

# IDENTIFICATION OF OLIGOSTILBENES FROM *Dipterocarpus semivestitus* THROUGH DEREPLICATION TECHNIQUE

## Article history

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## Graphical abstract



## Abstract

Three oligostilbenes have been identified in stems extract of *Dipterocarpus semivestitus* through dereplication strategy. The stems extract was injected to liquid chromatography tandem mass spectroscopy. Two tetramer, hopeaphenol and hemsleyanol D, were identified by comparing their mass fragmentation patterns with in-house library. A trimer,  $\alpha$ -viniferin was isolated by various separation techniques and its structure was confirmed by NMR analyses and comparison with published data.

Keywords: Dipterocarpaceae, *Dipterocarpus semivestitus*, resveratrol oligomers, tandem MS, dereplication

## Abstrak

Tiga oligostilbena telah berjaya dikenal pasti daripada ekstrak dahan *Dipterocarpus semivestitus* melalui strategi dereplikasi. Ekstrak dahan telah disuntik kepada kromatografi cecair spektroskopi jisim bertingkat. Dua tetramer, hopeafenol dan hemsleyanol D telah berjaya dikenal pasti melalui perbandingan pola jisim penyerpihan dengan perpustakaan dalaman. Satu trimer,  $\alpha$ -viniferin telah dipisahkan dengan pelbagai teknik pengasingan dan strukturnya telah disahkan melalui analisis data NMR serta perbandingan dengan data yang telah dilaporkan.

Kata kunci: Dipterocarpaceae, *Dipterocarpus semivestitus*, oligomer resveratrol, tandem MS, dereplikasi

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## 1.0 INTRODUCTION

Dipterocarpaceae family is known to produce high diversity of oligostilbene structures, mostly ranging from dimeric to octameric stilbenes. Various chemical and biological activities have been reported on this plant including anti-inflammatory, cytotoxicity, antimicrobial and antioxidant activities [1-3]. Most of the recent phytochemical studies on this family were on oligostilbenes due to their abundance in the plants which can be isolated from their leaves, bark, wood, heartwood and seeds.

*Dipterocarpus semivestitus* is one of the trees in the family of Dipterocarpaceae. The local name is 'keruing padi' or 'keruing dadih' and endemic to Peninsular Malaysia and Kalimantan (Indonesia). It inhabits lowland and swamp forest. The current distribution of this species is only in freshwater swamp forest in UiTM Kampus Seri Iskandar, Perak, with current population of 53 trees due to extensive logging activities in its original locations [4]. *D. semivestitus* is classified as critically endangered species by Red List and International Union for Conservation of Nature (IUCN) [5].

The research is about isolation and characterization of oligostilbenes from *D. semivestitus* using chromatographic and spectroscopic techniques. A dereplication procedure will be introduced prior to the isolation process to identify selected compounds, so that only targeted compounds will be isolated.

## 2.0 EXPERIMENTAL

### 2.1 Plant Materials

Plant sample of *D. semivestitus* was collected in freshwater swamp forest in UiTM Kampus Sri Iskandar, Perak. The sample was identified and confirmed by the botanist. The voucher specimen (DS1233) was deposited in herbarium at Atta-ur-Rahman Institute for Natural Products Discovery. The initial processing of cutting and drying was carried out on-site to avoid growth of fungi and other microorganism.

### 2.2 Extraction

Dried plant sample (2.0 kg) was extracted with hexane to remove non polar compound and then with acetone (3×24h) at room temperature to afford phenolic rich extract. The acetone extract was vacuum filtered and dried using rotary evaporator to afford 42 g of crude extract. The crude extract was then filtered through 0.45 µm PTFE filter before introduce into HPLC.

