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Phytochemical Profile of *Rhizophora apiculata*, *Bruguiera gymnorhiza*, and *Bruguiera cylindrica* for Wood Identification

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ABSTRACT

Identification of wood species that are difficult to distinguish from their anatomical structure can be done through phytochemical (extractive substance) profiling. This research aimed to analyze the phytochemical profile as a sorter for three species of mangrove wood from Indramayu and Cilacap Regencies, Indonesia, using the liquid chromatography-mass spectrum. The phytochemical markers of taxonomic were the dominant compounds only found in one wood species. The results showed that the three types of wood are dominated by phytochemicals dissolved in ethanol. The results of LCMS analysis of the ethanol extract showed that the phytochemical markers were triterpenoid, flavonoid, and fatty acyls glycoside groups for *Rhizophora apiculata*, steroid and naphthalene groups for *Bruguiera gymnorhiza*, and alkaloid as well as fatty amide group for *Bruguiera cylindrica*. The dominant compounds that characterize these can be used in sorting between mangrove wood species.

1. Introduction

Currently, it is feared that the condition of mangrove forests in Indonesia will continue to decline from year to year, both qualitatively and quantitatively (Mutik et al. 2022). This decline was due to illegal logging activities and the conversion of forest areas for various purposes such as plantations, agriculture, fisheries, industry, road infrastructure, ports, and settlements (Eddy et al. 2019; Harefa et al. 2024; Ikhsanudin et al. 2018; Konom et al. 2019; Luqman 2013; Narendra et al. 2018). In illegal logging cases in court, identifying the wood species as evidence is always a priority that must be proven. Identifying or recognizing species takes work because it requires special skills. In general, the identity of a wood species is determined by the anatomical structure of the cells that make it up because the anatomical structure of each species is different from one another. The presence of leaves, flowers, fruit, bark, and even sap is essential for wood, whose anatomical structure is relatively similar because it comes from the same genus (Ayala-Usma et al. 2019; Singh et al. 2015; Wangkhem et al. 2020). The species identification process will become increasingly complicated if the wood is already in sawn-sorted form.

The content of phytochemical compounds in mangrove wood can help identify and sort species if the species studied are similar in appearance and anatomical structure of the cells that

make up them. Identification and sorting of wood species can be carried out through an analysis approach of the phytochemicals contained in the wood. Phytochemicals or what is better known as extractive substances, are secondary metabolic products of plants whose type and composition in wood depend on the species, age of the tree, and growing conditions so that they have unique phytochemicals (Bandaranayake 2002; Das et al. 2020). Phytochemical profiles can be used as chemotaxonomy to identify wood, including wood originating from different growing locations. Fasciotti et al. (2015) and Flaig et al. (2023) proved that phytochemicals from the group of limonoid compounds such as khivorin, khayanolide A, and mexicanolide are characteristic compounds in mahogany wood from Africa and phragmalin type limonoids for mahogany from Brazil, as well as different polyphenols such as catechin and cinchonain derivatives which are characteristic phytochemical markers for both species.

Studies regarding the phytochemical and chemotaxonomic profiles of mangrove wood parts have yet to be widely reported. Ghalib (2011) reported the phytochemical profile of bark and wood of *Sonneratia caseolaris* gas chromatography time-of-flight mass spectrometer detected 32 compounds from bark and 28 from wood. Different chemical compounds found in wood and bark include alkanes, alkenes, aromatic compounds, phenolics, carboxylic acids, amides, and amines. Basyuni et al. (2021) reported the results of high-performance thin-layer chromatography analysis that the phytochemical dolichol can be used as chemotaxonomy in the leaf litter of *Avicennia* spp., *Bruguiera* spp., *Nypa fruticans*, and *Rhizophora* spp. from North Sumatra, Indonesia. Basyuni (2016) also from North Sumatra, Indonesia. Basyuni (2016) also reported that the leaves and roots of *Aegiciras ilicifolius*, *Bruguiera parviflora*, *Cerias tagal*, *Rhizophora apiculata*, *Sonneratia caseolaris*, and *Xylocarpus granatum* from North Sumatra are analyzed for non-saponified lipids (NSL) and the phytochemical compounds are identified using GC-FID.

