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Short notes

A CHEMOTAXONOMIC STUDY OF THE RESINS FROM THREE DIPTEROCARP SPECIES (*Shorea macrophylla, Shorea pinanga,* and *Shorea hopeifolia*)

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ABSTRACT

A chemotaxonomic study on the resins of *Shorea macrophylla*, *Shorea pinanga,* and *Shorea hopeifolia* was conducted. The dichloromethane extracts were separated into neutral and acidic fractions and then analyzed using GC-MS. The neutral fraction analysis revealed that spathulenol, caryophyllene oxide, aromadendrene oxide, and isoaromadendrene epoxide were the major constituents in all three species. Furthermore, the main compound in the neutral fraction of *Shorea hopeifolia* was isocaryophyllene which was undetected in the other two species. The major constituent of the acidic fraction of the three species was hexadecanoic acid, while pentadecanoic acid was the major constituent in the acidic fraction of *Shorea pinanga* and *Shorea hopeifolia*. Therefore, the presence of sesquiterpenes as well as fatty acids in *S. macrophylla*, *S. pinanga,* and *S. hopeifolia* was discovered to be a marker for identifying the genus Shorea.

KEYWORDS: Shorea, resin, sesquiterpenes, fatty acids, acidic, neutral.

INTRODUCTION

Timber is the primary use of trees from the Dipterocarpaceae family. This decreases the number of trees and threatens the sustainability of some species. *Shorea macrophylla* (de Vriese) Ashton, *Shorea pinanga* Scheff, and *Shorea hopeifolia* (Heim) Sym. are endemic mainland Boreal species of the Dipterocarpaceae family that are endangered. Although *Shorea* *pinanga* is still fairly easy to find locally, its population was reported to have decreased substantially (Gaveau et al. 2014). Based on the IUCN RED List, *Shorea macrophylla* is classified as vulnerable, while *Shorea hopeifolia* is an endangered species (Randi et al. 2019, Supartini et al. 2013). This is due to the clearing of forest land for agricultural and plantation purposes (Wiati et al. 2019). Therefore, alternative uses, such as for resin, are essential to transition from timber-oriented to non-timber.

Resin is a category of non-timber forest products (NTFPs) with significant commercialization potential. Indonesia's natural forests are one of the forces driving resin production, with high species diversity. Resin is a solid or semi-solid organic compound or a mixture of various natural polymer compounds called terpenes (Boer et al. 2001). Natural resin is produced by plant exudation that occurs naturally or artificially. It is solid, shiny, or clear-dull, brittle, melts when exposed to heat, and flammable by emitting smoke. The characteristic odor of natural resin results from a mixture of resin and essential oils. Furthermore, the resin is typically produced in the bark of trees, flowers of a herb, or other parts of plants (Kuspradini et al. 2016).

The Dipterocarpaceae family produces NTFPs such as resin and tengkawang oil. The resin produced majorly comes from the genera Shorea, Hopea, and Vatica, with the most common species being *Shorea javanica* K. et V., also known locally as 'damar mata kucing'. Other species from the Dipterocarpaceae family that produce resin include *Shorea macrophylla*, *Shorea pinanga*, and *Shorea hopeifolia*.

Shorea macrophylla, also known locally as 'tengkawang' is an endemic species of the Borneo region that belongs to the pink shorea group and produces a white resin (Randi et al. 2019). *Shorea pinanga*, locally known as 'kawang pinang', is also included in the pink shorea group and endemic to the plains of Borneo. The distribution of these species covers four main areas which include Indonesia (Kalimantan), Malaysia (Sabah and Sarawak), and Brunei Darussalam (Robiansyah et al. 2019). *Shorea Hopeifolia*, also known locally as 'damar asam', is a yellow Shorea species that is found in the plains of Borneo. There is still limited information available regarding the chemotaxonomy of these three resins, which has resulted in their not being widely used (Randi et al. 2019, Supartini et al. 2013, and Robiansyah et al. 2019) or traded compared to *Shorea javanica* resins. This study aimed to investigate the chemotaxonomy of *S. macrophylla*, *S. pinanga*, and *S. hopeifolia* of the genus Shorea. Furthermore, the chemical investigation of resin is essential because it can be used as fundamental knowledge or as a reference to the use of resins.

MATERIAL AND METHODS

Sample preparation

The samples used in this study are the resins of *Shorea macrophylla*, *Shorea pinanga*, and *Shorea hopeifolia*. These resins were collected from Labanan Special Purpose Forest in Berau District, East Kalimantan, Indonesia, at coordinates 117^{O} 10'–117^{O} 15'E and 1^{O} 52'-1^{O} 57'N, and an altitude of 125 - 275 asl. The temperature ranges between 26.9 - 28.9^{o}C, with an annual rainfall of 2.012 mm/year (Suryanto et al. 2010). All resin samples used in this experiment were extracted naturally from the peeling bark without any tapping process. As shown in Fig. 1, the resin lumps were hardened and irregularly shaped.

