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HPLC Profiles of Dipterocarpuscrinitus Extracts from Different Plant Organs and Geographical Locations

Dr. Nurhuda Manshoor, Abidul Hafidz Abdul Rahman

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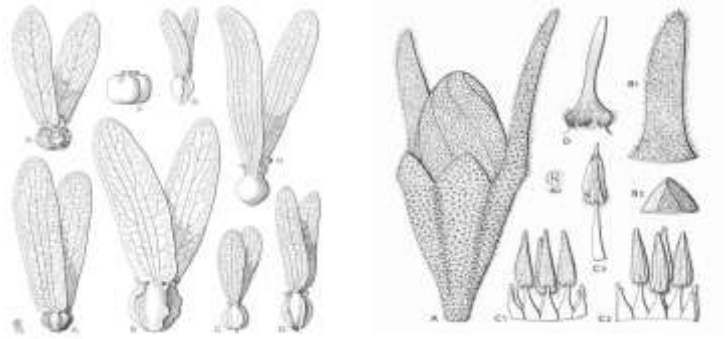
Abstract: *Dipterocarpuscrinitus* is a timber tree that belongs to Dipterocarpaceae family. Dipterocarpaceae is found to contain oligostilbenes, a polyphenolic group that have been widely studied as they show biological activities. To date, about one-fourth of all stilbene derivatives reported were isolated from Dipterocarpacae plants. Chromatographic profiles of 23 samples of *D. crinitus* extracts were recorded on UHPLC system. For all samples, the wavelengths were set at 215nm, the column temperature was 39°C, the flow-rate was 1 ml/min and the injection volume was 5 µl. The mobile phase was a gradient of acetonitrile: water (ACN: H_2O) 5:95 to 55:45 in a chromatographic run that was standardized for 15 minutes. The samples were of bark and wood from both trunk and buttress of *D. crinitus* collected from Selai, Jengka and Keniam. Analysis of all chromatograms showed that samples from the same species having similar chromatographic profiles regardless their plant parts and localities. In contract, extracts from different species show significant difference in their chromatographic patterns and composition of compounds. This study showed that the use of different HPLC techniques is an effective method to determine the differences and similarities of samples containing closely related chemical compositions.

Keywords: Dipterocarpaceae, Dipterocarpuscrinitus, Oligostilbenes, Chromatography, UHPLC.

INTRODUCTION

Dipterocarpuscrinitus is a species belongs to the Dipterocarpaceae family. It is characterized as medium-sized to large trees with thick, rounded, usually small and concave, sometimes tall and straight buttresses. *D. crinitus* is distributed in Malesia and widely spread on undulating land and low hills [1]. Figure 1 show some botanical illustration on Dipterocarpus species.



(a) **(b)**

Figure 1: (a) Fruits and (b) Flowers of Dipterocarpusspp.

Dr. Nurhuda Manshoor, Universiti Teknologi MARA, Atta-ur-Rahman Institute for Natural Product Discovery, Malaysia.
E-mail: nurhuda15@uitm.edu.my
Abidul Hafidz Abdul Rahman, Universiti Teknologi MARA, Faculty of Pharmacy, Malaysia.
E-mail: abidulhafidz95@gmail.com

Oligostilbenes are polyphenolic compounds that are synthesized as secondary metabolites in plants. They protect the plants from environmental stress. They also act as phytoalexin, which are produced in response to attacks by other organisms.The obvious challenge of isolating oligostilbenes from plants was to use open column, gravity chromatography. The nature of the complex structures of the oligostilbenes, many with the same skeleton, but having different stereochemical configuration makes their separation difficult using this method. This process is also time-consuming and requires a high volume of organic solvents. Various chromatographic techniques were used to isolate the compounds. High performance liquid chromatography (HPLC) analysis plays a major role in phytochemical analysis including identification of crude plant extracts [2].In isolation using a reverse-phase high performance liquid chromatography (HPLC), employment of non-polar stationary phase makes elution of polar compound more efficient and time-saving. For efficient isolation of compounds from a plant, a good chromatographic profile with acceptable resolution is required. Optimization of HPLC conditions and other important perspectives during method development are conducted to provide simple, precise, rapid and accurate analysis of plants.

