Metabolite Profiling of Ebony (*Diospyros celebica* Bakh) Leaves and Wood Extracts Using LC-MS/MS

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Received: August 19, 2021
Accepted: January 17, 2022

DOI: 10.22146/ijc.68529

Abstract: *Ebony (*Diospyros celebica *Bakh) is an endemic forest tree species found in Sulawesi whose basic information on its metabolite profile is still lacking. The objective of this research was to separate and identify putatively metabolite present in the leaves and wood of ebony across Sulawesi. Separation and identification of ebony metabolites were carried out using UHPLC-Q-Orbitrap HRMS analysis. Using ultrasonication with ethanol as the extracting solvent, we used powdered ebony leaves and wood. The results showed that the metabolites contained in the leaves and wood of ebony were 59 metabolites. About 14 compounds were found in the leaves and wood, 21 compounds in the wood, and 24 compounds in the leaves. The identified metabolites are flavonoids, terpenoids, amino aldehydes, alkaloids, quinones, steroids, amino acids, fatty acids, and saccharides. Clustering of ebony using principal component analysis obtained leaves and wood groups using peak area of known compounds as the variable.*

Keywords: Diospyros celebica *Bakh; ebony; metabolomics; UHPLC-Q-Orbitrap HRMS*

■ INTRODUCTION

Ebony (*Diospyros celebica* Bakh) is one of the endemic plants from Sulawesi, Indonesia. Ebony could be found in West Sulawesi, South Sulawesi, and Central Sulawesi [1]. Ebony wood is still a high economic value commodity made everyone seek the wood. Ebony is widely used as a material for luxury furniture, sculptures, carvings, fans, lathes, garnishing tools, brush bodies, luxury venire, wind instruments [2]. Another benefit of ebony wood was used as a non-synthetic preservative. It contains secondary metabolites that can be used in the health sector because it has several biological activities, such as antidiabetic, antibacterial, antifungal, and antiviral [3-6].

Utilization of ebony leaves may be an alternative to obtain secondary metabolites with various kinds of biological activities above. So, they would not cut down ebony because this wood is protected. However, those biological activities indeed come from secondary metabolites, which will differ in the composition and concentration in each part of the plant, such as leaves and stems. Therefore, research is needed to identify the different metabolites contained in ebony stems and leaves as initial information on the composition and concentration of the metabolites they have.

The different metabolites in the leaves and stems of ebony could be separated and identified using an LC-MS/MS-based metabolomics approach. LC-MS/MS is

used to analyze secondary metabolites' targeted and untargeted analysis. In addition, this instrument also has several advantages, such as high sensitivity and selectivity, can identify secondary metabolites in a relatively wide range, fast analysis time, and can separate large quantities of components with high resolution [7-8]. However, identifying metabolites using LC-MS/MS will produce complex data, so multivariate data analysis is needed to reduce data complexity [9].

In this study, the LC-MS/MS analysis results were evaluated using a multivariate analysis, such as the principal component analysis (PCA) method to describe the grouping pattern of metabolites contained in ebony leaves and wood extracts. A combination of LC-MS/MS with multivariate analysis has been reported to be able to classify metabolites based on plant parts extracted from the species *Andrographis paniculata* [10], *Chrysophyllum perpulchrum* [11], and *Harungana madagascariensis* [12], also used as an integrated strategy for quality control of *Dalbergia odorifera* [13]. However, no reported paper applies multivariate analysis to classify metabolites from ebony leaves and wood extracts. Therefore, this study aimed to identify metabolites from ebony leaves and wood extracts using LC-MS/MS and cluster them using PCA.

■ EXPERIMENTAL SECTION

Materials

The samples used in this study are ebony leaves and wood taken from three provinces in Sulawesi. We collected the ebony samples from several tree-growing sites in the three provinces, Batu Ampa and Sondoang from West Sulawesi, Pani Binangga, and Poso Pesisir from Central Sulawesi, Cani Sirenreng, and Bellabori from South Sulawesi. The collected samples were then composited in each location, so we have six ebony leaves and wood samples. All solvents (analytical or LC-MS grade) were purchased from Merck (Darmstadt, Germany).

