsamifera* leaves based on the differences in the growing location and solvent composition extraction. In this study, we used *B. balsamifera* from Bogor (180 masl (metres above sea level)) and Sukabumi (450 - 550 masl) district in West Java, Indonesia. Maceration using two solvents (70% ethanol and water) was performed to get the extracts of the leaves and then total phenolic and flavonoid contents, phytochemical profiling and antioxidant activity were determined. DPPH (2,2-diphenyl-1-picrylhydrazyl) (antioxidant activity), CUPRAC (cupric ion reducing antioxidant capacity) and FRAP (ferric-reducing antioxidant power) were used to determine the activities and the metabolites found in *B. balsamifera* leaves extracts were analysed using UHPLC-Q-Orbitrap HRMS.

Materials and Methods

Materials

B. balsamifera was obtained from Medicinal Plant Cultivation and Conservation Unit, Tropical Biopharmaca Research Centre, IPB University, Indonesia. *B. balsamifera* is grown and harvested in Bogor and Sukabumi, West Java, Indonesia. Plants were harvested at the age of 5 months. Sample identification was done by Mr. Taopik Ridwan (Trop-BRC, IPB University) with voucher sample number BMK0044052015. Ethanol, 7.5% sodium chloride, 10% aluminium chloride, Folin-Ciocâlteu, potassium acetate, acetate buffer, copper (II) chloride, $FeCl_3$ x $6H_2O$, hydrochloric acid and ammonium acetate were obtained from Merck (Darmstadt, Germany). Quercetin was obtained from Cayman Chemical (Michigan, USA). Gallic acid, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylate), DPPH (1,1-diphenyl-2-picrylhydrazyl), TPTZ (2,4,6-tripiridyl-triazine) and neocuproin were bought from Sigma Aldrich (St. Louis, USA).

Sample preparation and extraction

B. balsamifera leaves were cleaned and dried, then mashed and sieved with 40 mesh size. This material was then used for further analysis. Samples were macerated using water (ratio 1:30) and 70% ethanol (ratio 1:10). The samples were macerated in water for 3 x 24 hours and 70% ethanol for 2 x 24 hours. The results were concentrated using a rotary evaporator at 55 - 60°C until thick extracts were obtained. Furthermore, the extracts were determined by comparing the individual extract's weight and the weight of the initial sample used.

Determination of total phenolics

The Folin-Ciocâlteu method was used to determine the total phenolic content in *B. balsamifera* leaves extracts following the procedure described by Kruawan and Kangsadalampai [10]. Into a 96-well plate, it was pipetted 10 µL of each extract. Distilled water (160 µL), Folin-Ciocâlteu reagent 10% (10 µL) and Na_2CO_3 7.5% (20 µL) were then added to the extract, and the mixture was then treated to homogenize, and the homogenized mixture was incubated in the dark for 30 minutes. A microplate reader further read the absorbance of the mixture at 750 nm and gallic acid solution with five different concentrations: 25, 50, 75, 100, 150 and 175 mg/L were used to make a standard curve. TPC in the extract was expressed as milligram gallic acid equivalent *per* gram dry powder of *B. balsamifera* (mg GAE/g dry powder).

Determination of total flavonoids

Following the procedure described by Lee *et al*., [11], the aluminium chloride colorimetry method was used to determine the total flavonoid content (TFC). Into a 96-well plate, each 10 µL of the extract was added with 96% ethanol (60 µL), 10% $AlCl_3$ (10 µL), 1 M CH_3COOK (10 µL) and distilled water (120 µL). The mixture was then homogenized and incubated for 30 minutes at room temperature. Six concentrations of quercetin solution, 25, 50, 75, 100, 150 and 200 mg/L, were prepared to make a standard curve and the absorbance of the mixture and the standard were read by a microplate reader at 415 nm. TFC in the extract was expressed as milligram quercetin equivalent *per* gram dry powder of *B. balsamifera* (mg QCE/g dry powder).

DPPH radical scavenging activity assay

A method described by Adekola *et al*., [12] was used to measure the antioxidant activity of extracts. Into a microplate, it was added 50 µL extract with 150 µL of DPPH 125 µM solution and then homogenized and incubated in the dark for 30 minutes. The absorbance was then measured at 515 nm and a standard curve

using seven different concentrations of Trolox, 0, 25, 50, 75, 100, 150 and 200 µM, was prepared with ethanol as the blank. The antioxidant activity was expressed in µmol Trolox/g dry powder.