The analysis profile of phytochemicals can be carried out in several ways. Mass spectrometry, isotope composition (isotope stability), and near-infrared spectroscopy are some techniques to analyze chemotaxonomics (Flaig et al. 2023; Frezza et al. 2020). Murukesh (2014) analyzed the phytochemical compound of the leaves and bark of Rhizophoraceae using Liquid Chromatography by Tandem Mass Spectrometry (LCMS). This research aims to analyze the phytochemical profile as a sorter for three species of mangrove wood from Indramayu and Cilacap Regencies, Indonesia (*R. apiculata*, *B. gymnorhiza,* and *B. cylindrica*) using the LCMS spectrum.

2. Materials and Methods

2.1. Plant Materials

Research materials were collected from the mangrove forest areas in Indramayu, West Java, Indonesia, with the geographical coordinates 6° 20' 0" S, 108° 19' 0" E, and Cilacap, Central Java, Indonesia, with the geographical coordinates 7° 44' 0" S, 109° 0' 0" E. Confirmation of tree species names was based on herbarium test results at the National Research and Innovation Agency Herbarium (Indonesia). The primary research materials were wooden discs (slabs) taken from the trunks of *R. apiculata* from Cilacap and *B. gymnorhiza* from Indramayu and *B. cylindrica* (from Cilacap and Indramayu, respectively). The discs were taken from a height of 1.30 m above ground level (chest height) or 30 cm above the buttress. The sample characteristics are shown in **Table 1**. The phytochemical profile analysis used the heartwood of the wooden disc, which was made into powder measuring 40–60 mesh.

Table 1. Wood samples

No.	Species	Origin	Disk diameter (cm)	Tree age (year)	Number of tree sample
1	*R. apiculata*	Indramayu	15.5	25	3
2	*B. gymnorhiza*	Cilacap	11.5	16	3
3	*B. cylindrica*	Indramayu	14.5	25	3
4	*B. cylindrica*	Cilacap	15.0	25	3

2.2. Extraction

The wood powder was extracted using solvents with varying polarities. The successive extraction method was the soxhlet apparatus method, carried out for 2 hours for each solvent. Extraction began with n-hexane, ethyl acetate (EtOAc), ethanol (EtOH), and ended with water. The ratio of powder and solvent was 1:15. The filtrate obtained for each solvent was then concentrated using a vacuum rotary evaporator to get the concentrated extract and determine the extraction yield. The extraction was performed in 3 repetitions in each wood sample.

2.3. Phytochemical Analysis

Extracts with the highest extraction yield in each wood sample were further analyzed using UPLC-QToFMS/MS (Waters, US). The separation process was carried out with a C18 ACQUITYUPLC®BEH 1.7 µm column. The eluent system used a gradient with solvents A and B were water and acetonitrile containing formic acid 0.05%, respectively. The elution gradients used were 0–2 minutes (95% A), 2–3 minutes (75% A), 3–14 (75% A–100% B), 14–15 minutes (100% B), 15–19 minutes (100% B–95% A) and 19–23 minutes (95% A) with a flow rate of 0.2 mL/minute. Electrospray was used as an ionizer to fragment molecules (3 kV, capillary temperature 350°C, resolution 22000, and positive ionization mode). Fragment mass readings were carried out in the range of 30–1300 Da. Data was acquired using MassLynx V4.1 (Waters, US) and MSDial v4.9.221218 (RIKEN, Japan) software. Readings of compound names were searched based on the Pubchem, ChemSpider, and COCONUT databases. The relative abundance of the compounds was determined by comparing the percentage of the area of the compound to the percentage of the total area of the compound.