Instrumentation

Separation of ebony metabolites are using an LC-MS/MS Thermo Scientific Vanquish Flex UHPLC tandem Q Exactive Plus Orbitrap-High Resolution Mass Spectrometer (UHPLC-Q-Orbitrap HRMS) instrument (Thermo Fisher, Waltham, USA) equipped with ThermoXCalibur software and Compound Discoverer version 3.1 (Thermo Fisher, Waltham, USA). In addition, principal component analysis was carried out using the Unscrambler X version 10.1 (CAMO, Oslo, Norway).

Procedure

Extraction

Before being used for extraction, we pulverized all of the samples. The sample powder was extracted in ethanol at a ratio of 1:10 using ultrasonication for 30 minutes. The extract was then filtered, and the filtrate was analyzed using UHPLC-Q-Orbitrap HRMS to separate and identify metabolites.

Separation of ebony leaves and wood metabolites using UHPLC-Q-Orbitrap HRMS

Metabolites in ebony leaves and wood extracts were separated using the Vanquish Flex UHPLC-Q Exactive Plus Orbitrap-High Resolution Mass Spectrometer with Accucore C18 (100 × 2.1 mm, 1.5 m) as the column. The mobile phase used is 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) with a gradient elution system: 0.0–5.0 min (2–5 %B), 5.0–12.5 min (5–20 %B), 12.5–25.0 min (20–30 %B), 25.0–30.0 min (30–40 %B), 30.0-35.0 min (40–95 %B), 35.0–38.5 min (95 %B), 38.5–44.0 min (2 %B). The flow rate used was 0.25 mL/min with an injection volume of 2.5 L. The ionization source used in the MS system is ESI, with positive and negative ionization modes in the *m/z* range of 100–1500. The capillary temperature used was 320 °C, spray voltage 3.8 kV, sheath gas and auxiliary gas flow rate 15 and 3 mL/min, and automatic gain control (AGC) 3 × 106 and injection time set to 100 ms. The collision energy used is 18, 35, and 53 eV. The scan type used is full MS/dd MS2 and full scan data set with a resolving power of 70,000 FWHM.

Data analysis

UHPLC-Q-Orbitrap HRMS data were processed using Compound Discoverer 2.2 with an in-house database collected from information on compounds in

the genus *Diospyros* to identify ebony leaves and wood extracts metabolites. Metabolites were identified through several stages, namely selected spectra stage, alignment retention time, detected unknown compounds, grouping unknown compounds, predicting processes, searching mass lists, filling gaps, normalizing areas, and marking background compounds. Ebony leaves and wood extract were then grouped by PCA using The Unscrambler X version 10.1. The variable used for grouping is the peak area of the identified compounds.

■ RESULTS AND DISCUSSION

Putative Identification of Ebony Leaves and Wood Metabolites

UHPLC-Q-Orbitrap HRMS was used to separate and putative identification of ebony leaves and wood metabolites. Positive ionization mode obtained more detected metabolites than negative ionization mode. In addition, the ebony wood extract had a different chromatogram profile compared to the leaves extract, so the detected metabolites were also other, as shown in Fig. 1.

About 59 compounds were putatively identified in ebony leaves and wood extracts, consisting of 21 compounds identified only in the wood, 24 identified only in the leaves, and 14 identified in both wood and leaves (Fig. 2). The identified metabolites are flavonoids, terpenoids, amino aldehydes, alkaloids, phenolic acids, quinones, steroids, amino acids, fatty acids, and saccharides (Table 1).

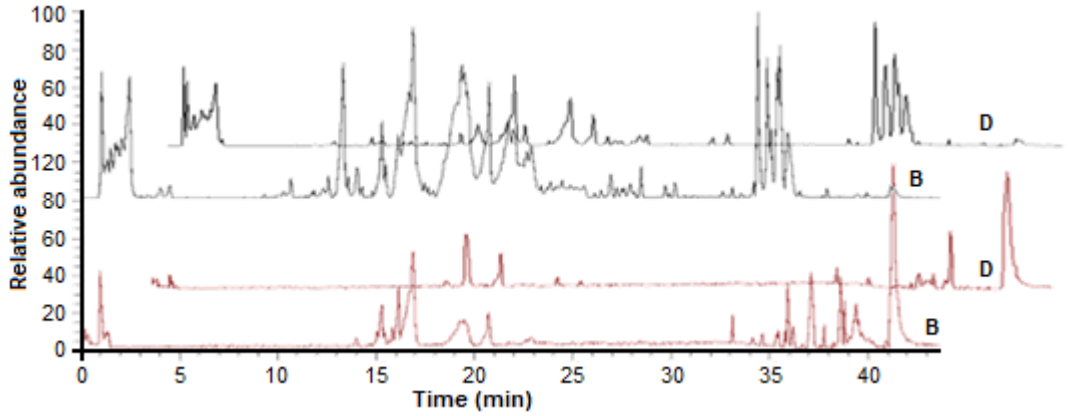

Fig 1. Representative chromatogram of ebony wood (B) and leaves (D) in positive ionization mode (black) and negative ionization mode (red)