Cupric ion reducing antioxidant capacity assay

The cupric ion-reducing antioxidant capacity was measured following the procedure described by Apak *et al*., (2007) [13]. Into 40 µL extract, it was added 10 mM of $CuCl_2$ (50 µL), 7,5 mM ethanolic neo-cuproine (50 µL) and 1 M NH_4CH_3COO (pH 7) (60 µL). The mixture was then homogenized and incubated for 1 hour. A microplate reader was used to read the absorbance at 450 nm and Trolox solution with nine different concentrations, 0, 150, 200, 250, 300, 350, 400, 500 and 600 µM, and was prepared to make a standard curve. The antioxidant activity was expressed in µmol Trolox/g dry powder.

FRAP method for antioxidant activity assay

The procedure described by Bolanos de la Torre *et al*., [14], was used to determine the ferric-reducing antioxidant power. The FRAP reagent was prepared with a mixture of 20 mM $FeCl_3$ x $6H_2O$, 10 mM TPTZ in 40 mM HCl and 300 mM acetate buffer pH 3.6 with a ratio of 10:1:1. Into a microplate, it was added 20 µL of extract and 180 µL of FRAP reagent and then homogenized and incubated for 30 minutes. A microplate reader was used to read the absorbance at 593 nm. Trolox solution with 8 different concentrations: 0, 50, 75, 100, 150, 200, 300, 400 µM were used to make a standard curve. The antioxidant activity was expressed in µmol Trolox/g dry powder.

Identification of metabolite in B. balsamifera extracts using UHPLC-Q-Orbitrap HRMS

The instrument used to analyse the metabolites in *B. balsamifera* leaves extract was Thermo Scientific Vanquish Flex Ultra-High-Performance Liquid Chromatography tandem Q Exactive Plus Orbitrap-High Resolution Mass Spectrometer (UHPLC-Q-Orbitrap HRMS) (Thermo Fisher, Waltham, MA, USA). The device was equipped with a diode array detector and the detection was carried out at 254 nm. The column used was Accucore™-Phenyl Hexyl LC Column, 2.1 mm × 100 mm, diameter 2.6 µm (Thermo Fisher, Waltham, MA, USA). This device was operated at 200°C. The mobile phase components were 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B), with a flow rate of 0.2 mL/minute under a gradient program. The sample injection volume was five µL with a high-pressure limit of 15000 psi and a seal wash period of 5.00 minutes. The resulting UHPLC-Q-Orbitrap HRMS data were collected in electrospray ionization (ESI) ± mode using the selected ion recording (SIR) function to maximize the analysis's sensitivity and in the m/z range 100 - 1500. Other parameters are set under the following conditions: capillary temperature 320ºC, spray voltage 3.8 kV, sheath and auxiliary gas were 15 and 3 mL/min, resolving power 70,000 FWHM. The scan type used is full MS/dd MS2.

An individual chromatogram was processed using Thermo Scientific Xcalibur ver. 4.2 (Thermo Fisher, Waltham, MA, USA) and MZmine ver. 2.53 [15] to see the differences in *B. balsamifera* leaves extract's metabolite profiles growing location and the type of the extracting solvent. The initial data processing step was done by reviewing the data, including the data format. Mass spectral alignment, signal normalization and metabolite identification were subsequently carried out [16]. The metabolite level was estimated by comparing the detected mass spectrum fragmentation patterns with the available databases.

Statistical analysis

The experimental data obtained were subjected to a one-way ANOVA test. The results were used to see the correlation between the total phenolic and flavonoid content on the antioxidant activity of each sample.

Results and Discussion

Extraction yield

The two samples of *B. balsamifera* were extracted by maceration using water and 70% ethanol as solvents. The yield of the 70% ethanol extract of *B. balsamifera* leaves was higher than the water extract (Figure 1).