In our previous study, we had successfully isolated oligostilbenes from *Neobalanocarpusheimii* [3,4] and *Dryobalanops* spp.[5] using HPLC. We also reported the isolation of four resveratrol oligomers, from the wood of *D. semivestitus*[6,7].In this report, we describe the chromatographicprofiles for the extracts of *D. crinitus.* The chromatograms were recorded on a UHPLC system for 23 samples of bark and wood from both trunk and buttress, collected from different locations in Malaysia, *i.e*. Selai, Jengka and Keniam.

MATERIALS AND METHODS

General Experimental Procedure

Solvent for extraction was of analytical grade, and chromatographic solvents are of HPLC grade from Fischer Scientific, Waltham, MA, USA. The ultra-pure water was purified at 18 $M\Omega.cm^{-1}$ by ELGA PURELAB® Option water purification system from Veolia Water Technologies, Paris, France. The LC-MS grade acetonitrile and water were from JT Baker, Center Valley, PA, USA.

The chromatographic system is a UHPLC by Dionex™ Ultimate® 3000 from Thermo Scientific™, Waltham, MA, USA. The system is equipped with an ultra-pressure pump, a degasser, an auto sampler and a diode array detector (DAD). The chromatographic profiles and the integrated data were recorded using Chromeleon™ Chromatography software. The separations were achieved through a Phenomenex® Luna 5 µm C18 column (150 X 4.6 mm) equipped with a guard column of similar chemistry.

Plant Materials and Sample Extraction

The plant samples of *Dipterocarpussemivestitus*was provided by Atta-ur-Rahman Institute for Natural Products Discovery (AuRIns), Universiti Teknologi MARA, Malaysia. The samples are the crushed powder from different parts of the plants, which are trunk bark (TB), trunk wood (TW), buttress bark (BB) and buttress wood (BW). The plants were collected from three different forest reserves in Malaysia, Selai, Jengka and Keniam. Samples from Selai and Keniam are duplicates whereas the one from Jengka is triplicate. The samples are numbered and labelled accordingly as shown in Table 1.

Table 1: Sample numbering and labelling according to their localities and the plant parts

Location	Sample replicate	Plant part	Label	Sample No.
Selai	*D. crinitus* 1	Trunk bark	SDC1 TB	1
		Trunk wood	SDC1 TW	2
		Buttress bark	SDC1 BB	3
		Buttress wood	SDC1 BW	4
	D. crinitus 2	Trunk bark	SDC2 TB	5
		Trunk wood	SDC2 TW	6
		Buttress bark	SDC2 BB	7
		Buttress wood	SDC2 BW	8
Jengka	*D. crinitus* 1	Trunk bark	JDC1 TB	9
		Trunk wood	JDC1 TW	10
		Buttress bark	JDC1 BB	11
		Buttress wood	JDC1 BW	12
	D. crinitus 2	Trunk bark	JDC2 TB	13
		Trunk wood	JDC2 TW	14
		Buttress wood	JDC2 BW	15
	D. crinitus 3	Trunk bark	JDC3 TB	16
		Trunk wood	JDC3 TW	17
		Buttress bark	JDC3 BB	18
		Buttress wood	JDC3 BW	19
Keniam	*D. crinitus* 1	Buttress wood	KDC1 BW	20
		Trunk wood	KDC1 TW	21
	D. crinitus 2	Buttress bark	KDC2 BB	22
		Buttress wood	KDC2 BW	23

Sample preparation

10 g of each sample was extracted using methanol by sonication for 1 hour. The samples were individually filtered through a 0.45µm polytetrafluoroethylene (PTFE) membrane into their respective HPLC vials.

