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Research Article

Dereplication of Oligostilbenes in The Crude Extracts of Dipterocarpaceae Plants from Kadamaian, Sabah

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ABSTRACT

Oligostilbenes are a class of polyphenolic compounds with notable bioactivities, predominantly produced by Dipterocarpaceae, a major tree family in Southeast Asian tropical rainforests. Given their ecological and pharmacological significance, efficient identification of oligostilbenes from natural sources is essential, particularly to avoid re-isolation of known compounds. This study aimed to apply an LC-ESI-MS^n-based dereplication approach for the rapid identification of known oligostilbenes directly from crude extracts of Dipterocarpaceae species collected from Kadamaian, Sabah. The selected species; *Parashorea tomentella*, *Dryobalanops lanceolata*, *Dipterocarpus caudiferus*, *Shorea xanthophylla*, and *Shorea seminist*, represent ecologically important flora from one of the most biodiverse forest regions in Malaysia. An in-house MS^1–MS^5 spectral database of authenticated oligostilbenes was used to match fragmentation profiles and retention times from ten crude extracts (bark and heartwood). A total of 11 known oligostilbenes were confidently identified, with species- and tissue-specific variations observed in their distribution. *P. tomentella* showed the richest profile in bark, while certain trimeric and tetrameric stilbenes were more prevalent in heartwood, suggesting tissue-specific biosynthetic patterns. Additionally, several unidentified peaks with consistent stilbene-like fragmentation were detected, indicating the presence of potentially novel oligostilbenes. This dereplication method significantly enhanced the speed and reliability of compound identification in complex matrices, demonstrating its utility in streamlining phytochemical workflows. The findings also provide valuable chemotaxonomic insights into the Dipterocarpaceae of Sabah and support their potential as reservoirs of bioactive natural products.

Keywords: Dereplication; LC-MS^n; oligostilbenes; Dipterocarpaceae; Kadamaian Sabah; natural products.

INTRODUCTION

Approximately 60% of Sabah's land area remains forested, representing one of the most significant expanses of tropical rainforest in Malaysia. However, much of this forest cover has been subjected to extensive logging activities, resulting in areas at various stages of ecological succession. A substantial portion of these forests comprise lowland dipterocarp rainforest, which is recognized as one of the most species-rich and biologically diverse terrestrial ecosystem. These forests are predominantly composed of species from the Dipterocarpaceae family, which plays a vital role in Sabah's forestry sector, an important contributor to the state's economy and revenue generation (Eschenbach et al., 1998).

Members of the Dipterocarpaceae family are well known for producing a wide range of oligostilbenes. Notably, nearly one-third of all stilbene derivatives reported to date have been isolated from this family (Shen et al., 2009). Previous work by this laboratory led to the isolation of four novel resveratrol oligomers, along with thirteen known oligostilbenes, from the heartwood of *Neobalanocarpus heimii* (Jalal et al., 2018). As analytical methodologies have advanced, particularly in the areas of chromatographic and spectroscopic techniques, the discovery of new oligostilbenes from various plant sources have continued to increase (Lim et al., 2023). However, this also raises the likelihood of re-isolating previously identified compounds.

The structural complexity of natural products necessitates the use of sophisticated spectrometric methods and considerable analytical expertise. Given the time and resources involved, there is a critical need for rapid and efficient methods to identify known compounds directly from crude extracts. Such dereplication strategies are essential to prevent redundant isolation of previously characterized metabolites, thereby allowing researchers to focus on the discovery of novel or pharmacologically relevant compounds.

A previous study demonstrated the application of liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS) for the dereplication of oligostilbenes, including its ability to distinguish closely related diastereoisomers (Manshoor & Weber, 2015a, b). The method, originally optimized for a triple quadrupole mass spectrometer (MS^2), has since been adapted for use with ion trap instrumentation. This study builds upon that work by employing a targeted dereplication strategy using a curated library of MS fragmentation data for known oligostilbenes.

METHODOLOGY

Plant materials and extraction method

A field expedition was conducted from 21st to 25th October 2019 in Kadamaian, Kota Belud, as part of the Borneo Geographic Expedition. This initiative was jointly organized by the Institute for Tropical Biology and Conservation (ITBC), Universiti Malaysia Sabah (UMS), and Sabah Parks. The primary aim was to collect bark and heartwood samples from selected Dipterocarpaceae species. Sampling sites included areas near a waterfall adjacent to the expedition base camp, along the Pinolobu and Meliawa rivers, and near Melangkap Noriou. Ten samples, including bark and heartwood from five dipterocarp species were collected.

Bark samples were obtained by carefully scraping the tree trunks at breast height (approximately 1.3 meters above ground level) using sterile knives to prevent contamination.

The underlying heartwood was then accessed and sliced from the area beneath the removed bark.

The species collected, all members of the Dipterocarpaceae family, were identified based on morphological characteristics and referenced to taxonomic descriptions by Meijer and Wood (1964) and Cockburn (1980):
1. *Parashorea tomentella* (Urat mata beludu); a very large tree, the height can exceed 60 m, with a dense crown.
2. *Dryobalanops lanceolata* (Kapur paji); A large tree with a diameter of up to 160 cm at breast height and a dense, oval crown, endemic to Borneo and widely distributed except in the south.
3. *Dipterocarpus caudiferus* (Keruing puteh, white Seraya); A large, lowland species common in Sabah, endemic to Borneo.
4. *Shorea xanthophylla* (Seraya kuning); Found only in northern Borneo (Sabah, Sarawak, Brunei), this species grows up to 28 m and commonly occurs in lowland forests.
5. *Shorea seminis* (Selangan batu terendak); Reaches heights of up to 50 m and is typically found along slowly flowing rivers; widely distributed in the lowland forests of Sabah.