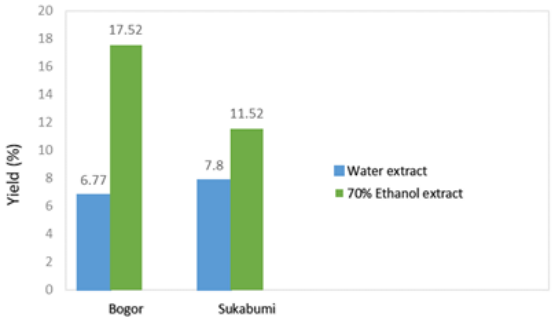

Figure 1.
Extract yield of *B. balsamifera* leaves

The selection of polar solvents, ethanol and water is related to solvents permitted by drug and food agencies. Differences in solvent composition were carried out in this study as an initial study to observe changes in metabolite composition on antioxidant activity. The selection of solvents for extraction plays an important role in the quantity and quality of extracts. Ethanol is a polar organic solvent with a non-polar side, so it is more efficient in degrading cell walls and dissolving chemical components. Therefore, more polyphenol compounds in the cells are extracted by the solvent. Accordingly, more yield was obtained from the extraction of *B. balsamifera* leaves using 70% ethanol.

Total phenolics and total flavonoids of B. balsamifera extracts

The total phenolics content of *B. balsamifera* leaves extracts was determined using the Folin-Ciocâlteu

method. The reagent used was Folin-Ciocâlteu as a coloured complex and Na_2CO_3 as a base alkaline. The standard used was gallic acid. The TPC values obtained can be seen in Table I. TPC decreased in each extract: Bogor-70% ethanol extract > Sukabumi-70% ethanol extract > Bogor-water extract > Sukabumi-water extract. The levels are 62.68 mg GAE/g dry powder for 70% ethanol extract of *B. balsamifera* from Bogor and 13.01 mg GAE/g dry powder for water extract of *B. balsamifera* from Sukabumi.

TFC was determined by using the $AlCl_3$ method. $AlCl_3$ was used as a complex formation with flavonoid groups so that the wavelength shifted towards the visible light and CH_3COOK was used as a complex stabilizer so that the complex remained at visible light wavelengths. The standard used was quercetin. The value of TFC from each sample of *B. balsamifera* leaves extract can be seen in Table I. TFC decreased in each extract: Bogor-70% ethanol extract > Sukambumi-70% ethanol extract > Sukabumi-water extract > Bogor-water extract. The levels ranged from 19.06 mg QE/g dry powder for 70% ethanol extract from Bogor and 4.03 mg QE/g dry powder for water extract from Bogor. Each value was reported as a mean of five measurements followed by the ± SD (standard deviation) value as a statistical value, which was used to see how the data was distributed and how close the data points were to the mean value so that the measurement data can be said to be good.

The values of TPC and TFC of the ethanol extract were 70% higher than the water extract (Table I). Ethanol has more advantages over water as a solvent since ethanol can suppress the activity of the polyphenol oxidase enzyme. High polarity is also provided by 70% ethanol to dissolve flavonoids with glycoside bonds. Apart from the different solvents used, other factors, such as differences in growing locations, also affect the components' levels. Different growing locations can affect the plant's metabolic system, so the compounds produced also differ in quantities [17, 18]. The decrease in flavonoid level occurs with the increasing height of the growing area. In this study, the statistical tests showed that each extract's total phenolic and flavonoid levels were significantly different at $p > 0.05$. This showed that the difference in the growing location of the plant and the type of extracting solvent could affect the plant's total phenolic and flavonoid contents.

Table I

Total phenolic and flavonoid contents of *B. balsamifera* leaves extract

Growing location of sample	Extraction solvent	Total phenolics (mg GAE/g dry powder)	Total flavonoids (mg QE/g dry powder)
Bogor	Water	17.41 ± 0.52^a	4.03 ± 0.16^a
	Ethanol 70%	62.68 ± 0.99^d	19.06 ± 0.41^c
Sukabumi	Water	13.01 ± 0.48^b	4.07 ± 0.20^a
	Ethanol 70%	19.87 ± 0.68^c	10.27 ± 0.37^b

The mean ± SD within each measurement in the same column, followed with different lowercase letters, represent significant differences at $p < 0.05$

Antioxidant activity of B. balsamifera extract

The antioxidant activity of *B. balsamifera* leaves in this study was determined using DPPH, CUPRAC and FRAP methods. The standard used was Trolox. The standard was chosen because it has a high antioxidant capacity index to determine a sample's antioxidant potential, which is stated as Trolox equivalent (TE) [19]. The methods used in determining the antioxidant activity have different mechanisms of action. In principle, the DPPH method works by capturing hydrogen from the antioxidant by DPPH. Proton donation causes the purple DPPH free radical to become a DPPH-H non-radical compound marked by the fading purple colour. One that can influence this method is the use of solvent. The transfer of electrons and hydrogen atoms will work best when using ethanol or methanol as a solvent, whereas water or acetone will perform poorly [20].