Fig. 1: Resin lumps of Shorea pinanga, Shorea hopeifolia, and Shorea macrophylla.

Extraction and neutral-acidic fractionation

Each resin was ground, and 1 g of it was dissolved in 100 ml dichloromethane (CH_2Cl_2) for a day at room temperature. The dichloromethane extract of the resin was then transferred to a 500 mL separation funnel, and 100 mL of aqueous 1% sodium hydroxide (NaOH) was added to bind the free acid that had dissolved in it. Subsequently, the acidic and neutral fractions were separated as usual.

Gas chromatographic analysis of neutral and acidic fractions

The chromatographic spectrum of neutral and acidic fractions was collected using Shimadzu gas chromatographic-mass spectrometry GCMS-QP 2010 equipped with the RTX –5 MS column type, Restek corp (30 m length). The conditions observed include a column temperature from 50^{o}C (1 min) to 320^{o}C at 5^{o}C/min, injection temperature of 290^{o}C, detection temperature of 320^{o}C, and acquisition mass range from 35 - 665 amu using helium as the carrier gas. Furthermore, neutral fraction samples were prepared for direct injection with two replicates each, using dichloromethane (CH_2Cl_2) as the solvent. The acidic fraction samples were derivatized before injection using the method described by Preciado et al. (2016). A total of 1 mg of the soluble part of dichloromethane was then diluted in 50 mL of pyridine and 100 mL of a mixture of BSA/N,O-bis (trimethylsilyl) acetamide and TMCS/ trimethylchlorosilane (99/1, v/v). The resultant mixtures were then heated for 30 min at 100^{0}C. The constituents of the fractions were determined by comparing the experimental data to the database (NIST MS library) and calculating based on the percentage of the peak area.

RESULTS AND DISCUSSION

GC-MS analysis of neutral and acidic fraction

Tabs. 1 and 2 show the fractionation results of the resins from *S. macrophylla*, *S. pinanga*, and *S. hopeifolia*. Tab. 1 shows that all compounds were identified as sesquiterpenes. Furthermore, all three species contained spathulenol, caryophyllene oxide, aromadendrene oxide, isoaromadendrene epoxide, and cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1.alpha., 2.beta.,4.beta.)]- as the major constituents. Isocaryophyllene was the main compound in the neutral fraction of *S. hopeifolia*. This is consistent with previous studies that reported the presence of sesquiterpene in the *Shorea* species, namely *S. javanica* (Sari et al. 2004, Ukiya et al. 2010), *S. robusta* (Sushma et al. 2017), and *Dipterocarpus* species such as *D. kerrii* (Richardson et al. 1989), *D. retusus*, *D. intricatus*, *D. hasseltii*, and *D. grandiflorus* (Messer et al. 1990).

Tab. 1: Neutral fraction of dichloromethane extracts from three Shorea genus.

No	Retention time (min)	Constituents	Percentage (%)			Similarity index (%)
			S. macrophylla	*S. pinanga*	*S. hopeifolia*	
1	15.67	gamma.-Elemene	-	-	1.62	92
2	16.62	Germacrene D	5.81	2.66	4.8	90
3	16.98	Cyclohexane,1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-	8.95	3.23	9.72	94
4	17.27	Cadina-3,9-diene	-	-	6.41	88
5	17.64	Isocaryophyllene	-	-	13.65	93
6	18.40	cis-.alpha.-Bisabolene	-	-	3.93	91
7	19.02	Germacrene D (isomer)	tr	tr	7.75	93
8	20.78	Methyl 4,7-octadecadiynoate	1.97	-	-	80
9	21.29	(-)-Spathulenol	7.82	4.75	10.83	92
10	21.33	Caryophyllene oxide	8.84	4.68	6.45	90
11	21.96	Hexane, 1-(isopropylidenecyclopropyl)-	1.91	-	-	82
12	21.92	Andrographolide	3.82	2.82	2.5	82
13	22.84	Aromadendrene oxide		-	2.59	80
14	23.42	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol	2.13	2.15	-	82
15	25.05	cis-Z-.alpha.-Bisabolene epoxide	1.49	-	-	83
16	25.82	(-)-Spathulenol (isomer)	tr	2.19	tr	73
17	26.32	Isoaromadendrene epoxide	6.21	7.31	8.42	81
18	26.75	Isoaromadendrene epoxide (isomer)	6.88	9.66	tr	84

Note: (-): not detected, (tr): trace.

The major compound of the acidic fraction of the three species was fatty acids. *S. macrophylla* and *S. hopeifolia* contained a high percentage of palmitic acid or hexadecanoic acid. However, pentadecanoic acid was detected in higher concentrations in *S. pinanga*. Octadecanoic acid and its derivatives were detected in the acidic fraction of *S. hopeifolia* but not in *S. macrophylla* and *S. pinanga*. In previous studies, the detection of fatty acids also was reported in *Shorea* spp. (Blicher-Mathiesen 1994).