Freshly collected bark and heartwood samples were chopped and oven-dried at 40°C until a constant weight was achieved. Bark samples (300 g each) were first defatted with *n*-hexane by maceration (1 L, overnight at room temperature) to remove non-polar constituents. The defatted material was then extracted with acetone by maceration and lixiviation (1 L, 3 cycles × 24 hours) at room temperature to obtain phenolic-rich extracts.

Heartwood samples (300 g each) were also delipidated with *n*-hexane and subsequently extracted using a water:acetone mixture (30:70, v/v). The extraction involved maceration for 20 hours followed by lixiviation with fresh solvent for 4 hours. The combined extracts were concentrated under reduced pressure and subjected to liquid–liquid partitioning using water and a methanol:ethyl acetate (1:1, v/v) mixture. The organic phase was collected and evaporated to dryness to yield the crude extracts. All extractions were done at RT, without mechanical shaking or centrifuging. All extracts were filtered through 0.45 µm PTFE membranes before chromatographic analysis. The extraction yields are shown in Table 1.

Table 1: Extraction yields of crude bark and heartwood extracts from five Dipterocarpaceae species collected in Kadamaian, Sabah. Yields were calculated based on the dry weight of starting material (300 g per sample).

No	Plant species	Bark g (% w/w)	Heartwood g (% w/w)
1	*Parashorea tomentella*	8.77 (2.92)	4.71 (1.57)
2	*Dryobalanops lanceolata*	7.22 (2.41)	3.06 (1.03)
3	*Dipterocarpus caudiferus*	5.89 (1.96)	5.33 (1.77)
4	*Shorea xanthophylla*	5.22 (1.74)	3.12 (1.04)
5	*Shorea seminis*	6.25 (2.08)	5.15 (1.72)

Reference standards

Pure compounds were isolated from the wood extract of *Neobalanocarpus heimii* through successive chromatographic separation techniques. Fractionated samples were subjected to repeated purification steps until chemical homogeneity was achieved. For each fraction, appropriate chromatographic parameters and solvent systems were carefully optimized based on its unique composition. The structures of the purified compounds were subsequently

elucidated using advanced spectroscopic techniques, including nuclear magnetic resonance (NMR) and mass spectrometry (MS), ensuring accurate identification and structural confirmation. The compounds were identified as heimiol A, heimiol B, balanocarpol, copaliferol A, vaticanol A, vaticaphenol A, heimiol D, heimiol E, hemsleyanol D, hopeaphenol, and isohopeaphenol (Bayach et al., 2015).

Chromatography

High-performance liquid chromatography (HPLC) and ultra-high-performance liquid chromatography (UHPLC) analyses were carried out using a Thermo Scientific™ UltiMate™ 3000 system (Thermo Fisher Scientific, Waltham, MA, USA). Both systems were equipped with a quaternary pump, autosampler, column oven, and a diode array detector (DAD-3000). The detector was set to monitor UV absorbance at 270 nm, which corresponds to the maximum absorbance of oligostilbenes. The DAD parameters were as follows: sampling rate of 5 Hz, slit width of 4 nm, and bandwidth of 20 nm. Column temperature was maintained at 35°C for both systems. The solvent systems and gradient profiles were optimized as described earlier to ensure effective separation of oligostilbenes.

HPLC Analysis. High-performance liquid chromatography (HPLC) was conducted using an ODS Hypersil® column (150 × 4.6 mm, 5 µm particle size; Thermo Fisher Scientific, Waltham, MA, USA) maintained at 35 °C. The mobile phase consisted of acetonitrile (ACN) and ultrapure water (H_2O), delivered through a combination of isocratic and gradient elution as follows: 5% ACN (0–3 min), 5–16% ACN (3–6 min), isocratic at 16% ACN (6–36 min), followed by a linear gradient to 34% ACN (36–39 min). At the end of each run, the column was flushed with 85% ACN for 6 minutes and re-equilibrated with the initial solvent composition for 10 minutes. The total run-time was 50 minutes, including pre-/post-equilibration, and the injection volume was 10 µL, with a flow rate of 1.0 mL/min.

UHPLC Analysis. Ultra-high-performance liquid chromatography (UHPLC) was carried out using an ODS Hypersil® column of the same stationary phase but with reduced internal diameter and particle size (150 × 2.1 mm, 3 µm particle size; Thermo Fisher Scientific). The solvent gradient was adapted from the HPLC protocol. Injections were made at 2 µL with a flow rate of 0.2 mL/min.

Mass spectrometry

Mass spectrometric analyses were performed using an Agilent 6300 Series Ion Trap LC/MS system (Agilent Technologies, Santa Clara, CA, USA) equipped with an electrospray ionization (ESI) source operating in positive ion mode. Nitrogen served as both the nebulizing and drying gas, with the nebulizer temperature set at 350 °C. Spectra were acquired in positive ion mode across a mass range of *m/z* 100–1000. MS^n experiments were carried out up to MS^5, using helium as the collision gas. The isolation width was set to 2.0 *m/z*, and the fragmentation amplitude was maintained at 0.90 V.