The CUPRAC and FRAP methods work on the oxidation-reduction mechanism between the sample and the metal complex in their respective reagent solutions. The CUPRAC method works based on the reduction of Cu^{2+} metal in the blue complex of Cu^{2+}-neocuproine ($Cu(Nc)_2^{2+}$) into the yellow complex of Cu^{+}-neocuproin ($Cu(Nc)^{2+}$). The NH_4CH_3COO, pH 7, functions to stabilize the pH of the solution because the complex ($Cu(Nc)_2^{2+}$) will work optimally at that pH [13]. The FRAP method reduces Fe^{3+} metal in the Fe^{3+}-TPTZ complex into a blue complex of Fe^{2+}-TPTZ. The Fe^{3+}-TPTZ complex was formed by adding $FeCl_3$ x $6H_2O$ and tripyridyl triazine (TPTZ) to the preparation. Adding acetate buffer pH 3.6 stabilizes the complex formed and facilitates the reduction of Fe^{3+} by antioxidant agents [21]. The results for determining antioxidant activity using these three methods can be seen in Table II.

Table II

Antioxidant activity of *B. balsamifera* leaves extract

Growing location of sample	Extraction Solvent	Antioxidant capacity (µmol Trolox/g dry powder)		
		DPPH	CUPRAC	FRAP
Bogor	Water	17.21 ± 0.15^a	430.98 ± 6.93^a	93.29 ± 1.76^a
	70% Ethanol	60.35 ± 0.19^d	1815.25 ± 19.83^d	309.91 ± 3.56^c

Growing location of sample	Extraction Solvent	Antioxidant capacity (µmol Trolox/g dry powder)		
		DPPH	CUPRAC	FRAP
Sukabumi	Water	19.59 ± 0.28^{b}	486.58 ± 13.87^{b}	103.01 ± 2.21^{b}
	70% Ethanol	39.90 ± 0.15^{c}	505.98 ± 8.98^{c}	144.28 ± 0.89^{d}

The mean ± SD within each measurement in the same column, followed with different lowercase letters, represent significant differences at $p < 0.05$

The results indicate that the 70% ethanol extract of *B. balsamifera* from Bogor has the highest antioxidant activity, while the water extract from Bogor has the lowest. As for the three methods used, the CUPRAC method provides the highest activity value compared to the other methods. Therefore, data analysis was done using one-way ANOVA to see the difference between the three methods. The results of the variance analysis showed that the F counts were greater than the F values (0.05, 2 and 12). So that the null hypothesis (H_0) was rejected, meaning that at least a pair of methods gave different values for antioxidant activity, which needed further Duncan tests. The results showed a different subset for each sample of the three methods used. This showed that the DPPH, CUPRAC and FRAP methods differed significantly. The DPPH method gave the lowest results compared to the other two methods. This may occur because the DPPH reagent can only dissolve in polar organic solvents such as methanol and ethanol. Besides, its use is limited to lipophilic antioxidants [19, 20]. The CUPRAC method gave the highest results compared to the other methods. This method can measure hydrophilic and lipophilic antioxidants and is stable at a pH of around 7. The FRAP method showed lower results than the CUPRAC method. In the FRAP method, the reagent cannot react with the antioxidant thiol and glutathione. In addition, the reduction potential of Fe^{3+} to Fe^{2+} (0.77 V) is higher than that of Cu^{2+} to Cu^{+} (0.159 V). Therefore, it is less selective about the potential effects of sugar and citric acid [22].

Correlation between metabolite composition of B. balsamifera extract and antioxidant activity

The quantitative correlation between the total phenolic and flavonoid levels of *B. balsamifera* leaves and the antioxidant activity was determined using the linear regression equation model and Pearson correlation. The two methods were used because each method of determining antioxidants showed different relationship dynamics. Pearson correlation can determine the linear relationship between the two variables. The correlation is high if the correlation coefficient (r) is high. The significance test is based on the assumption that the residual value of the y variable follows a normal distribution, and the variability of the residual value is the same for all x variables [23]. The highest yield was obtained by 70% ethanol extract of *B. balsamifera* from Bogor with a value of 0.824 (Table III). This value shows a positive relationship between phenol levels and antioxidant activity as tested by the DPPH method.