### 2.3 Evaluation of MS Data

The crude extract was subjected to HPLC system to obtain optimized analytical chromatographic conditions. The system, Agilent 1200 series was fitted with vacuum degasser, binary pump, auto sampler and thermostat column compartment using LUNA C18 column (150×4,6 mm, particle size 5 µm) equipped with guard column cartridge. Mobile phase flow rate of 1 mL/min was used and started with gradient of

acetonitrile-water (5:95 to 34:66) for 15 minutes followed by isocratic of acetonitrile-water (34:66) for the next 5 minutes. It was then continued with gradient increment of acetonitrile-water (85:15) for 5 minutes. The column flushing was performed at acetonitrile-water (85:15) for 3 minutes. The analysis was detected at UV 215, 254 and 283 nm. Using the optimized conditions, chemical screening of stem extract was done using ion trap mass spectrometer where MS/MS data of crude extract was compared to the previously isolated oligostilbenes that stored in system's library.

### 2.4 Isolation

A small portion of the crude extract (10 g) was subjected to MPLC system. The system, Yamazen W-Prep 2XY was fitted with binary pump, column compartment, UV detector and parallel fraction FR-260. Hi-Flash 5 L silica gel (40 µm) column was used and the extract was fractioned with normal phase gradient elution of *n*-hexane-ethyl acetate (3:2, 3:7, 1:4, 10:0) and ethyl acetate-methanol (95:5, 4:1) to give 17 fractions (Fr A- Fr Q). Fr G (975 mg) was then introduced to preparative HPLC using reverse phase gradient elution of acetonitrile-water (3:7 to 2:3) for 15 minutes and further purification was applied using reversed phased recycling HPLC with isocratic elution of acetonitrile and water (1:1) to give the major compound,  $\alpha$ -viniferin (**1**) (325 mg).

## 3.0 RESULTS AND DISCUSSION

Using the optimized conditions from HPLC analysis, chemical screening of the stem extract conducted using ion trap mass spectrophotometer shows similar chromatographic profiles as the HPLC. From the Base Peak Chromatogram (BPC) (Figure 1), mass spectra of each compound were extracted from their chromatographic peaks. The fragmentation patterns from mass data were compared with an in-house library, which were developed in our laboratory.

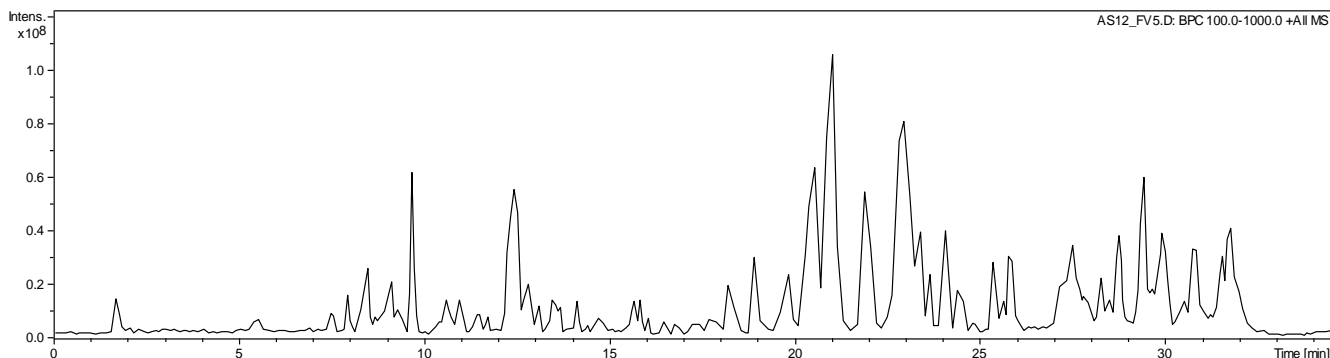


Figure 1 Base peak chromatogram of *D. semivestitus* crude extract

Two significant peaks at RT 14.4 and 18.2 min showed similar fragmentation patterns with the data in the library. A comparison between MS data of the crude extract and in-house library confirmed that the peaks were corresponding to two tetramers, hopeaphenol (**2**) and hemsleyanol D (**3**) respectively. Both oligostilbenes showed  $[M+H]^+$  peak at  $m/z$  907. Upon inspection of further MS fragmentation ( $MS^2 - MS^5$ ), fragmentation pattern for peak at RT 14.4 min corresponding to (**2**) as shown in Figure 2, whereas, peak at RT 18.2 min was attributed to (**3**) (Figure 3). Hopeaphenol (**2**) was first reported by Coggon *et al.* in 1965 which was isolated from the heartwood extract of *Hopea odorata* and *Balanocarpus heimii* [6]. Hemsleyanol D (**3**), a tetramer oligostilbene was