Development of Chromatographic Method on UHPLC

A good chromatographic condition for each sample was achieved by adjusting the slope of the gradient elution. The slopes were systematically adjusted by gradually changing the solvent composition at the end of a chromatographic run while maintaining the initial solvent composition, and vice versa. In a preliminary analysis, the initial solvent composition was maintained at ACN:H_2O (5:95), while at the end of the chromatographic run, the solvents were adjusted as ACN:H_2O (95:5, 85:15, 75: 25, 65:35 and 55:45). The chromatographic run was 15 minutes, followed by a column flushing at 95% ACN for 5 minutes and a post-run column condition for another 5 minutes. The chromatographic profiles for all samples were analyzed individually by examining their peak resolutions.

RESULTS AND DISCUSSION

A fast gradient method of ACN:H_2O (5:95 to 95:5 for 15 minutes) at 1.0 ml/min, which covered a full range of polarity from the very low to high polarity, was chosen as anorigin of a method development. This chromatographic condition shows peaks correspond toall compounds in the sample. The gradient slope was gradually changed by adjusting the solvent composition at the end of the chromatographic-run until a base-line resolution is achieved. These adjustments, however, resulted in longer retention time for the compounds to be eluted. The gradient was again adjusted, this time by changing the solvent composition at the beginning of the chromatographic run. This adjustment caused the compounds to retain less in the column and prompt the elution time.Figure 2 shows the gradual improvements of the retention time and peak resolutions with the change of the solvent composition.