Table III

Correlation between phenolic and flavonoid contents and antioxidant activity

Growing Location	Extraction Solvent	Variable	DPPH	CUPRAC	FRAP
Bogor	Water	Phenol	0.650	0.547	-0.451
		Flavonoid	-0.611	-0.388	0.480
	70% ethanol	Phenol	0.824	0.124	0.795
		Flavonoid	0.395	-0.850	0.539
Sukabumi	Water	Phenol	0.302	0.392	0.118
		Flavonoid	0.937	-0.385	0.821
	70% ethanol	Phenol	-0.179	-0.267	-0.718
		Flavonoid	-0.142	-0.232	-0.642

The correlation of total flavonoid level with antioxidant activity can be acknowledged by analysing Table III. The water extract of *B. balsamifera* obtained the highest yield from Sukabumi, with a value of 0.937. This value indicates a positive relationship between the flavonoid level and antioxidant activity as tested by the DPPH method. According to Apak *et al.*, (2007) [13], flavonoid activity highly depends on the number and location of -OH to neutralize free radicals. The ability of flavonoids to neutralize free radicals is also related to their ability to donate electrons. The higher the total phenolic and flavonoid levels in the water and 70% ethanol extract of *B. balsamifera* leaves, the higher the antioxidant activity given by donating electrons to suppress free radicals.

Putative identification of metabolites in B. balsamifera extracts

The metabolite profile in the negative ionization mode of *B. balsamifera* leaves extract can be observed in Figure 2. *B. balsamifera* extracts from Bogor and Sukabumi showed similar chromatogram patterns with different intensities. *B. balsamifera* from Bogor and Sukabumi showed differences in peak intensity at 4 - 8 (minutes) retention times. The intensity of the extracts from Bogor is higher than those from Sukabumi. The difference could come from the growing location,

which can affect the distribution of metabolite components of a plant. The location where a plant grows is influenced by soil fertility, climate and altitude above sea level. The higher the place, the lower the average air temperature. These geographical conditions can affect plants in their metabolic processes. The difference in the chromatogram can be seen from the different solvents used. The 70% ethanol extract of *B. balsamifera* leaves has a higher intensity than the water extract at a 4 - 8 (minutes) retention time. This suggests that the metabolites contained in *B. balsamifera* are probably semi-polar. This principle is based on like dissolve like, the extracting solvent can only extract compounds with similar polarity [24].