Table 1. Putative identification of metabolite in ebony leaves and wood extracts

No	Name of Metabolites	RT	Formula	MW	Error mass (ppm)	Ion mode	MS-MS
	Flavonoids						
1	Ethyl 1,3-dihydroxy-2-naphthoate	24.70	$C_{13}H_{12}O_4$	232.0734	-0.86	$[M+H]^+$	233, 218, 190,161
2	Gibberellin A5*	15.01	$C_{19}H_{22}O_5$	330.1456	-3.33	$[M+H]^+$	331, 313
3	3-Methylplumbagin*	21.90	$C_{12}H_{10}O_3$	202.0627	-1.48	$[M+H]^+$	203, 188, 160
4	Rutin *	23.39	$C_{27}H_{30}O_{16}$	610.1503	-5.08	$[M+H]^+$	611, 423, 329, 167
5	Kaempferol 3-(2''-galloylglucoside)	20.79	$C_{28}H_{24}O_{15}$	600.0863	-1.67	$[M+H]^+$	601, 403, 287, 153
6	Diosindigo A*	34.48	$C_{24}H_{20}O_6$	404.1252	-1.98	$[M+H]^+$	405,387,373,345
7	Apigenin*	14.34	$C_{15}H_{10}O_5$	270.0521	-2.59	$[M+H]^+$	271, 253, 225, 211
8	Palomid 529*	35.37	$C_{24}H_{22}O_6$	406.1413	-0.74	$[M+H]^+$	405, 390, 358, 357
9	Daidzein*	15.31	$C_{15}H_{10}O_4$	254.0572	-2.76	$[M+H]^+$	255, 214, 185, 172
10	Kaempferol**	16.08	$C_{15}H_{10}O_6$	286.0477	-2.45	$[M+H]^+$	287, 253, 201

Table 1. Putative identification of metabolite in ebony leaves and wood extracts (*Continued*)