previously isolated from *Shorea hemsleyana* and *Dipterocarpus grandifloras* [7, 8].

Further evaluation of the rest of the peaks in the chromatogram reveals a few significant peaks which are well resolved. The major peak was found at RT 22.8 min with  $[M+H]^+$  peak at  $m/z$  679.2 (Figure 4) suggested that the compound is a trimer. A data search with in-house database did not show any match. The  $^1H$  and  $^{13}C$  NMR data of the compound were obtained and comparison with the published data confirmed it as  $\alpha$ -viniferin (**1**) [9]. The compound was previously isolated from five *Dipterocarpus* species which were *D. grandiflorus*, *D. retusus*, *D. hasseltii* [10], *D. elongates* [11] and *D. verrococus*.

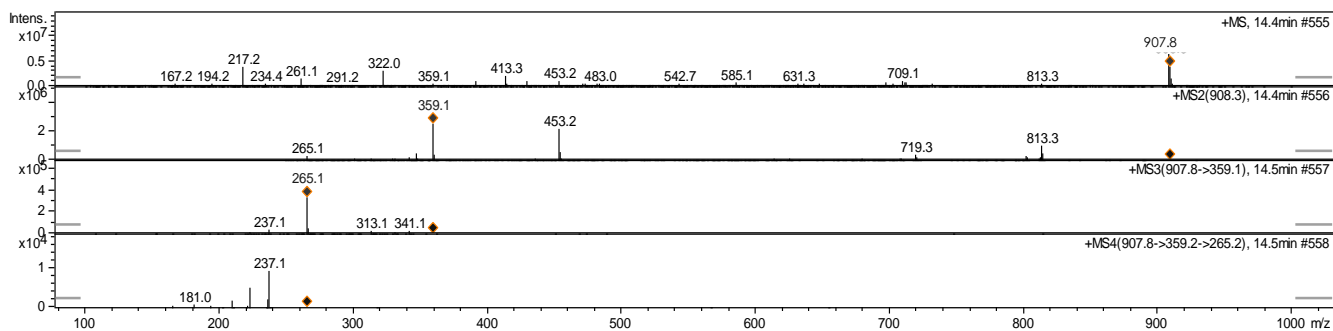


Figure 2 Mass spectra of hopeaphenol (**2**)

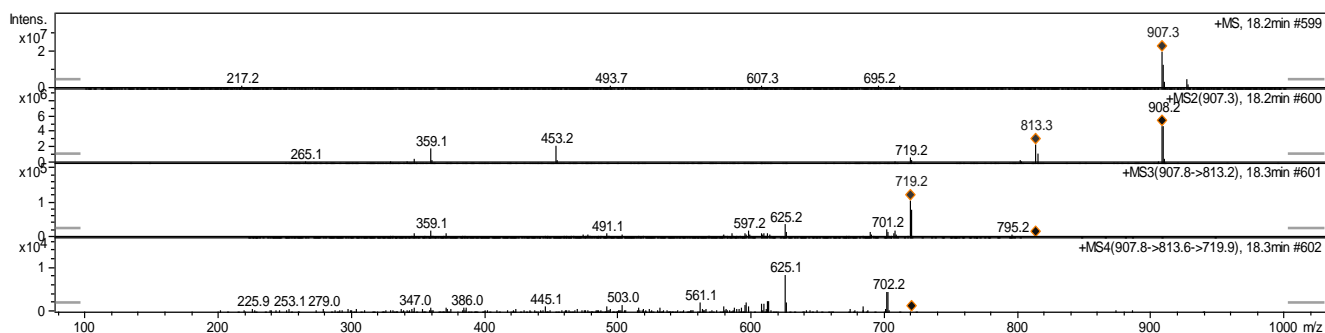


Figure 3: Mass spectra of hemsleyanol D (**3**)