2.4. Data Analysis

The extract yield values were analyzed descriptively after obtaining the average yield from three replications. Data on the relative concentration of chemical compounds resulting from LCMS analysis were also analyzed descriptively.

3. Results and Discussion

3.1. Extractive Substance Levels

The solvent extraction influences the yield of all samples. Ethanol extracts had the highest yield compared to other extracts (**Table 2**). At the same time, water extracts had the lowest yield. It can be stated that polar extractive compounds dominated all wood samples since ethanol is a polar solvent with a dielectric constant of 24.30 (Sirwutubun 2016). According to Mutik et al. (2022), a solvent's polarity level greatly influences extractive substances in extracts.

Table 2. Wood extractive substance levels based on extraction results with varying solvent polarities

Species	Origin	Yield (%)				
		***n*-hexane**	**EtOAc**	**EtOH**	**water**	**Total**
R. apiculata	Indramayu	0.05 ± 0.006	0.94 ± 0.10	8.33 ± 0.11	0.45 ± 0.29	9.75
B. gymnorhiza	Cilacap	0.73 ± 0.06	0.58 ± 0.11	2.81 ± 0.07	0.76 ± 0.02	4.86
B. cylindrica	Indramayu	1.07 ± 0.24	1.32 ± 0.16	5.58 ± 0.04	0.39 ± 0.07	8.33
B. cylindrica	Cilacap	0.82 ± 0.08	1.98 ± 0.69	5.67 ± 0.12	0.63 ± 0.01	9.07
Average		0.6 ± 0.44	1.20 ± 0.60	5.60 ± 2.26	0.56 ± 0.17	

Note: The extract yield values were the average of 3 repetitions.

This study indicates that wood species and genus influence the yield of extractive substances. Comparing the genus of Rhizopora to Bruguiera, the genus of Rhizopora had a higher yield of extractive substances than the genus of Bruguiera (**Table 2).** These phenomenons were confirmed by previous research of Anggraini and Khabibi (2022), showing that different species and genus, namely tembesu (*Fragraea fragrans*), rengas (*Gluta renghas*), and medang (*Litsea* sp.), have different extractive yield. In addition, Suhendry et al. (2017) reported the different genus and species using *R. apiculata* bark extract, *R. mucronata*, and *Avicennia officinalis* wood species showed different yields of extractive substances. Moreover, *B. gymnorhiza* and *B. cylindrica* also showed different extractive yields. However, this phenomenon was not affected only by the different species since the *B. gymnorhiza* and *B. cylindrica* had different tree ages (**Table 1**). The tree's different age is presumed to be the one factor that affected the extractive content.

The different geographical origins result in different yields of extractive substances. The *B. cylindrica* from Cilacap had a higher yield of extractive substance than the *B. cylindrica* from Indramayu (**Table 2**). It showed that the different species in the same genus affected the extractive content. Aulia et al. (2022) and Prayogo et al. (2021) prove that even though they are from the same genus, the wood and bark of different species contain different extractive content. The levels of dissolved wood extractive substances methanol from *Acacia auriculiformis*, *A. mangium*, *A. leucophloea*, *A. decurrens*, and *A. crassicarpa* wood were 8.59%, 7.66%, 3.88%, 3.60%, and 3.01%, respectively. The levels of *A. mangium* and *A. auriculiformis* bark extractive substances were 13.41% and 7.64%, respectively. The same phenomenon was also reported by Sari et al. (2022) that the extractive substance content of gaharu leaves (*Gyrinops versteegi*) from Bogor, West Java (25.43%) is higher than that from Purworejo, Central Java (18.59%).