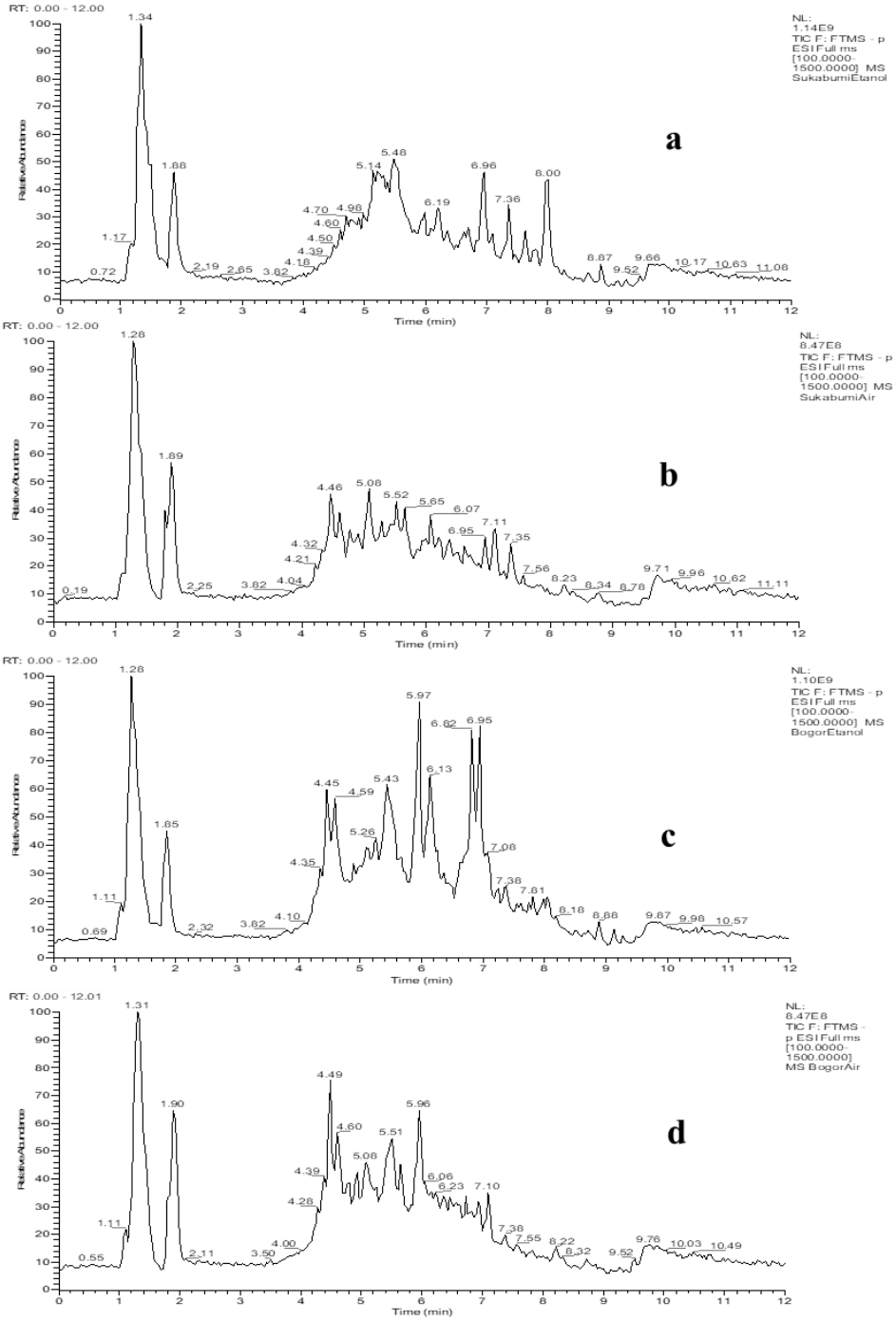

Figure 2.

Total ion chromatogram (negative ionization mode)

(a) Sukabumi-ethanol extract, (b) Sukabumi-water extract, (c) Bogor-ethanol extract, (d) Bogor-water extract

The total metabolite identified in *B. balsamifera* extracts was 47 compounds (Table IV). A total of 29 compounds were present in Bogor-water extract (BW), 31 compounds in Bogor-ethanol extract (BE) and 33 compounds in each Sukabumi-water extract (SW) and Sukabumi-ethanol extract (SE), respectively. The

results were obtained from data processing using MZmine, which were then confirmed using MS2 and compared with the literature review. Further studies on plant cultivation and essential oil standardization are needed to determine the varying chemical composition of the extracts of *B. balsamifera* [25,26].