Compound identification

An in-house compound library was constructed using mass spectral data of authenticated reference standards. Fragmentation patterns (MS^1–MS^5) of each pure compound were recorded and stored using Agilent MassHunter software. Subsequently, LC-MS data from crude plant extracts were acquired and analysed. The identification of known compounds was accomplished by matching the MS^n spectra of sample peaks with those in the reference in-house library, enabling confident dereplication (Ramli et al., 2015).

RESULTS

As part of the Borneo Geographic Expedition in Kadamaian, Sabah, five Dipterocarpaceae species were selected for analysis: *Parashorea tomentella*, *Dryobalanops lanceolata*, *Dipterocarpus caudiferus*, *Shorea xanthophylla*, and *Shorea seminis*. For each species, both bark and heartwood were sampled to assess tissue-specific variations in oligostilbene content. Figure 1 presents photographs of the trees to provide visual context for the samples used in this study.

Figure 1: Representative photographs of Dipterocarpaceae species sampled during the Borneo Geographic Expedition in Kadamaian, Sabah. The trees selected for this study include: **A.** *Parashorea tomentella*, **B.** *Dryobalanops lanceolata*, **C.** *Dipterocarpus caudiferus*, **D.** *Shorea xanthophylla*, and **E.** *Shorea seminis.* Bark and heartwood samples were collected from each tree species for chemical profiling and dereplication analysis.

Chromatographic profile of the crude extracts

A polar heartwood extract was obtained via liquid–liquid partitioning of an aqueous–acetone extract using a methanol: ethyl acetate (1:1) and water biphasic system. Chromatographic profiles of all crude extracts were first generated using conventional high-performance liquid chromatography (HPLC) prior to liquid chromatography-mass spectrometry (LC-MS) analysis.

The resulting chromatograms revealed the chemical complexity of the extracts, characterized by numerous overlapping peaks, particularly among metabolites present in low abundance. Optimal dereplication requires baseline separation of individual compounds; however, the method must also remain time-efficient and robust.

The complexity of the crude extracts, particularly the presence of structurally similar oligostilbenes with varying degrees of polymerization, posed a significant challenge for chromatographic separation. These compounds often differ only slightly in polarity and molecular weight, leading to co-elution if the chromatographic conditions are not carefully optimized.

To improve resolution, we adjusted the solvent gradient to achieve a balance between polar and non-polar interactions with the stationary phase. A reversed-phase ODS column was selected to exploit hydrophobic interactions, allowing better separation of the phenolic stilbenes based on their increasing hydrophobicity with higher oligomerization. The early

gradient phase (5–16% acetonitrile) allowed more polar compounds to elute slowly, enhancing separation, while the later gradient (up to 34%) gradually increased elution strength to resolve more hydrophobic trimers and tetramers.

The 35-minute run time was chosen as an optimal point to allow sufficient separation without unnecessarily extending the analysis duration, maintaining throughput for multiple sample runs. An additional 10-minute high-acetonitrile flush ensured removal of strongly retained compounds and re-equilibration of the column, preventing carryover and preserving reproducibility. These adjustments aimed to ensure baseline separation of closely related metabolites, thereby increasing confidence in compound identification and enhancing dereplication efficiency.

Mass spectrometric analyses for the crude extracts

All extract samples were further analyzed using LC-MS, equipped with a diode array detector and an ion trap mass spectrometer operating with an electrospray ionization (ESI) interface. The chromatographic separation was performed using an ultra-high-performance liquid chromatography (UHPLC) system, with minor adjustments made to accommodate the narrower column dimensions and higher pressure requirements relative to conventional HPLC.

Compound identification was based on MS fragmentation patterns, which were compared against an established in-house spectral library (Table 2). Detected peaks corresponding to known compounds were assigned numerical labels, corresponding to the standard compounds in the library (Fig. 2).

Table 2: The fragment ions at each MS level obtained from LC-ESI-ion trap-MS spectral data of reference compounds.

No	Compound	MS	MS2	MS3	MS4	MS5
1	Heimiol A	471	453, 349, 243	359, 241	331	-
2	Heimiol B	471	453, 349, 243	243	215	-
3	Balanocarpol	471	377, 243	349, 243	173	-
4	Copaliferol A	681	587, 453, 331	313, 239	-	-
5	Vaticanol A	681	557, 453, 359	359, 265	265	239
6	Vaticaphenol A	907	813, 707, 513	479, 371	409	-
7	Heimiol D	907	813, 709, 347	709, 707, 625	689, 613, 479	-
8	Heimiol E	907	813, 719	719, 635	701, 625	-
9	Hemsleyanol D	907	813, 719	719, 625	701, 625, 515	607, 531, 409
10	Hopeaphenol	907	453, 359	341, 265	237	-
11	Isohopeaphenol	907	813, 453, 359	359, 265	265	-

Dereplication of known oligostilbenes in the crude extracts

LC-MS analysis of the bark extract of *Parashorea tomentella* led to the identification of seven oligostilbenes, matched against a reference compound from in-house library. All detected peaks were well-resolved, enabling confident identification based on retention time and fragmentation profiles. Notably, compound 9 was detected despite its low abundance, demonstrating the sensitivity of the method. The wood extract of *P. tomentella* exhibited a distinct oligostilbene profile compared to its bark counterpart, with differing retention times for most compounds except compound 3. Among four major peaks observed between 17.0 and 21.0 minutes, three matched known oligostilbenes. An additional peak at 17.0 minutes,

consistent with a stilbene trimer, did not correspond to any known entry in the database. Overall, the peaks displayed good resolution, facilitating dereplication.