No	Name of Metabolites	RT	Formula	MW	Error mass (ppm)	Ion mode	MS-MS
	Terpenoids						
15	Hemigossypolone	16.24	$C_{15}H_{14}O_5$	274.0838	-2.55	$[M-H]^-$	272, 151, 125
16	Betulinic acid	37.91	$C_{30}H_{48}O_3$	456.3602	-3.07	$[M-H]^-$	455, 439, 421
17	(6aR,11aR)-3,9-Dihydroxypterocarpan	19.02	$C_{15}H_{12}O_4$	256.0730	-2.73	$[M+H]^+$	257, 239, 211
18	(2S)-Naringenin	15.19	$C_{15}H_{12}O_5$	272.0676	-3.31	$[M+H]^+$	273, 255, 197
19	Betulinaldehyde	39.52	$C_{30}H_{48}O_2$	440.2891	-2.27	$[M+H]^+$	441, 315, 286
20	Pomolic acid*	37.68	$C_{30}H_{48}O_4$	472.3899	-2.54	$[M+H]^+$	473, 437, 409, 313
21	Phlorisobutyrophenone *	11.46	$C_{10}H_{12}O_4$	196.0733	-1.53	$[M+H]^+$	197, 151, 109, 72
22	Lupeol*	39.70	$C_{30}H_{50}O$	426.3847	-3.52	$[M+H]^+$	427, 409, 229, 217
23	Ursa-12,18-dien-3-ol**	39.54	$C_{30}H_{48}O$	424.3693	-2.83	$[M+H]^+$	426, 425, 407, 271
24	Marsformosanone**	36.86	$C_{30}H_{46}O$	422.3535	-3.32	$[M+H]^+$	423, 269, 243, 229
	Amino Aldehydes						
25	Histidinal*	40.85	$C_6H_9N_3O$	139.0744	-1.44	$[M+H]^+$	140, 122, 81
	Alkaloids						
26	2-Phenylethylamine**	5.37	$C_8H_{11}N$	121.0891	0	$[M+H]^+$	122, 105, 80
	Phenolic acid						
27	Benzaldehyde	16.94	C_7H_6O	106.0419	0	$[M+H]^+$	107, 79, 77
28	Gallic acid	2.44	$C_7H_6NO_5$	170.0205	-5.88	$[M-H]^-$	169,125, 97, 81
29	4-Hydroxy-2-oxopentanoate	40.95	$C_5H_8O_4$	132.0424	-1.94	$[M-H]^-$	131,113, 88
30	2-Dehydropantoate	40.74	$C_6H_{10}O_4$	146.0582	2.05	$[M-H]^-$	145, 118, 77
31	Methyl gallate*	8.30	$C_8H_8O_5$	184.0362	-5.43	$[M-H]^-$	168, 139, 123
32	Ellagic acid*	15.03	$C_{14}H_6O_8$	302.0059	-1.32	$[M+H]^+$	300, 283, 230
33	(S)-4-Hydroxymandelate	10.61	$C_8H_8O_4$	168.0419	-2.38	$[M+H]^+$	169, 151, 109, 93
34	(R)-3-(3,4-Dihydroxyphenyl) lactate*	13.63	$C_9H_{10}O_5$	198.0521	-3.53	$[M-H]^-$	197, 169, 140
35	4-Hydroxyphenylacetate*	12.69	$C_8H_8O_3$	152.047	-1.97	$[M+H]^+$	153, 125, 111
36	(R)-2-Benzylsuccinate**	16.27	$C_{11}H_{12}O_4$	208.0732	-1.9	$[M+H]^+$	209, 191, 177
37	p-Hydroxybenzoic acid**	14.01	$C_7H_6O_3$	138.0314	-2.17	$[M+H]^+$	139, 111, 93, 65
38	p-Coumaric acid**	13.30	$C_9H_8O_3$	164.0464	-5.49	$[M-H]^-$	163, 147, 119, 93
	Quinone						
39	6-Methyl-8-hydroxy-1,4-naphthoquinone*	24.26	$C_{13}H_{12}O_5$	248.0680	-2.02	$[M+H]^+$	249, 217, 161, 143
40	Xylospyrin*	19.65	$C_{22}H_{18}O_9$	426.0937	-3.29	$[M+H]^+$	427, 275, 153, 108
41	3',5'-Diacetoxyacetophenone	16.08	$C_{12}H_{12}O_5$	236.0683	-0.42	$[M+H]^+$	237, 193, 177
42	Demethylphylloquinol**	38.24	$C_{15}H_{20}O_4$	438.3486	-2.74	$[M+H]^+$	439, 393, 249, 201
43	4-Prenylphlorisobutyrophenone**	10.21	$C_{30}H_{46}O_2$	264.1356	-2.27	$[M+H]^+$	265, 247, 203, 187
44	Eremopetasinorone A **	12.29	$C_{13}H_{18}O_2$	206.1303	-1.94	$[M+H]^+$	207, 189, 174, 149
45	Carnocin CP 5**	16.55	$C_{23}H_{19}N_3O_5S$	449.1030	-2.00	$[M-H]^-$	448, 327, 284, 255
	Steroids						
46	4α-Hydroxymethyl-4β-methyl-5α-cholesta-8-en-3β-ol	39.97	$C_{29}H_{50}O_2$	428.3645	-2.56	$[M-H]^-$	429, 219, 191, 165
47	3-Dehydroteasterone	39.04	$C_{28}H_{46}O_4$	446.3383	1.51	$[M+H]^+$	447, 351, 191
48	4,4-Dimethylzymosterol	40.97	$C_{29}H_{48}O$	412.3695	-2.91	$[M+H]^+$	413, 395, 241

Table 1. Putative identification of metabolite in ebony leaves and wood extracts (*Continued*)