Table IV

Putative identification of metabolite in *B. balsamifera* leaves extract

No.	t_R	$[M-H]^-$	Formula	Compound	BW	BE	SW	SE
1	1.26	160.0971	$C_5H_7NO_5$	α-Butenoic acid, 3-methoxy-4-nitro	-	-	✓	-
2	1.29	195.0687	$C_{10}H_{12}O_4$	Xanthoxylin	-	✓	✓	✓
4	1.36	284.1402	$C_{16}H_{12}O_5$	4',5-Dihydroxy-7-methyletherflavanone	-	-	✓	-
5	1.46	133.0869	$C_{10}H_{14}$	1,3,4,5,6,7-hexahydro-2,5,5-trimete-hyl-2H2,4A-et hanonaphthalene	-	✓	✓	✓
6	2.91	145.9982	$C_5H_9NO_4$	O-acetyl-1-serine	-	✓	-	✓
7	4.19	317.1469	$C_{16}H_{14}O_7$	Dihydroquercetin-4'-methylether	-	✓	-	-
8	4.55	343.1841	$C_{18}H_{16}O_7$	3,5-Dihydroxy-3',4',7-trimethoxyflavone	-	✓	✓	-
10	4.84	305.0695	$C_{15}H_{14}O_7$	Epigallocatechin	-	✓	✓	✓
11	5.16	287.2309	$C_{15}H_{12}O_6$	Eriodctyol	-	✓	✓	✓
12	5.28	301.1475	$C_{16}H_{14}O_6$	Blumeatin (5,3',5'-trihydroxy-methoxydihydroflavone)	-	✓	✓	-
13	5.38	463.2448	$C_{21}H_{20}O_{12}$	Isoquercetin	-	✓	✓	✓
14	5.56	337.0919	$C_{16}H_{18}O_8$	Chlorogenic acid	✓	✓	✓	✓
15	5.57	153.1179	$C_{10}H_{18}O$	L-borneol	-	-	✓	-
16	5.60	151.1021	$C_{10}H_{16}O$	Perillyl alcohol	-	-	✓	-
17	5.64	153.1179	$C_{10}H_{18}O$	Isoborneol	-	-	✓	-
18	5.88	353.0865	$C_{16}H_{18}O_9$	2-Ocaffeoylquinic acid	✓	✓	✓	✓
19	5.93	193.0495	$C_{10}H_{10}O_4$	Ferulic acid	✓	✓	✓	✓
20	5.95	153.0179	$C_7H_6O_4$	Protocatechuic acid	✓	-	✓	✓
21	6.06	331.1734	$C_{17}H_{16}O_7$	Dihydroquercetin-7,4'-dimethylether	✓	✓	-	✓
22	6.07	137.0231	$C_7H_6O_3$	Benzoic acid	✓	✓	✓	✓
23	6.11	289.1368	$C_{15}H_{14}O_6$	Catechin	✓	✓	-	✓
24	6.18	135.1027	$C_{10}H_{16}$	(+)-Limonene	✓	-	-	-
25	6.21	309.2107	$C_{16}H_{22}O_6$	Blumealactone C	✓	✓	-	✓
26	6.23	177.0106	$C_9H_6O_4$	5,7-Dihydroxychromone	✓	✓	✓	✓
27	6.41	257.1380	$C_{15}H_{14}O_4$	Davidigenin	✓	-	-	✓
28	6.45	303.1204	$C_{15}H_{12}O_7$	3,3',5,5',7-Pentahydroxyflavanone	-	-	✓	✓
29	6.51	147.0705	$C_{10}H_{12}O$	Cuminaldehyde	✓	✓	✓	✓
30	6.56	395.1375	$C_{19}H_{24}O_9$	5,7-Hydroxyl2-(1methylpropyl) isopropylchroone-8-βD-glucoside	✓	✓	✓	✓
31	6.58	169.1173	$C_{10}H_{18}O_2$	Trans-linalool oxide	✓	✓	✓	✓
32	6.77	181.0836	$C_9H_{10}O_4$	3,4-Dihydroxyhydrocinnamic acid	✓	✓	✓	✓
33	6.91	301.0708	$C_{15}H_{10}O_7$	Quercetin	✓	✓	✓	✓
34	7.30	315.2529	$C_{16}H_{12}O_7$	3,5,3',4'-Tetrahydroxy-7-methoxyflavone	✓	✓	✓	✓
35	7.37	179.0339	$C_9H_8O_4$	Caffeic acid	✓	✓	✓	✓
36	7.43	208.1086	$C_8H_3NO_6$	3-Nitrophthalic acid	✓	-	-	-
37	7.51	359.1835	$C_{18}H_{16}O_8$	Chrysosplenol C	✓	✓	✓	✓
38	7.56	329.1576	$C_{17}H_{14}O_7$	3,5,3'-Trihydroxy-7,4-dimethoxyflavone	✓	✓	✓	✓
39	7.61	299.2584	$C_{16}H_{12}O_6$	Luteolin -7-methyl ether	✓	✓	✓	✓
40	8.15	191.0547	$C_7H_{12}O_6$	Quinic acid	✓	✓	✓	✓
41	8.57	285.1052	$C_{15}H_{10}O_6$	Luteolin	-	-	-	✓
42	8.90	149.0863	$C_{10}H_{14}O$	Chrysanthenone	-	✓	✓	-
43	9.46	195.1545	$C_{12}H_{20}O_2$	Bornyl acetate	✓	-	-	-
44	9.71	161.0112	$C_9H_6O_3$	Umberlliferone (7-hydroxycoumarin)	✓	-	-	✓
45	9.78	191.0371	$C_{10}H_8O_4$	Hydrangetin	✓	-	-	-
46	9.82	417.3053	$C_{22}H_{26}O_8$	Syringaresinol	-	✓	-	✓
47	10.36	365.1880	$C_{17}H_{18}O_9$	3,4'5-Trihydroxy-3'7-dimethoxyflavanone	✓	-	✓	✓
48	10.71	127.0807	$C_8H_{16}O$	1-Octen-3-ol	✓	-	✓	✓
49	10.92	183.1438	$C_{11}H_{20}O_2$	Trans-2-undecenoic acid	✓	✓	✓	✓