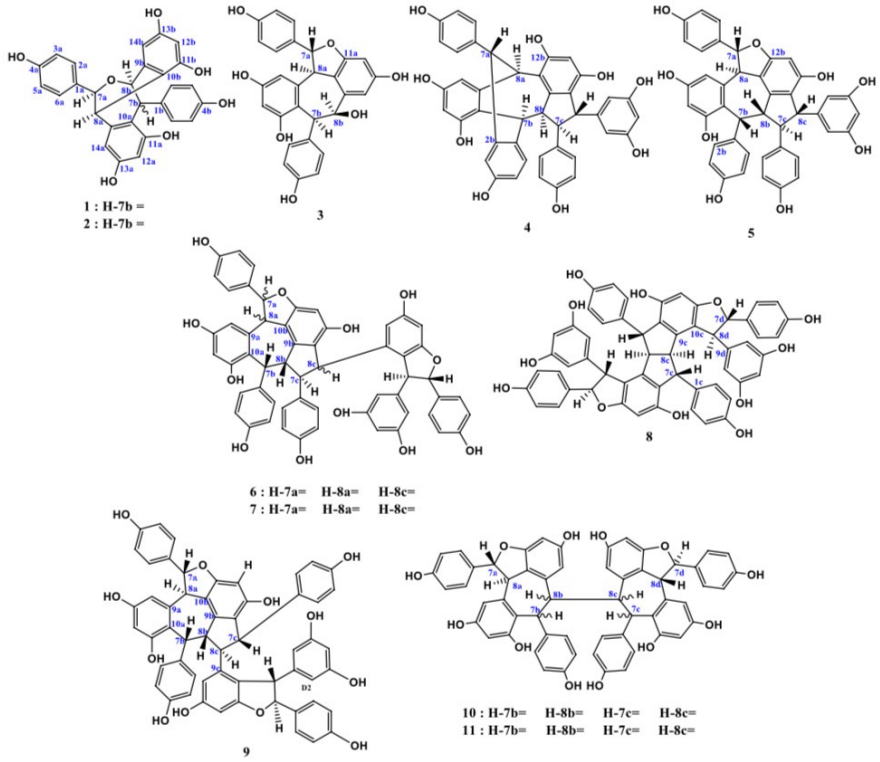

Figure 2: Structures of the oligostilbenes 1–11 used to generate the MS data reference library.

The methanolic bark extract of *Dryobalanops lanceolata* produced ten prominent peaks, though most eluting before 15 minutes did not correspond to known oligostilbenes. A broad, unresolved peak prior to 5.0 minutes was excluded due to incompatible fragmentation data. A minor peak at 9.8 minutes (*m/z* 502) suggested the presence of a stilbene dimer lacking one phenolic ring, possibly a degradation product. Peaks at 11.2 and 12.7 minutes were identified as stilbene dimers, while those at 14.9 and 17.2 minutes corresponded to trimeric stilbenes, although these were not present in the compound database. In total, five known compounds were identified. The chromatographic profile of the wood extract mirrored that of the bark extract, though variations in peak intensity were observed. Several unidentified peaks between 11 and 18 minutes displayed fragmentation patterns consistent with previously detected oligostilbenes. Compound 7, which was too low in abundance in the bark to be detected, was clearly observed here. Five oligostilbenes were identified based on MS fragmentation.

Analysis of the bark extract of *Dipterocarpus caudiferus* revealed a major peak at 13.4 minutes (*m/z* 681), confirmed via MS as compound 4, a stilbene trimer. Three additional peaks at 16.9, 21.2, and 23.5 minutes showed *m/z* values of 907 and were identified as compounds 6, 9, and 10, respectively. Peaks at 9.5 (*m/z* 469.2) and 10.2 (*m/z* 679.2) suggested the presence of dimeric and trimeric stilbenes, although no matches were found in the database. A broad

unresolved peak at 20.0 minutes could not be identified due to overlapping signals. The wood extract of *D. caudiferus* showed three distinct, well-resolved peaks at 13.5, 18.5, and 25.2 minutes, identified as compounds 4, 7, and 11, respectively. Compound 5, which co-eluted with several unknowns, was identified based on its intensity, *m/z* value (680), and fragmentation data as a stilbene trimer. A cluster of peaks between 22.0 and 26.0 minutes was partially resolved; however, only the peak at 24.7 minutes matched a known compound (compound 10). The remaining peaks could not be assigned due to lack of reference data.

The bark extract of *Shorea xanthophylla* displayed eight well-resolved peaks between 7.0 and 19.0 minutes. Five peaks, at 7.5, 9.1, 10.0, 15.8, and 16.9 minutes, corresponded to compounds 1, 2, 3, 5, and 6, respectively. The remaining peaks did not match any known compounds in the database. Similar to the bark extract, the wood extract of *S. xanthophylla* exhibited a series of well-defined peaks, though with a distinct chromatographic profile. A peak at 4.2 minutes was excluded due to incompatible fragmentation data. Two major peaks at 9.5 and 17.1 minutes (*m/z* 471 and 907) were identified as compounds 2 and 6, consistent with the bark extract. Additionally, peaks at 17.0, 21.9, and 23.7 minutes (all *m/z* 907) were identified as compounds 7, 9, and 10, respectively, indicative of stilbene tetramers.