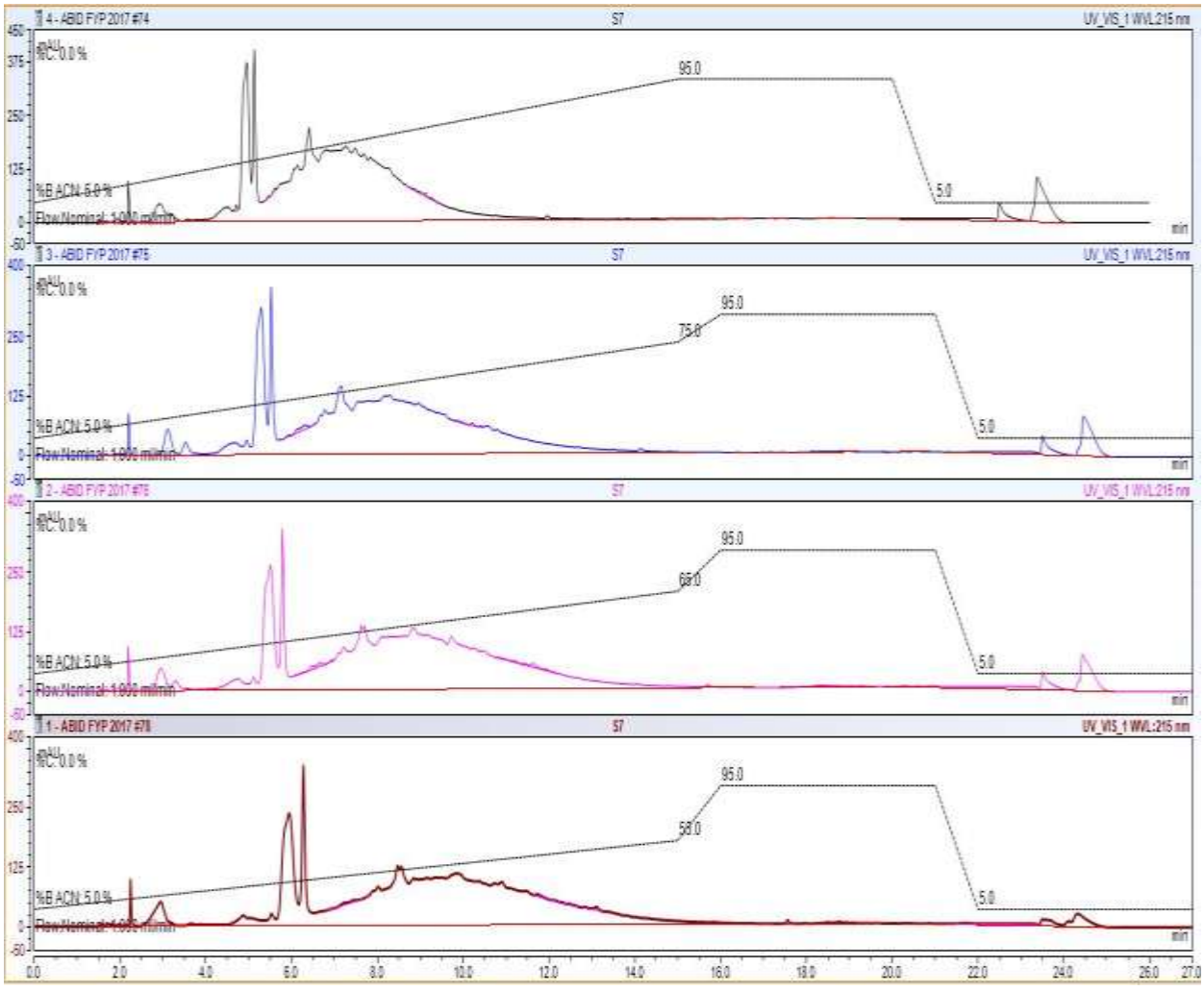


Figure 2: Chromatographic profiles of model sample in method development

The initial chromatogramshowstwo majorpeaks at a narrow range of retention time (≈1 min), followed by a hump that covers over 7 minutes of chromatographic run. The hump correspond to a group of unresolve compounds that are coeluted within a close-range of retention time. This clump could also due to the presence of mucilage or resin in the extracts. Dipterocarpaceae, as described by Ashton,[1] is resinous and contains mucilage cell, which is able to produce mucilage, a polysaccharide that exhibit many degrees of polymerization. It can be derived from various kind of monomers, linear or highly branced, different length and sizes. Due to variation of polymers that exhibit similar property, the presence of mucilage may explain the hill-like shape in the chromatogram.

Based on this chromatographic profile, further analyses were carried out by maintaining the selected solvent composition at the begining of the chromatographic run, while adjusting the end composition.The acceptable condition for this sample is a gradient of acetonitrile:water (ACN:H_2O) 5:95 to 55:45 in 15 minutes. This solvent composition and gradient slope is used as a standardise condition in recording the chromatographic profiles of all *D. crinitus* samples.

Apart from some minor differences, analysis of all chromatograms showed that samples from *D. crinitus* having similar chromatographic profiles regardless their plant parts and localities. The following discussion is based the differences in chromatographic profiles of samples from different plant parts within their localities.

Crinitus from Selai

*D. crinitus*collected in Selai represented by two replicates. For each replicate, the sample was collected from trunk bark (TB), trunk wood (TW), buttress bark (BB) and buttress wood (BW). Table 2 (excerpt from table 1) shows the eight samples of *D. crinitus* from Selai.

Table 2: Numbers and labels for samples from Selai

Location	Sample replicate	Plant part	Label	Sample No.
Selai	*D. crinitus* 1	Trunk bark	SDC1 TB	1
		Trunk wood	SDC1 TW	2
		Buttress bark	SDC1 BB	3
		Buttress wood	SDC1 BW	4
	D. crinitus 2	Trunk bark	SDC2 TB	5
		Trunk wood	SDC2 TW	6
		Buttress bark	SDC2 BB	7
		Buttress wood	SDC2 BW	8

A close examination of the two replicates (Figure 3 and 4) shows very high similarities in both replicates for the same plant parts. The differences only can be seen from different plant parts in the same sample replicate.