Tab. 2: Acidic fraction of dichloromethane extracts from three Shorea genus.

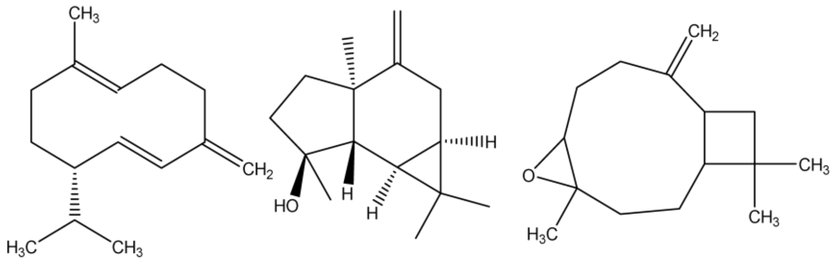
No	Retention time (min)	Constituents	Percentage (%)			Similarity index (%)
			S. macrophylla	*S. pinanga*	*S. hopeifolia*	
1	27.32	Pentadecanoic acid	-	16.28	20.08	95
2	28.13	Hexadecanoic acid	58.17	4.93	21.68	95
3	29.20	Methyl hexadecanoate	4.54	-	-	87
4	30.10	9,12-Octadecadienoic acid	-	-	4.25	96
5	30.14	11-Octadecenoic acid	-	-	10.87	96
6	30.45	7-Hexadecenoic acid	-	10.22	-	90
7	30.58	Octadecanoic acid	-	-	1.96	93

Note: (-): not detected.

Chemotaxonomic analysis

Sesquiterpene was identified as the dominant compound in the neutral fraction of resins from *S. macrophylla*, *S. pinanga*, and *S. hopeifolia* and then subjected to chemotaxonomic elucidation. *S. hopeifolia* contained more compounds than *S. macrophylla* and *S. pinanga*, indicating its evolution. The absence of gamma-elemene, cadina-3,9-diene, isocaryophyllene, cis-alpha-bisabolene, and aromadendrene oxide in *S. macrophylla* and *S. pinanga* suggests that these sesquiterpenes could be used as chemotaxonomic markers to distinguish *S. hopeifolia*. However, germacrene D, (-)-spathulenol, and caryophyllene oxide were present in three species (Fig. 2). Chemically, the compounds in *S. macrophylla*, *S. pinanga*, and *S. hopeifolia* are similar to those found in the other species of the Shorea and Dipterocarpaceae family.

Previous studies reported that germacrene D was detected in *Dipterocarpus* and *Shorea* genera namely *D. retusus*, *D. hasseltii*, (Messer et al. 1990), and *S. acuminata* (Muhammad et al. 2011). In addition, (-)-spathulenol was observed in *Dryobalanops aromatica* (Kamariah et al. 2011), *D. alatus* (Yongram et al. 2019), and *D. cornutus* (Salleh et al. 2020). Caryophyllene oxide was also identified in *D. retusus* (Messer et al. 1990), *S. acuminata* (Muhammad et al. 2011), *D. gracilis* (Fernandes and Maharani 2019), and *Hopea odorata* (Paul et al. 2016). The discovery of germacrene D, (-)-spathulenol, and caryophyllene oxide in *S. macrophylla*, *S. pinanga*, *S. hopeifolia*, and other species suggested that sesquiterpenoid could be used as a biomarker in chemotaxonomic studies.



(a) *(b)* *(c)*

Fig. 2: a) Chemical structure of germacrene D, b)(-)-spathulenol, and c) caryophyllene oxide.

Ecological significance

The presence of terpenoids in the trees was associated with resistance to external attacks such as weather and organisms. Previous studies showed that terpenoids such as sesquiterpenoids can act as anti-termite, anti-ant, and inhibitors of ant-associated fungi (Howard et al. 1988, Richardson et al. 1989), diterpenoid compounds can act as a tissue barrier against harm and chemical deterioration (Seki et al. 2012), while triterpenoid compounds act as antifungal agents against white-rot (Masendra et al. 2020). The presence of terpenoids or sesquiterpenoids in *S. macrophylla*, *S. pinanga*, and *S. hopeifolia* could have ecological significance. Therefore, a bioassay test of the extracts will be reported in a subsequent study.

CONCLUSIONS

The resins of three species of *S. macrophylla*, *S. pinanga*, and *S. hopeifolia* were analyzed using GC-MS. The neutral fraction of dichloromethane extracts of the sample contained sesquiterpene as the major compound, while the acidic fraction was dominated by fatty acids.

The detection of sesquiterpene in *S. macrophylla*, *S. pinanga*, and *S. hopeifolia* could be used as a chemotaxonomic marker for the genus Shorea and Dipterocarpaceae family. Furthermore, this is the first chemotaxonomic study on *S. macrophylla, S. pinanga,* and *S. hopeifolia* resins.

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