3.2. Phytochemical Profile

Fig. 1 shows the phytochemical profile of the ethanol extract of the four wood species, shown by LCMS chromatograms with varying retention times and relative abundances. These different profiles show differences in the type of compound and composition in each wood species. However, there are also similar retention times but different peak heights. It shows the same compound content, but the abundance is different. Based on a database search of the chromatogram, there were eight similar compounds contained in the four species of wood studied, namely one compound from the coumarin group at a retention time of 1.23 minutes with a relative abundance of 0.22–6.24%; two alkyl amine compounds at a retention time of 10.85 minutes with a relative abundance of 2.43–11.64%, two benzopyran compounds at a retention time of 11.81 and 13.47 minutes with a relative abundance of 0.63–13.39%, two flavonoid compounds (anthocyanins) at a retention time of 13.19 and 13.54 with a relative abundance 0.79–4.29%, and

one compound from the terpenoid group at a retention time of 12.25 minutes with a relative abundance of 0.88–13.47% (**Table 3**). Gouda (2015) stated that phytochemicals from the phenolic and terpenoid groups were identified in mangrove vegetation.

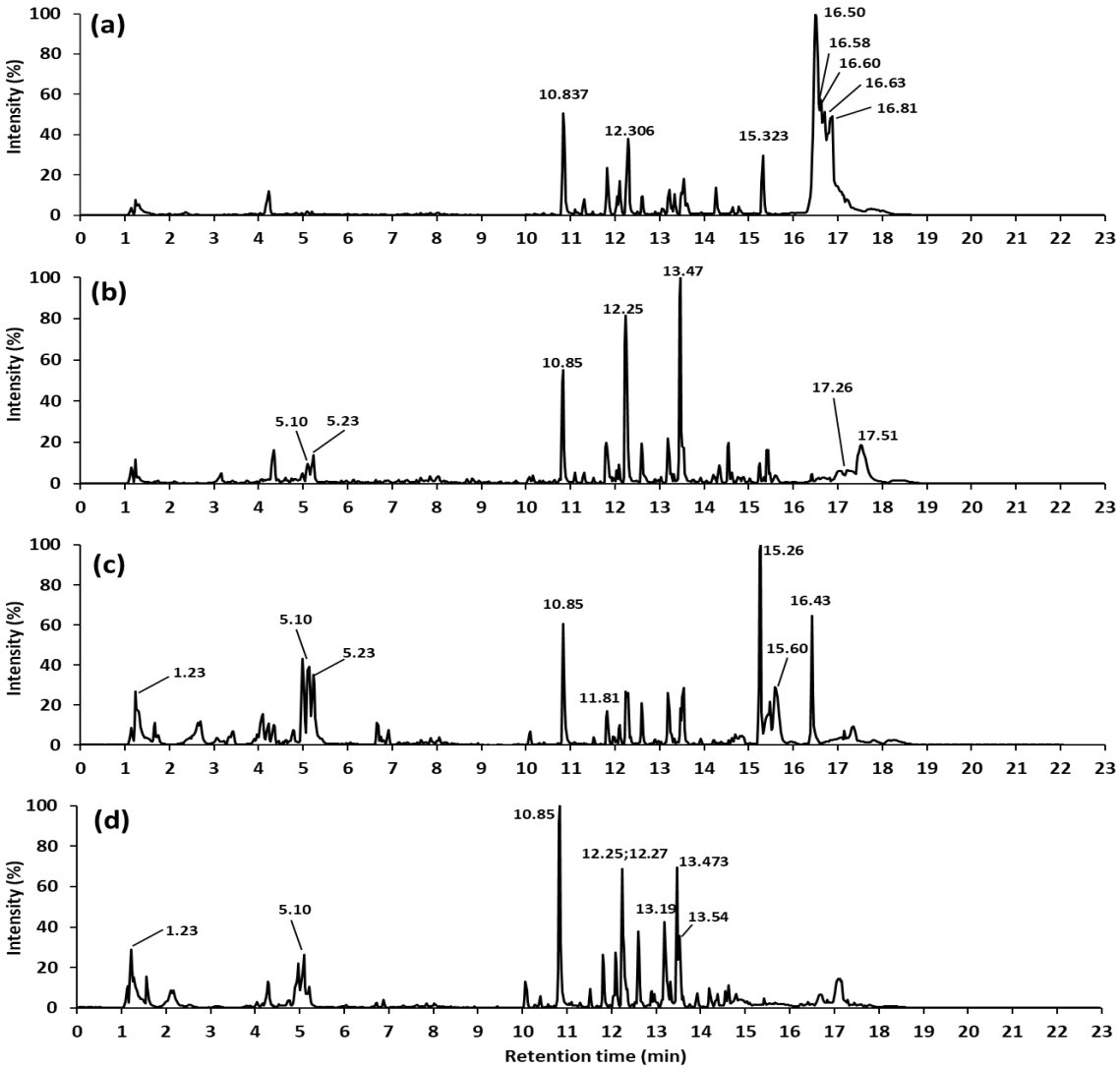

Fig. 1. Chromatogram profiles of (a) *R. apiculata* from Indramayu: 16–18 mins (flavonoid and triterpenoid), 10 – 14 mins (benzopyran and alkyl amine); (b) *B. gymnorhiza* from Cilacap: 10 – 14 mins (terpenoid, benzopyran, alkyl amine); (c) *B. cylindrica* from Indramayu: 15 – 17 mins (alkaloid and fatty amide), 5 – 6 mins (coumarin), 10 – 11 mins (alkyl amine); (d) *B. cylindrica* from Cilacap: 10 – 14 mins (alkyl amine, terpenoid, benzopyran derivatives (including flavonoid).

Differences in species in the same genus also impact differences in their phytochemical profiles. **Fig. 1** shows that there were similar retention times but different peak heights. Based on database searches, the same eleven compounds were found in *B. gymnorhiza* and *B. Cylindrica* woods with different abundances (**Table 3**). The chromatogram of *B. gymnorhiza* from Cilacap showed the highest peak intensity at a retention time of 12.25 minutes, while *B. cylindrica*, which also came from Cilacap, had the highest peak intensity at a retention time of 10.85 minutes (**Fig. 1**). The phytochemical content of wood *B. gymnorhiza* from Cilacap was the highest in terpenoid compounds, with a relative abundance of 13.47%.