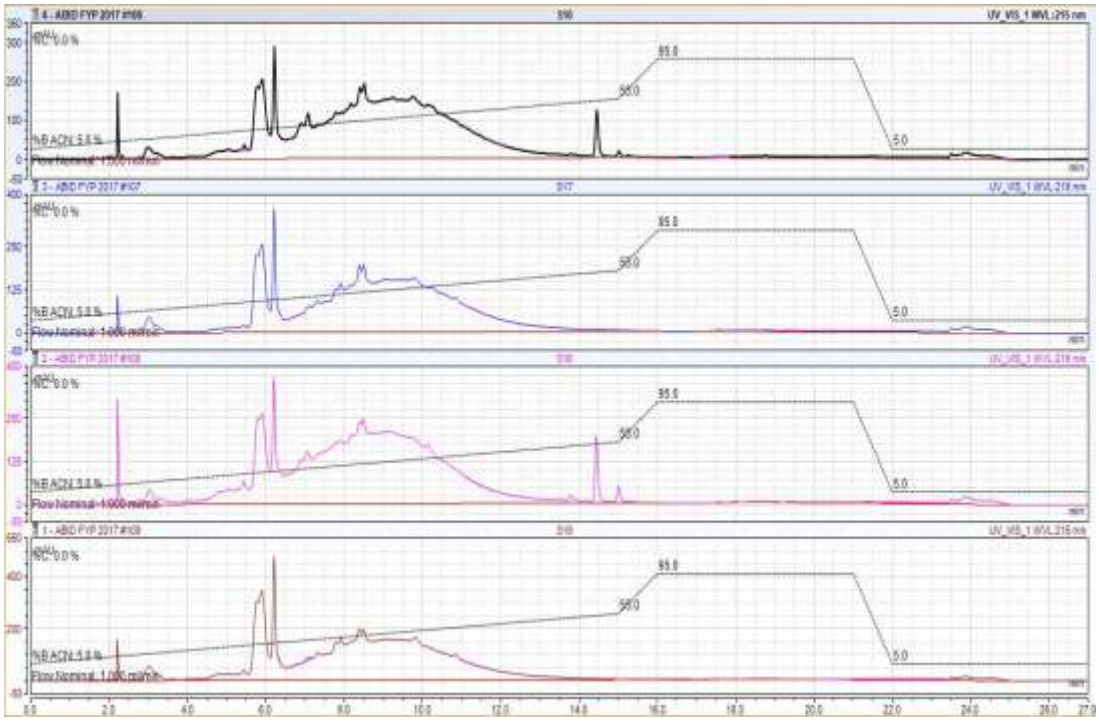


Figure 3: HPLC profiles of (from top) SCD1 TB, SDC1 TW, SDC1 BB and SDC1 BW

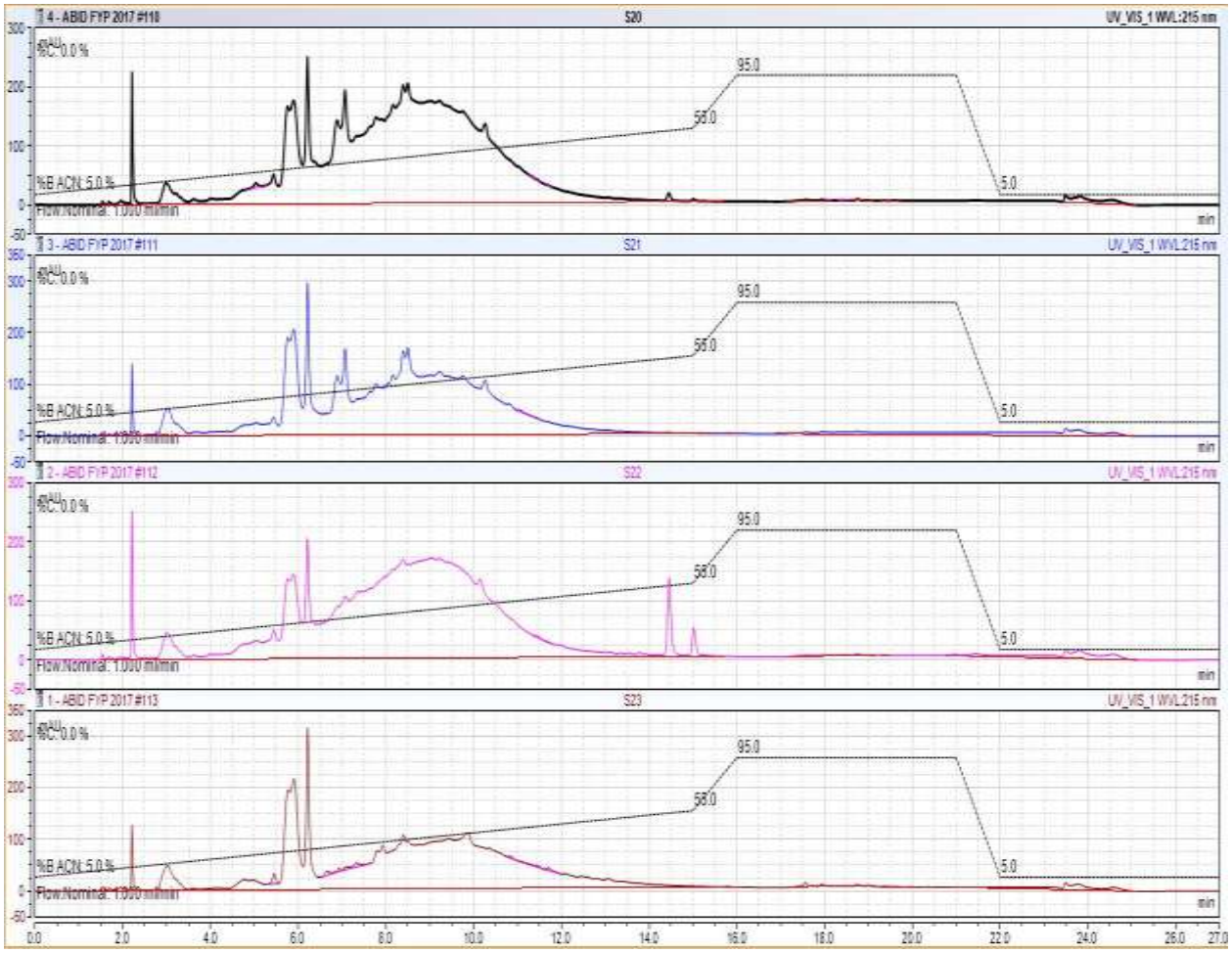


Figure 4: HPLC profiles of (from top) SDC2 TB, SDC2 TW, SDC2 BB and SDC2 BW

Profile of samples from trunk (both bark and wood) shows almost complete similarity, both in shape and number of peaks. Sample from buttress bark shows two additional peaks at the end of the chromatographic run, which are not observed in the other samples. A comparison between samples from bark and wood (both trunk and buttress) shows bigger hump on the bark profile as compared to wood. This is due to richer component of chemicals in the bark than the wood. Considering that the bark is placed at the outer layer of a tree trunk, it is more reasonable for the bark to produce more secondary metabolites to protect the tree from external predator.

Crinitus from Jengka

*D. crinitus*collected in Jengka represented by three replicates. For each replicate, in exception of replicate 2, the sample was collected from trunk bark (TB), trunk wood (TW), buttress bark (BB) and buttress wood (BW). Replicate 2 consists of all the sample except for buttress bark (BB). Table 3 (excerpt from table 1) shows the eight samples of *D. crinitus* from Jengka.

Table 3: Numbers and labels for samples from Jengka

Location	Sample replicate	Plant part	Label	Sample No.
Jengka	*D. crinitus* 1	Trunk bark	JDC1 TB	9
		Trunk wood	JDC1 TW	10
		Buttress bark	JDC1 BB	11
		Buttress wood	JDC1 BW	12
	D. crinitus 2	Trunk bark	JDC2 TB	13
		Trunk wood	JDC2 TW	14
		Buttress wood	JDC2 BW	15
	D. crinitus 3	Trunk bark	JDC3 TB	16
		Trunk wood	JDC3 TW	17
		Buttress bark	JDC3 BB	18
		Buttress wood	JDC3 BW	19

The chromatographic profiles for all replicates are shown in figures 5, 6 and 7. A quick look at all the chromatogram shows similar characteristic, with two significant peaks at the begining of the profiles, follows by a hump for the rest of the chromatographic run.A close examination however shows a significant different in the intensity of the hump for sample from trunk wood of replicate 2 (JDC2 TW). The profile also show two additional peaks at the end of the chromatographic run, which could not be seen in other similar samples. This observation cannot be explained by exermining the crude profile, unless further investigation is initiated.