The bark and wood extracts of *S. seminis* shared highly similar chromatographic profiles, with well-resolved peaks throughout. All compounds identified in the bark extract were also present in the wood extract, except for compound 5. Several minor peaks were below the detection threshold for mass spectral analysis. In total, eight oligostilbenes (compounds 1–8) were successfully identified from both extracts. The chromatograms with all identified peaks for all sample extracts are shown in Fig. 3.

The presence of broad or unresolved peaks, such as the one observed at 20.0 minutes in *Dipterocarpus caudiferus*, likely reflects the complex chemical nature of the sample matrix and the structural characteristics of oligostilbenes. One possible explanation is the presence of co-eluting isomers or oligomeric species with very similar polarity and molecular weight, which can be difficult to separate under standard chromatographic conditions. Oligostilbenes often share core structural features with subtle differences in linkage type, degree of polymerization, or hydroxylation pattern, leading to overlapping retention behaviours. Additionally, strong matrix effects may interfere with chromatographic resolution or ionization efficiency, particularly in heartwood extracts, which are known to contain dense mixtures of phenolic compounds and polymeric substances. Chemical instability, such as oxidation or partial degradation during extraction or analysis, may also contribute to peak broadening or shifting. These factors, individually or in combination, can complicate both chromatographic separation and confident spectral interpretation. Future studies may address this by incorporating sample clean-up techniques, targeted isolation, or orthogonal chromatographic methods to improve resolution and aid in the identification of such ambiguous features.

The observed variations in oligostilbene profiles between bark and wood extracts, as well as among species, appear to be primarily driven by differences in the degree of polymerization, as reflected by the distinct *m/z* values and fragmentation patterns obtained from LC-MS^n analysis. Bark extracts generally exhibited a higher proportion of dimeric oligostilbenes (e.g.,

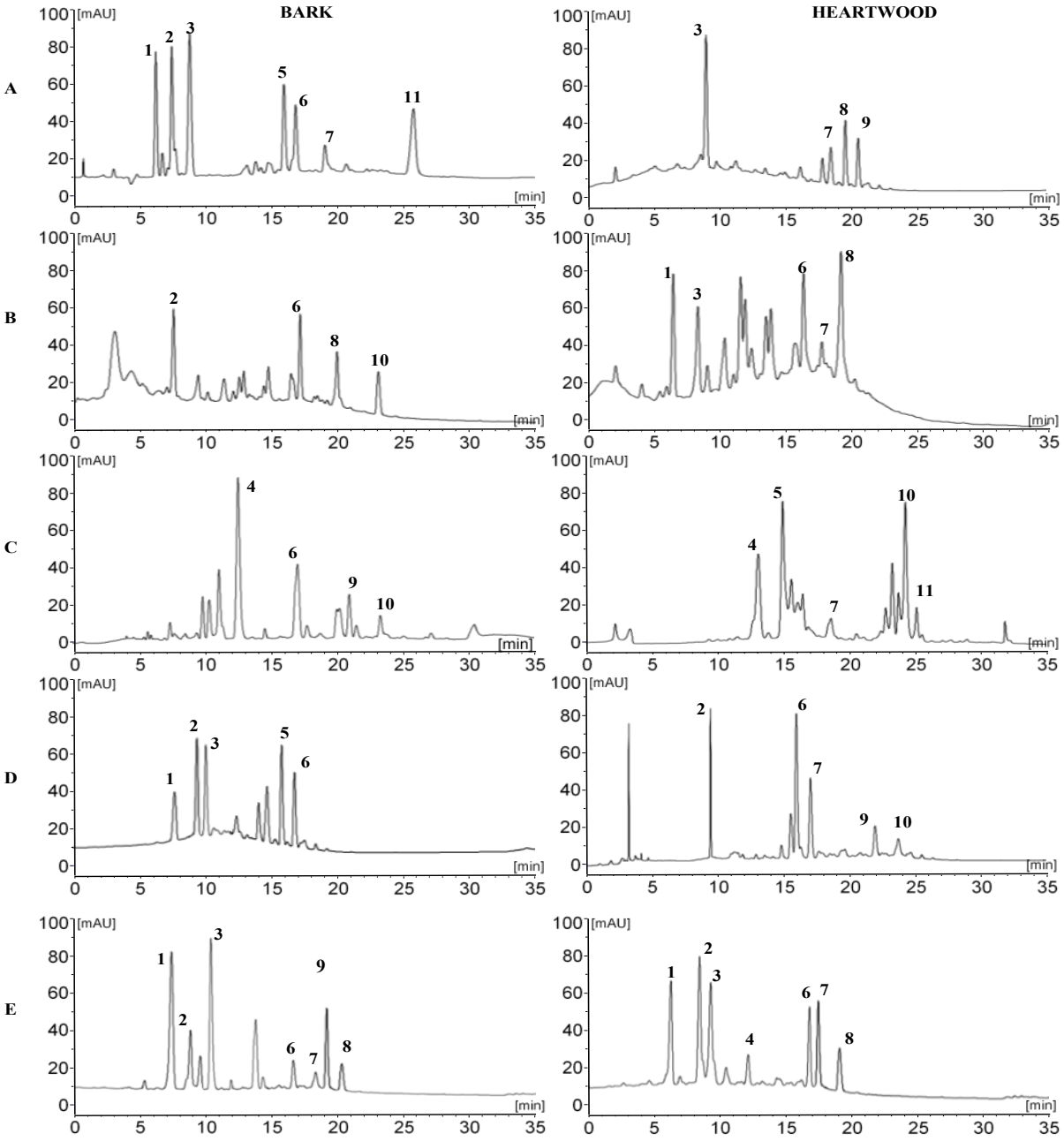

Figure 3: Chromatograms of crude methanolic extracts; **A.** *Parashorea tomentella*, **B.** *Dryobalanops lanceolata,* **C.** *Dipterocarpus caudiferus*, **D.** *Shorea xanthophylla, and* **E.** *Shorea seminis* recorded at 270 nm. Respective peaks are labelled with their corresponding identifying MS characteristics from the data library.