No	Name of Metabolites	RT	Formula	MW	Error mass (ppm)	Ion mode	MS-MS
49	3-(4-Ethylphenoxy)-4-oxo-4H-chromen-7-yl 2-furoate**	26.99	$C_{22}H_{16}O_6$	376.0938	-2.39	$[M+H]^+$	377, 257, 173
	Amino acid						
50	5-Aminopentanoate**	1.23	$C_{15}H_{11}NO_2$	117.0789	-0.85	$[M+H]^+$	118, 101, 70
51	L-Tryptophan**	7.40	$C_{11}H_{12}N_2O_2$	204.0895	-1.96	$[M+H]^+$	205, 188, 170, 149
52	L-Isoleucine**	1.90	$C_6H_{13}NO_2$	131.0944	-4.58	$[M+H]^+$	132, 116, 86, 69
53	L-Phenylalanine**	3.26	$C_9H_{11}NO_2$	165.0788	-1.20	$[M+H]^+$	166, 149, 131, 103
54	Ethyl 3,5-bis[(4-nitrobenzoyl) amino] benzoate**	14.32	$C_{23}H_{18}N_4O_8$	478.1105	-3.76	$[M-H]^-$	477, 205, 163
	Fatty acid						
55	(9S)-HPODE	34.84	$C_{18}H_{32}O_4$	312.2298	-1.60	$[M-H]^-$	311, 275, 223
56	Colneleate**	35.48	$C_{18}H_{30}O_3$	294.2190	-1.70	$[M-H]^-$	293, 275, 235, 183
57	Linoleate**	36.22	$C_{18}H_{32}O_2$	280.2395	-2.50	$[M+H]^+$	281, 263, 245, 175
58	9,10-Epoxy-10,12Z,15Zoctadecatrienoate**	33.63	$C_{18}H_{28}O_3$	292.2032	-2.05	$[M+H]^+$	293, 278, 277, 249
	Saccharide						
59	Galactitol*	1.39	$C_6H_{14}O_6$	182.0782	-3.30	$[M-H]^-$	181, 163, 131, 113

*) Only identified in the ebony wood extract

**) Only identified in ebony leaves extract

Fig 2. Venn diagram of the putatively identified compound in the ebony leaves and wood

Flavonoid

Flavonoids commonly give yellow color to higher plants, including ebony. In addition, flavonoids also play an important role as natural antioxidants and anticancer [14]. In ebony leaves and wood extracts, we identified ethyl 1,3-dihydroxy-2-naphthoate (1), a flavonoid compound detected at a retention time of 24.7 min. This compound was identified in the positive ionization mode with *m/z* 233 $[M+H]^+$, 218 $[M+H-CH_3]^+$, 190 $M+H-CH_3-CO]^+$, and 161 $[M+H-CH_3-CO-COH]^+$.

Other flavonoid compounds putatively identified only in the ebony wood extracts were gibberellin A5 (2), 3-methylplumbagin (3), rutin (4), kaempferol 3-(2''-galloylglucoside) (5), diosindigo A (6), apigenin (7), palomid 529 (8), daidzein (9). While those identified only in the leaves extracts were kaempferol (10), Kaempferol 3-glucuronide (11), (+)-dihydrokaempferol (12), quercetin 3-O-rhamnoside (13), epicatechin gallate (14).

Terpenoid

As we know, terpenoids function as a protector against microbes and insects [15]. So, maybe because of this, ebony wood is not easily damaged even if buried in the ground for decades. A total of five terpenoid compounds were identified in the leaves and wood extracts of ebony, consisting of hemigossypolone (15), betulinic acid (16), (6aR,11aR)-3,9-dihydroxypterocarpan (17), (2S)-naringenin (18), and betulinaldehyde (19).

Compound (15) was identified at a retention time

of 35.8 and fragmented at *m/z* 272 $[M-H]^-$, 219 $[M-H-OH-CH-CH_2]^-$, and 203 $[M-H-OH-CH_2-CH_2-CH_3]^-$. In addition, three compounds were only identified in the wood extract, namely pomolic acid (20), phlorisobutyrophenone (21), and lupeol (22), as well as two compounds that were only identified in the leaf extract, namely ursa-12,18-dien-3-ol (23), marsformosanone (24).

Amino Aldehydes

In this study, histidinal (25) is a compound from the amino aldehyde group identified in the wood extract of ebony. This compound was detected at 40.9 min and fragmented at *m/z* 140 $[M+H]^+$, 122 $[M+H-H_2O]^+$, and 81 $[M+H-H_2O-C_2-NH_3]^+$.

Alkaloid

One compound from alkaloids that have been putatively identified in ebony leaves is 2-phenylethylamine (26). Compound (26) was detected at a retention time of 5.37 min. This compound was identified based on its fragmentation in the positive ionization mode with *m/z* 122 $[M+H]^+$, 105 $[M+H-NH_2]^+$, and 80 $[M+H-NH_2-C_2]^+$ [16].