Based on the identified compounds, *B. balsamifera* extract was dominated by flavonoid and phenolic acid. The content of flavonoids and phenolic acids in *B. balsamifera* from Bogor and Sukabumi has differences, possibly due to the location of growth or post-harvest processes, such as the drying, cutting and extraction process. The flavonoid and phenolic acid compounds in the *B. balsamifera* extract comprises 4',5-dihydroxy-7-methyletherflavanone, Dihydroquercetin-4-methyl-ether, epigallocatechin, catechin, luteolin -7-methyl-ether, quercetin, chlorogenic acid, ferulic acid, 3,4-dihydrocinnamic acid and caffeic acid.

The compound 4',5-dihydroxy-7-methyletherflavanone (4) is detected at 1.36 min with m/z 284.1402 $[M-H]^-$. Dihydroquercetin-4-methyl ether (7) was identified at m/z 317.1469 $[M-H]^-$, compound **7** was fragmented by releasing H_2O molecules at m/z 299 $[MH-H_2O]^-$ and followed by loss of ring B of flavonoids at m/z 152 and ring A at m/z 165. Epigallocatechin (10) was identified at a retention time of 4.84 min with m/z 305.0695 $[M-H]^-$. Catechins (23) were identified at m/z 289.1368 $[M-H]^-$ and fragmented by releasing A ring of flavonoids followed by CH_3 and OH with m/z 133. Luteolin-7-methyl ether was identified at m/z 299.2584. Fragmentation of this compound by releasing CH_3 molecules on m/z 284, the loss of flavonoid ring B at m/z 165 and the loss of flavonoid A ring at m/z 135. Quercetin (33) was detected with a retention time of 6.91 min at m/z 301.0708. This compound fragmented by the loss of flavonoid B ring followed by OH release at m/z 135.

Chlorogenic acid (14) was identified as m/z 337.0919 and fragmented at m/z 179, 163 and 145. Ferulic acid (19) is a phenolic acid group identified at m/z 193.0495 and fragmented by releasing CH_3 as indicated by the presence of m/z at 178 and followed by the release of CO_2 molecules at m/z 134. Caffeic acid (35) was identified at m/z 179.0339. This compound is fragmented by releasing the CO_2 molecule indicated by m/z 135. In addition to the flavonoid and phenolic acid class compounds, *B. balsamifera* extract contains carboxylic acid, lignans, furans, phthalic acid, a sesquiterpene lactone and chalcone compounds.

Conclusions

DPPH, CUPRAC and FRAP methods can determine the total phenolic, flavonoid and antioxidant activity of *B. balsamifera* leaves extract. Of the three methods, the CUPRAC method showed the highest results with a total phenolic content value of 62.68 mg GAE/g dry powder, total flavonoid content of 19.06 mg QE/g dry powder and antioxidant activity value of 1815.25 µmol Trolox/g dry powder. Total phenolic and flavonoid contents of *B. balsamifera* leaves positively correlate with antioxidant activity. Other compounds in *B. balsamifera* leaves extracted with 70% ethanol and water as solvents were identified using LC-MS/MS, apart from phenolics and flavonoids. The difference in growing location and the extracting solvent affect the metabolite composition of this plant. The compounds identified from *B. balsamifera* extracts from Bogor with water and 70% ethanol as the extracting solvents were 29 and 31, respectively. The number of compounds identified from *B. balsamifera* extracts from Sukabumi with water and 70% ethanol as solvents were 33 compounds each. These compounds are monoterpenes, flavonoids, coumarin, phenolic acids, carboxylic acids, lignans, furans, phthalic acids, sesquiterpene lactones and chalcones.

Use of AI tools declaration

The authors declare that there are no used Artificial Intelligence (AI) tools in the creation of this article.

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Conflict of interest

The authors declare no conflict of interest.

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