m/z ~470), which are likely biosynthesized rapidly in response to environmental stimuli such as UV exposure, microbial invasion, or herbivory. In contrast, wood extracts, particularly heartwood, contained a greater abundance of trimeric and tetrameric stilbenes (e.g., *m/z* ~680–907), suggesting long-term accumulation and structural reinforcement roles. Minor but consistent differences in fragmentation profiles also point to variations in interflavonoid linkage types (e.g., C–C vs. C–O–C bonds) and hydroxylation or methoxylation patterns on the aromatic rings. These structural variations not only influence chromatographic behaviour

and ionization efficiency but also likely reflect species-specific metabolic adaptations. For instance, the frequent occurrence of highly polymerized oligostilbenes in *Dipterocarpus caudiferus* wood may indicate a strategy for durable heartwood defence, while the diversity of dimers in *Parashorea tomentella* bark suggests a more dynamic defensive chemistry. Such differences underscore the chemical diversity and ecological specialization among Dipterocarpaceae species.

The chromatograms revealed that heartwood extracts exhibited a higher degree of peak co-elution and non-baseline separation compared to bark extracts. This can be attributed to the inherently more complex chemical matrix of heartwood, which tends to accumulate a broader range of secondary metabolites over time, including higher order oligostilbenes, lignans, and other phenolic polymers. These compounds often possess similar physicochemical properties, such as molecular weight, polarity, and functional group composition leading to overlapping retention times and compromised chromatographic resolution.

Additionally, the dense and lignified nature of heartwood may result in the co-extraction of structurally related but unresolved oligomeric compounds, further contributing to peak broadening and reduced separation. Matrix effects in heartwood extracts may also affect ionization efficiency, making minor components harder to detect and resolve.

To improve chromatographic clarity in future analyses, further clean-up steps such as solid-phase extraction (SPE) or liquid-liquid partitioning with more selective solvents could help reduce matrix complexity. More targeted extraction protocols focusing on specific polarity ranges may also enrich for particular classes of stilbenes. Alternatively, employing orthogonal separation techniques, such as two-dimensional chromatography or different stationary phase chemistries (e.g., phenyl-hexyl or biphenyl columns) could enhance the resolution of closely eluting compounds. Highlighting and addressing these challenges is important to fully appreciate the chemical richness of heartwood and optimize future dereplication strategies.

DISCUSSION

This study successfully demonstrated the application of LC-MS-based dereplication for the rapid identification of oligostilbenes in bark and wood extracts from five Dipterocarpaceae species. A total of eleven known compounds were identified, revealing both species-specific and tissue-specific variations in metabolite profiles. These findings provide valuable insights into the chemotaxonomic characteristics of the family and highlight the biosynthetic diversity of oligostilbenes across different plant tissues.

Parashorea tomentella exhibited the most diverse oligostilbene profile among the studied species, particularly in its bark, which contained eight compounds: Heimiols A, B, and D, balanocarpol, vaticanol A, vaticaphenol A, hemsleyanol D, and isohopeaphenol. This richness suggests that the bark serves as a major site of stilbenoid biosynthesis, likely in response to environmental exposure. The wood extract showed a reduced but overlapping profile, containing balanocarpol, heimiols D and E, and hemsleyanol D. The recurrence of compounds such as balanocarpol and hemsleyanol D across both tissues suggests a core set of metabolites that are systemically distributed within the species (Lim et al., 2023).

In *Dryobalanops lanceolata*, five compounds were identified in the bark extract, including heimiols A, D, and E, balanocarpol, and vaticaphenol A. These overlap partially with the

compounds found in *P. tomentella*, indicating shared biosynthetic capabilities within the family. The wood extract of *D. lanceolata* contained copaliferol A, vaticaphenol A, hemsleyanol D, and hopeaphenol, with vaticaphenol A being the only compound common to both tissue types. The exclusive presence of copaliferol A and hopeaphenol in wood highlights tissue-specific metabolite accumulation, potentially linked to heartwood maturation and defence (Chong et al., 2009).

The bark of *Dipterocarpus caudiferus* yielded four compounds, copaliferol A, vaticaphenol A, hemsleyanol D, and hopeaphenol, while the wood extract presented a broader chemical profile. Additional compounds, including vaticanol A and isohopeaphenol, were identified only in the wood, suggesting that the heartwood may act as a reservoir for certain trimeric and tetrameric stilbenes. This extended profile may result from long-term metabolic accumulation or adaptive responses to biotic stress, such as fungal pathogens or decay (Tiwari et al., 2025).

Shorea xanthophylla also displayed substantial oligostilbene diversity. Its bark extract included heimiols A and B, balanocarpol, vaticanol A, and vaticaphenol A, while the wood extract contained heimiols B and D, vaticaphenol A, hemsleyanol D, and hopeaphenol. The consistent detection of vaticaphenol A in both tissues suggests a central role in the plants metabolic or defensive functions. Similarly, the presence of heimiol derivatives in both extracts reflects the continuity of stilbenoid biosynthesis across developmental stages or tissue types (Huong et al., 2025).