Table 3. Dominant compounds for each wood species based on LCMS

RT (min)	m/z (Da)	Tentative identification	Molecular formula	References	Class	Relative abundance (%)*			
						A	B	C	D
1.23	217.07	10-hydroxy-2H,6H-pyrano[3,2-g]chromen-2-one	$C_{12}H_8O_4$	Cmaup, Unpd, Pubchem	Coumarin	0.22	1.79	1.52	6.24
5.10	584.27	2-{2-amino-5-[2-(3-ethylazetidin-1-yl)-2-oxoethyl]pyridin-4-yl}-6-methyl-7'-oxo-3',7'-dihydrospiro[cyclohexane-1,2'-furo[3,2-g]chromen]-3-en-6-yl 2-methylbut-2-enoate	$C_{34}H_{37}N_3O_6$	Zincnp	Coumarin	0.00	1.92	6.10	4.58
5.23	249.11	Olivetonide	$C_{14}H_{16}O_4$	MzCloud	Coumarin	0.00	3.83	6.61	0.73
10.85	214.25	N,N-Dimethyldodecylamine	$C_{14}H_{31}N$	Pubchem	Alkyl amine	3.99	**8.53**	6.29	**11.64**
11.81	318.3	Pestalotiopsone C	$C_{18}H_{22}O$	KnapSack	Benzopyran	1.27	3.84	1.55	2.69
12.25	304.3	ent-kaur-16-en-13,19-diol	$C_{20}H_{32}O_2$	KnapSack	Terpenoid	0.88	**13.47**	2.62	6.89
12.27	242.29	dimethyl(tetradecyl)amine	$C_{16}H_{35}N$	Pubchem	alkyl amine	2.69	2.43	2.72	5.05
13.19	496.34	Oenin	$[C_{23}H_{27}O_{12}]+$	KnapSack	Flavonoid	0.79	2.78	2.32	4.92
13.47	332.33	Pestalotiopsone A	$C_{19}H_{24}O_5$	KnapSack	Benzopyran	0.63	**13.39**	1.55	5.95
13.54	522.36	Pelargonidin 3-o-(6-o-malonyl-beta-d-glucoside)	$[C_{24}H_{25}O_{13}]+$	KnapSack	Flavonoid	1.24	3.02	2.63	4.21
15.26	256.27	1,3,5-Tributyl-1,3,5-triazinane	$C_{15}H_{33}N_3$	ChemSpider	Alkaloid	0.00	1.32	**9.44**	0.16
15.60	282.28	Oleamide	$C_{18}H_{35}NO$	MzCloud	Fatty amide	0.00	1.19	**7.07**	0.00
16.43	284.3	N-(cyclohexylmethyl)-N-[3-(dimethylamino)propyl]-N',N'-dimethyl-1,3-propanediamine	$C_{17}H_{37}N_3$	ChemSpider	Alkaloid	0.00	0.66	**5.30**	0.00
16.50	663.45	3a,7,8-trihydroxy-1-[10-hydroxy-10-(3-hydroxypropyl)-8-(propan-2-yl)tetracyclo[7.7.1.0^{2},^{7}.0^{13},^{17}]heptadeca-1(17),7-dien-11-yl]-9a,11a-dimethyl-1H,2H,3H,3aH,5H,5aH,6H,7H,8H,9H,9aH,9bH,10H,11H,11aH-cyclopenta[a]phenanthren-5-one	$C_{42}H_{62}O_6$	Ttcmdb_taiwan, Supernatural2	Triterpenoid	**16.77**	0.00	0.00	0.00
16.58	607.39	Kaempferol 3-(3''-Acetyl-Alpha-L-Arabinofuranoside)-7-Rhamnoside	$C_{28}H_{30}O_{15}$	Supernatural2, Tcmid, KnapSack, Unpd, Tipdb	Flavonoid	**10.86**	0.00	0.00	0.00
16.60	551.33	6-Hydroxyluteolin 7-(6''-Malonylglucoside)	$C_{24}H_{22}O_{15}$	KnapSack	Flavonoid	**6.95**	0.00	0.00	0.00
16.60	439.21	4-[(3-hydroxyoct-1-en-1-yl)oxy]-6-{[(3,4,5-trihydroxyoxan-2-yl)oxy]methyl}oxane-2,3,5-triol	$C_{19}H_{34}O_{11}$	Supernatural2, Unpd	*Fatty acyl glycoside*	**3.62**	0.00	0.00	0..00
16.63	494.27	8-Hydroxytricetin 7-Glucuronide	$C_{21}H_{18}O_{14}$	Supernatural2, KnapSack, Unpd, Tipdb	Flavonoid	**12.94**	0.00	0.00	0.00
16.81	663.45	3-(5,7-dihydroxy-4-oxo-4H-chromen-2-yl)phenyl heptacosanoate	$C_{42}H_{62}O_6$	Unpd, Pubchem	Flavonoid	**12.71**	0.00	0.00	0.00
17.26	832.24	1-(5-ethyl-6-methylheptan-2-yl)-9a,11a-dimethyl-7-{[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy}-1H,2H,3H,3aH,3bH,4H,6H,7H,8H,9H,9aH,9bH,10H,11H,11aH-cyclopenta[a]phenanthren-5-yl hexadecanoate	$C_{51}H_{90}O_8$	Supernatural2, Unpd	Steroid	0.00	**6.04**	0.00	0.00
17.51	684.2	2-{[3-(3,4-dihydroxy-7-{3',4,5-trihydroxy-[1,1'-biphenyl]-2-yl}naphthalen-1-yl)prop-2-enoyl]oxy}-4-(ethylamino)-3-(3,4,5-trihydroxyphenyl) butanoic acid	$C_{37}H_{33}NO_{12}$	Tcmdb_taiwan, Supernatural2, Zincnp	Naphthalene	0.00	**11.04**	0.00	0.00