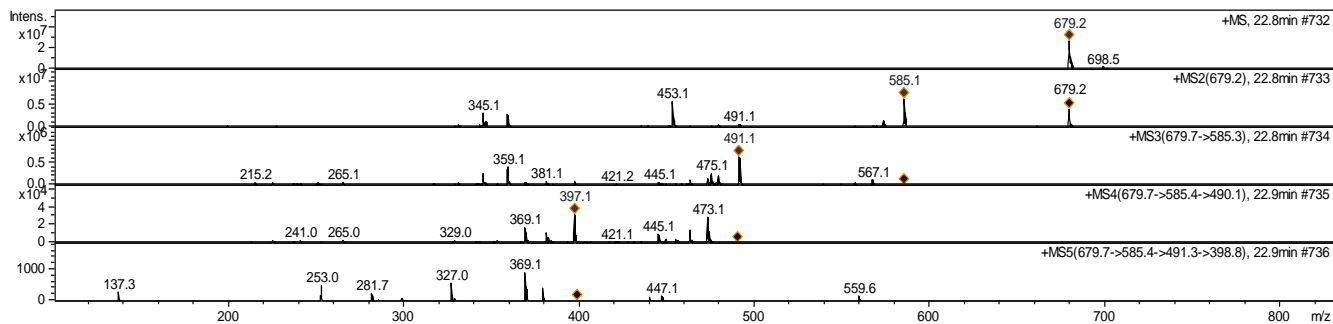
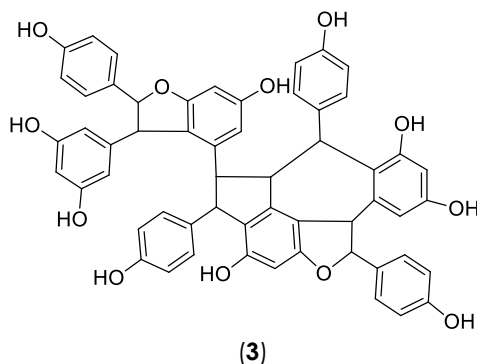
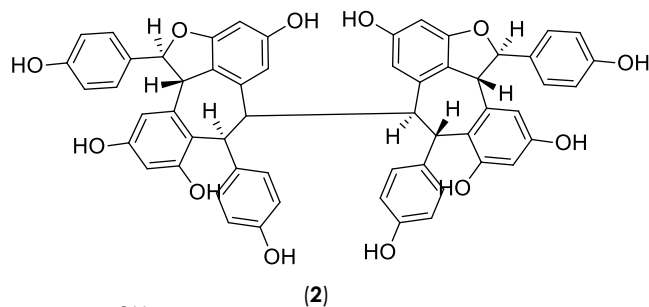
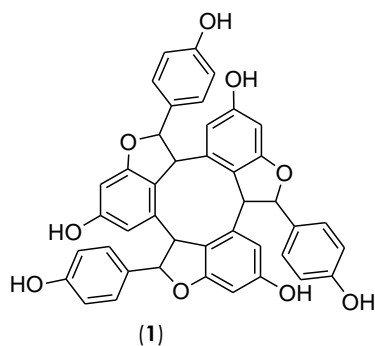


Figure 4: Mass spectra of  $\alpha$ -viniferin (**1**)



## 4.0 CONCLUSION

Identification of hopeaphenol (2) and hemsleyanol D (3) were successfully conducted through dereplication technique using liquid chromatography tandem mass spectroscopy together with a known compound but new to our collection,  $\alpha$ -viniferin (1).

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