Shorea seminis demonstrated the highest degree of overlap between bark and wood extracts, with eight oligostilbenes detected in the bark and seven in the wood. Shared compounds included heimiols A, B, D, and E, balanocarpol, copaliferol A, and vaticaphenol A. Vaticanol A was identified exclusively in the bark. This high metabolite redundancy suggests that *S. seminis* is a metabolically rich species with strong potential for yielding bioactive stilbenes.

Across all species, certain compounds such as vaticaphenol A, balanocarpol, and heimiol derivatives were frequently encountered. Vaticaphenol A was especially widespread, detected in all species except in the wood of *P. tomentella*. The consistent presence of heimiol variants across genera and tissues further underscores their significance in Dipterocarpaceae secondary metabolism (Deng et al., 2017).

Interestingly, some compounds demonstrated tissue-specific distribution. Hopeaphenol, isohopeaphenol, and copaliferol A were predominantly found in wood extracts, suggesting a functional role in heartwood physiology, possibly related to structural defence or long-term storage of antimicrobial agents. In contrast, bark extracts generally exhibited broader chemical diversity, likely reflecting their direct interaction with environmental stressors, including UV radiation, pathogens, and herbivores (Mattio et al., 2020).

This study highlights the utility of LC-MS-based dereplication as a rapid and effective approach for profiling complex plant extracts. The identification of 11 known oligostilbenes across five Dipterocarpaceae species reveals distinct yet overlapping chemical signatures that are influenced by both species and tissue type. These results contribute to our understanding of the chemical ecology and taxonomic relationships within this important tropical family. Furthermore, the findings provide a foundation for future studies into the bioactivity, ecological functions, and pharmacological applications of stilbene derivatives. A summary of the oligostilbenes identified in each species and tissue type is presented in Table 3.

Table 3: Identified oligostilbenes in the bark and wood extracts of Dipterocarpaceae plants collected from Kadamaian, Kota Belud, Sabah.

	Plant (extract)	**Compound**
1	*Parashorea tomentella* (Bark)	Heimiols A, B, D, Balanocarpol, Vaticanol A, Vaticaphenol A, Hemsleyanol D, Isohopeaphenol
2	*Parashorea tomentella* (Wood)	Balanocarpol, Heimiols D, E, Hemsleyanol D
3	*Dryobalanops lanceolata* (Bark)	Heimiols A, D, E, Balanocarpol, Vaticaphenol A
4	*Dryobalanops lanceolata* (Wood)	Copaliferol A, Vaticaphenol A, Hemsleyanol D, Hopeaphenol
5	*Dipterocarpus caudiferus* (Bark)	Copaliferol A, Vaticaphenol A, Hemsleyanol D, Hopeaphenol
6	*Dipterocarpus caudiferus* (Wood)	Copaliferol A, Vaticanol A, Heimiol D, Hopeaphenol, Isohopeaphenol
7	*Shorea xanthophylla* (Bark)	Heimiols A, B, Balanocarpol, Vaticanol A, Vaticaphenol A
8	*Shorea xanthophylla* (Wood)	Heimiols B, D, Vaticaphenol A, Hemsleyanol D, Hopeaphenol
9	*Shorea seminis* (Bark)	Heimiols A, B, D, E, Balanocarpol, Copaliferol A, Vaticanol A, Vaticaphenol A
10	*Shorea seminis* (Wood)	Heimiols A, B, D, E, Balanocarpol, Copaliferol A, Vaticaphenol A

The application of LC-MS^n-based dereplication proved especially valuable in navigating the inherent chemical complexity of Dipterocarpaceae crude extracts. The ability to generate and compare multi-stage fragmentation data (MS^2–MS^5) allowed for the confident identification of closely related oligostilbenes, even when present in low abundance or embedded within dense matrices. In particular, the reproducibility of key fragmentation pathways, such as losses of phenolic groups, stilbene units, or characteristic neutral fragments, provided diagnostic clues to differentiate compounds that share similar molecular weights but differ in structural connectivity or substitution patterns. Retention time consistency, when interpreted alongside MS^n fragmentation, added another layer of confidence in compound identification, especially in distinguishing positional or stereoisomers. For peaks that could not be dereplicated, the most probable reasons include low signal intensity resulting in incomplete MS^2 spectra, absence of distinctive fragmentation features, or the presence of oligomeric structures not yet included in the reference library, potentially representing novel stilbenoid scaffolds. These ambiguous features were acknowledged but excluded from detailed interpretation to maintain the methodological rigor of this dereplication-focused study.

Furthermore, the tissue- and species-specific distribution patterns of identified oligostilbenes appear to reflect adaptive biochemical strategies. Compounds predominantly found in bark, such as dimers and certain hydroxyl-rich stilbenes, often possess structural features (e.g., free phenolic groups, lower degrees of polymerization) associated with higher chemical reactivity and rapid mobilization in response to environmental stressors such as pathogens or UV radiation. These features support the hypothesis that bark serves as a frontline defence compartment in Dipterocarpaceae species. Conversely, heartwood extracts tended to contain more polymerized, structurally complex oligostilbenes, such as trimers and tetramers that are chemically more stable and less prone to oxidative degradation. Such compounds are likely to function as long-term protective agents, contributing to the durability and resistance of heartwood tissues against microbial decay and structural weakening. These structure-activity relationships not only help explain the observed chemical profiles but also provide insight into the ecological roles of oligostilbenes within tropical forest species.