Notes: *based on the top 30 highest peak areas, RT (retention time), A (*R. apiculata*), B (*B. gymnorhiza*), C (*B. cylindrica* from Indramayu), and D (*B. cylindrica* from Cilacap)

In comparison, compounds from the alkyl amine compound group have the highest relative abundance in *B. cylindrica* from Cilacap (11.64%) (**Table 3**). **Table 3** also showed that two

compounds were only found in *B. gymnorhiza* from Cilacap and were not found in *B. cylindrica* from Indramayu, and *B. cylindrica* from Cilacap, namely compounds from the steroid group that was identified at a retention time of 17.26 minutes with a relative abundance of 6.05% and naphthalene at a retention time of 17.51 minutes with a relative abundance of 11.04%. Differences in the type and composition of extractive substances in different species of the same genus also occurred in the context of two Acacia wood species, namely *A. crassicarpa* and *A. auriculiformis*. The abundance of flavonoid compounds was more dominant in *A. auriculiformis*, whereas in *A. crassicarpa*, apart from flavonoids, anthraquinone, xanthonoid, and flavonoid compounds are also detected (Prayogo et al. 2021). The content of secondary metabolite compounds differs for each species of mangrove because these compounds are not always produced in every plant but will be produced when needed by the plant or in certain phases (Akasia 2021).

Differences in growing locations also result in differences in phytochemical profiles. These differences can be seen in *B. cylindrica* wood from Indramayu and Cilacap. Wood dominant compound *B. cylindrica* from Indramayu was an alkaloid compound identified at retention times of 15.26 and 16.43 minutes with a relative abundance of 9.44% and 5.30%. However, at this retention time, the abundance of alkaloid compounds in *B. cylindrica* wood decreased from Cilacap by only 0.16% and 0% (**Table 3**). Likewise, compounds from the fatty amide group were identified in *B. cylindrica* wood from Indramayu at a retention time of 16.43 minutes with a relative abundance of 7.07% not identified in *B. cylindrica* wood from Cilacap. At a retention time of 10–14 minutes, the relative abundance of the alkyl amine, benzopyran, terpenoid, and flavonoid compounds in *B. cylindrica* wood from Cilacap was higher than that from Indramayu. The difference in geographical origin affected the phytochemical compound since Cilacap and Indramayu had different climates, kinds of soil, and the tides of the sea zone of the mangrove growing location. Another previous research has confirmed this phenomenon. The phytochemical profile of *B. cylindrica* leaf extract from Goa is different from the phytochemical profile of leaf extract derived from Tamilnadu, even though both locations are located in India (Dahibhate and Kumar 2021; Revathi 2014).

4. Conclusions

R. apiculata wood from Indramayu, *B. gymnorhiza* wood from Cilacap, *B. cylindrica* wood from Indramayu, and *B. cylindrica* wood from Cilacap were dominated by phytochemicals (extractive substances) dissolved in ethanol. The phytochemical profiles of the four mangrove woods varied. The ethanol extracts of the four wood species contain the same eight compounds from the coumarin, alkyl amine, benzopyran, flavonoid, and terpenoid groups with varying abundances. There are six dominant compounds from the triterpenoid, flavonoid, and fatty acyls glycoside groups, which were only identified in *R. Apiculata* wood, two dominant compounds from the steroid and naphthalene groups, which are only found in *B. gymnorhiza* from Cilacap, two dominant compounds from the alkaloid group and one compound from fatty amide in *B. cylindrica* wood from Indramayu whose abundance is deficient in *B. cylindrica* wood from Cilacap. The dominant compounds only found in a wood species can be used as phytochemical markers to assist in identifying and sorting wood species.

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