Phenolic Acids

A total of five compounds from the phenolic acid group were putatively identified in the extracts of ebony leaves and wood. The five compounds are benzaldehyde (27), gallic acid (28), 4-hydroxy-2-oxopentanoate (29), 2-dehydropantoate, and (30). Benzaldehyde (27) was detected at a retention time of 16.9 min, with its fragmentation producing peaks at *m/z* 107 $[M+H]^+$, 79 $[M+H-CO]^+$, and 77 $[M+H-CO-H_2]^+$.

In addition, five other phenolic acid group compounds that were only identified in the ebony wood extract, namely methylgallate (31), ellagic acid (32), (S)-4-hydroxymandelate (33), (*R*)-3-(3, 4-dihydroxyphenyl) lactate (34), 4-hydroxyphenylacetate (35), and three compounds identified only in leaf extracts, namely (R)-2-benzylsuccinate (36), p-hydroxybenzoic acid (37), p-coumaric acid (38).

Quinone

A quinone is a group of compounds that gives a unique color to ebony leaves and wood. Three compounds were identified in the wood extract, namely 6-methyl-8-hydroxy-1,4-naphthoquinone (39), xylospyrin (40), and 3',5'-diacetoxyacetophenone (41). Compound (42) was identified at a retention time of 24.26 min and fragmented to give *m/z* at 249 $[M+H]^+$, 217 $[M+H-CH_3-OH]^+$, 161 $[M+H-CH_3-OH-C_2O_2]^+$, and 143 $[M+H-CH_3-OH-C_2H_2O_2-O]^+$. At the same time, the ebony leaves extract contains four compounds from the quinone group consisting of demethylphylloquinol (43), 4-prenylphlorisobutyrophenone (43), eremopetasinorone A (44), carnocin CP 5 (45).

Steroid

Three compounds belonging to the steroid group were successfully detected and identified in the extracts of ebony leaves and wood, namely 4α-hydroxymethyl-4β-methyl-5αcholesta-8-en-3β-ol (46), dehydrotasterone (47), 4,4-dimethylzymosterol (48). The compound 4α-hydroxymethyl-4β-methyl-5αcholesta-8-en-3β-ol (46) was detected at a retention time of 40 min and fragmented at *m/z* 429 $[M-H]^-$, 219 $[M-H-C_{14}H_{25}-OH]^-$, 191 $[M-H-C_{14}H_{25}-OH-C_2H_4]^-$, and 165 $[M-H-C_{14}H_{25}-OH-C_4H_6]^-$ [11]. In addition, the compound 3-(4-ethylphenoxy)-4-oxo-4H-chromen-7-yl 2-furoate (49) was also identified, which was only detected in the ebony leaves extract.

Amino Acid, Fatty Acid, and Saccharide

Ebony leaves extract contains five amino acid group compounds, namely 5-aminopentanoate (50), L-tryptophan (51), L-isoleucine (52), L-phenylalanine (53), and ethyl 3,5-bis[(4-nitrobenzoyl) amino] benzoate (54). 5-aminopentanoate (55) was detected at a retention time of 1.23 min with its fragmentation yielding *m/z* at 118 $[M+H]^+$, 101 $[M+H-OH]^+$, and 70 $[M+H-OH-CO-H_2]^+$ [17].

(9S)-HPODE (56) was identified in ebony leaves and wood extracts. Linoleate (57) and 9,10-epoxy-10,12Z,15zoctadecatrienoate (58) were fatty acids identified only in ebony leaf extract, while colneleate (56) was identified in ebony stem and leaf extracts. The colneleate compound 56 was detected at a retention time of 35.48 min. The fragmentation that occurred in this

compound (56) were *m/z* 293 $[M-H]^-$, 275 $[M-H-H_2O]^-$, 235 $[M-H-H_2O-CH_2-H_2]^-$, and 183 $[M-H-H_2O-CH_2-H_2-C_4H_4]^-$ [18].

Galactitol (59) is a saccharide group identified only in an ebony wood extract with a retention time of 1.39 min and fragmented at *m/z* 181 $[M-H]^-$, 163 $[M-H-H_2O]^-$, 131 $[M-H-H_2O-CH_3-OH]^-$, and 113 $[M-H-2H_2O-CH_3-OH]^-$.