CONCLUSIONS

This study demonstrated the effective use of LC-ESI-MS^n-based dereplication for profiling oligostilbenes in the bark and heartwood extracts of five Dipterocarpaceae species: *Parashorea tomentella*, *Dryobalanops lanceolata*, *Dipterocarpus caudiferus*, *Shorea xanthophylla*, and *Shorea seminis*. Through comparison with an in-house MS^1–MS^5 data library, a total of 11 known oligostilbenes were confidently identified. The method also enabled the recognition of previously uncharacterized stilbenes based on their fragmentation patterns and condensation levels. The approach proved particularly valuable for distinguishing structurally similar compounds within complex mixtures, even at low concentrations. Unlike traditional methods that rely heavily on chromatographic conditions, the tandem MS analysis provided consistent and interpretable spectral data, reducing the dependency on precise retention times. This highlights LC-MS^n as a robust tool for streamlining phytochemical workflows and minimizing unnecessary re-isolation of known metabolites. In summary, LC-MS-based dereplication not only accelerates the identification of bioactive natural products but also enhances the strategic focus of phytochemical research. The oligostilbene diversity uncovered in these Dipterocarpaceae species reinforces their significance as promising reservoirs of pharmacologically relevant compounds, supporting further investigation into their bioactivity and conservation value.

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DECLARATIONS

Research permit(s). This study was conducted with the approval of the Sabah Biodiversity Council Access License Ref. - JKM/MBS.1000-2/1JLD.3.
Ethical approval/statement. Not applicable.
Generative AI use. The authors declare that generative AI has been used in compliance with the JTBC policies, and that we have reviewed and edited the content after using this service and we take full responsibility for the content of the publication.

REFERENCES

Bayach I, Manshoor N, Sancho-Garcia JC, Choudhary MI, Trouillas P, Weber JFF (2015) Oligostilbenoids from the heartwood of *Neobalanocarpus heimii* and insights in their biogenesis. Chemistry – An Asian Journal 10(1): 198–211. https://doi.org/10.1002/asia.201403028.

Chong J, Poutaraud A, Hugueney P (2009) Metabolism and roles of stilbenes in plants. Plant Science 117 (3): 143–155. https://doi.org/10.1016/j.plantsci.2009.05.012.

Cockburn PF (1980) Trees of Sabah, Vol. 2. Sabah Forest Record No. 10. Borneo Literature Bureau for Forest Department, Sabah.

Deng N, Liu C, Chang E, Ji J, Yao X, Yue J, Bartish IV, Chen L, Jiang Z, Shi S (2017) High temperature and UV-C treatments affect stilbenoid accumulation and related gene expression levels in *Gnetum parvifolium*. Electronic Journal of Biotechnology 25: 43–49. https://doi.org/10.1016/j.ejbt.2016.11.001.

Eschenbach C, Glauner R, Kleine M, Kappen L (1998) Photosynthesis rates of selected tree species in lowland dipterocarp rainforest of Sabah, Malaysia. Trees 12(6): 356–365. https://doi.org/10.1007/s004680050162.

Huong NT, Hop NQ, Duy DA, Son NT (2025) The genus Gnetum: traditional use, phytochemistry, nutritional value, biosynthesis, pharmacology, toxicology, synthetic advances, and pharmacokinetics. Fitoterapia 182: 106461. https://doi.org/10.1016/j.fitote.2025.106461.

Jalal RS, Weber JFF, Manshoor N (2018) Dereplication of oligostilbenes in dipterocarpaceous plants using LCMS-ESI-ion trap-database. Journal of Liquid Chromatography & Related Technologies 41(4): 161–169. https://doi.org/10.1080/10826076.2018.1428200.

Lim PC, Ramli R, Manshoor N (2023) Polyphenolic profiling of Dipterocarpus species using LC-MS. Biochemical Systematics and Ecology 110: 104685. https://doi.org/10.1016/j.bse.2023.104685.

Manshoor N, Weber JFF (2015a) Mass spectrometric analysis for discrimination of diastereoisomers. Mass Spectrometry Letters 6(4): 99–104. https://doi.org/10.5478/msl.2015.6.4.99.

Manshoor N, Weber JFF (2015b) Mass fragmentation patterns as fingerprints for positive identification of polyphenolic compounds in a crude extract. Mass Spectrometry Letters 6(4): 105–111. https://doi.org/10.5478/msl.2015.6.4.105.

Mattio LM, Catinella G, Pinto A, Dallavalle S (2020) Natural and nature-inspired stilbenoids as antiviral agents. European Journal of Medicinal Chemistry 202: 112541. https://doi.org/10.1016/j.ejmech.2020.112541.

Meijer W, Wood GHS (1964) Dipterocarps of Sabah (North Borneo). Sabah Forest Record No. 5.

Ramli R, Ismail NH, Manshoor N (2015) Identification of oligostilbenes from *Dipterocarpus semivestitus* through dereplication technique. Jurnal Teknologi 77:2 85–88.

Shen T, Wang XN, Lou HX (2009) Natural stilbenes: An overview. Natural Product Reports 26: 916–935. https://doi.org/10.1039/b905960a.

Tiwari RK, Goswami SK, Gujjar RS, Kumar R, Kumar R, Lal MK, Kumari M (2025) Mechanistic insights on lignin-mediated plant defense against pathogen infection. Plant Physiology and Biochemistry 228: 110224. https://doi.org/10.1016/j.plaphy.2025.110224.