Clustering of Ebony Leaves and Wood Extracts

Chromatogram profiles of ebony leaves and wood extracts from the three provinces in Sulawesi had relatively different separation profiles. However, it is not easy to distinguish it using eye visualization. Therefore, multivariate analysis, such as PCA, is needed to classify or differentiate leaves and wood extracts of ebony. The basic principle of clustering with PCA is to simplify variables by reducing dimensions and providing an overview of sample grouping through principal components (PC).

Fig. 3 shows the PCA score plot of the ebony extract using the peak area of identified compounds as the variable. The two main components often used in PCA analysis are PC1 and PC2. The two PCs show a diversity of data that can be explained by the variables used. In this study, the PCA results showed that leaves and wood extract of ebony could be separated with total variants from the two PC, which is about 85% (PC1 = 48% and PC2 = 37%). The two PCs can already explain most of the data variability (at least 70%) [19]. The grouping of the ebony leaves and the wood extract was obtained in the PC2.

Evaluation of Analytical Performance

This study also evaluates the analytical performance to ensure the method used has good repeatability. The evaluated parameters are the repeatability of the retention time and the area of the peak numbers 40 and 7 analyzed for three days with three replications. The evaluation results show that the method used has good consistency based on the repeatability of retention time and band area. It has a % relative standard deviation (RSD) of less than 5% [20], as shown in Table 2.

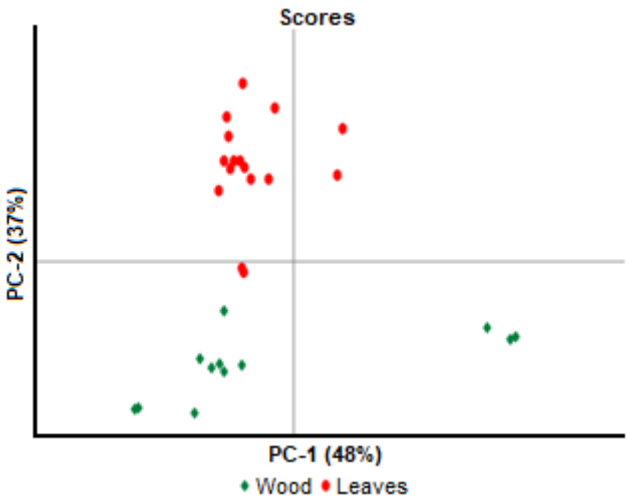

Fig 3. PCA plot of ebony leaves and wood extracts

Table 2. Evaluation of the analytical performance of the method (retention time and peak area of compound number 40 for wood and 7 for leaves)

Replication	Day 1		Day 2		Day 3	
	Wood	Leaves	Wood	Leaves	Wood	Leaves
Retention time						
1	19.86	14.12	19.73	14.15	19.81	14.09
2	19.74	14.12	19.76	14.12	19.85	14.07
3	19.75	14.09	19.82	14.06	19.78	14.09
Mean	19.78	14.11	19.77	14.11	19.81	14.08
% RSD	0.34	0.12	0.23	0.32	0.18	0.08
Peak area						
1	63462836	184356116	634798083	172252497	49589761	192765330
2	63218698	188160978	638253596	172375975	49769599	195513112
3	61695175	189204522	657139855	176205074	48694268	191175169
Mean	62792236	187240539	643397178	173611182	49351209	193151204
% RSD	1.53	1.26	1.87	1.23	1.17	1.14

■ CONCLUSION

Separation and putative identification of ebony leaves and wood extracts from three different provinces in Sulawesi, Indonesia, using LC-MS/MS, were successfully identified about 59 compounds. Approximately 14 compounds were identified in leaves and wood, 24 were identified only in leaves, and 21 in wood. Those compounds come from flavonoids, terpenoids, amino aldehydes, alkaloids, quinones, phenolic acids, steroids, amino acids, fatty acids, and saccharides. Therefore, PCA could cluster ebony leaves and wood extract with the total variants from PC-1 and PC-2 about 85% using the peak area of the identified metabolites.

■ ACKNOWLEDGMENTS

The authors gratefully acknowledged World Resources Institute (WRI) Forest Program grant (project code: 04682 periods 1 March 2019–30 September 2020) with the title "Timber tracking project IPB-WRI: a collection of physical timber reference material and setting up a reference data building pipeline for DNA of commercial timber species (*Diospyros celebica*. Bakh)" provided by Norwegian International Climate and Forest Initiative (NICFI).

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