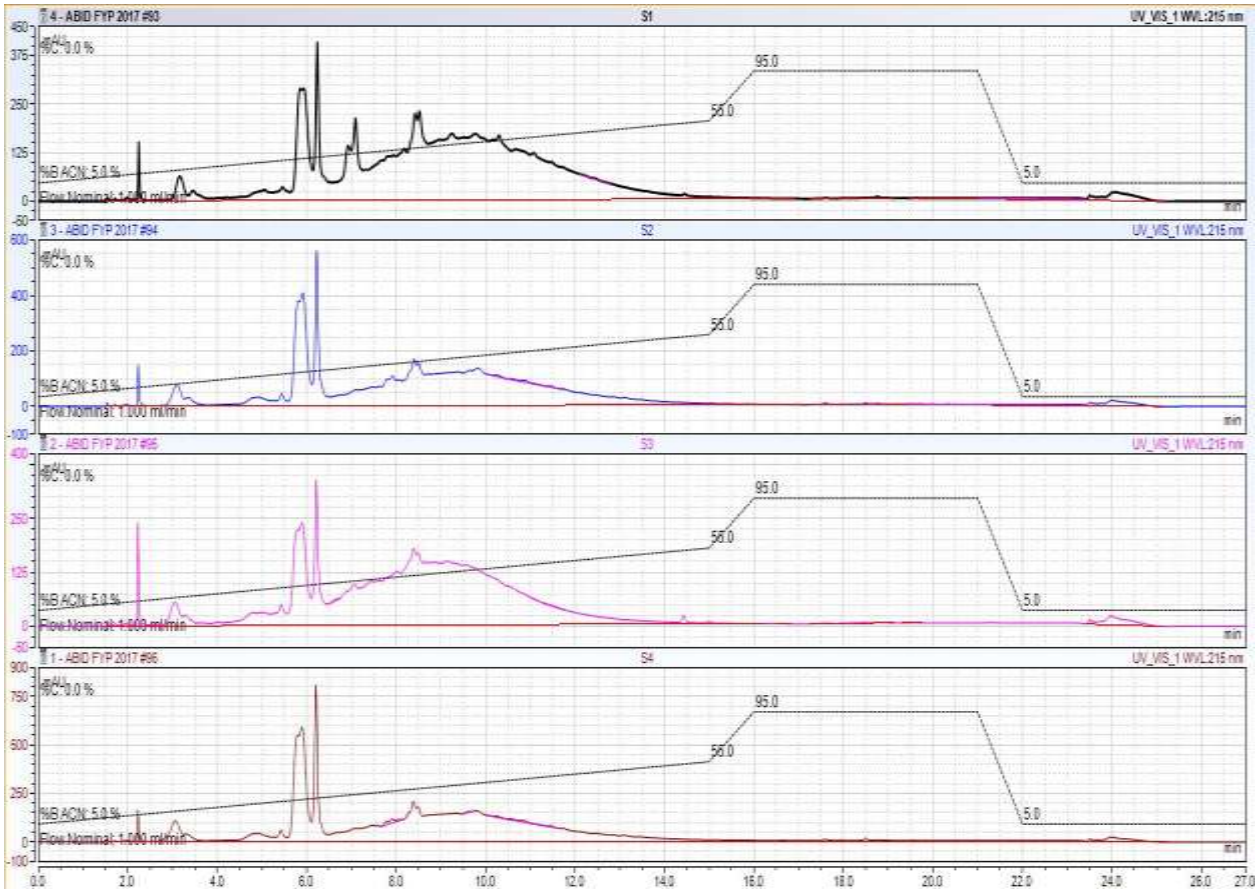


Figure 5: HPLC profiles of (from top) JDC1 TB, JDC1 TW, JDC1 BB and JDC1 BW

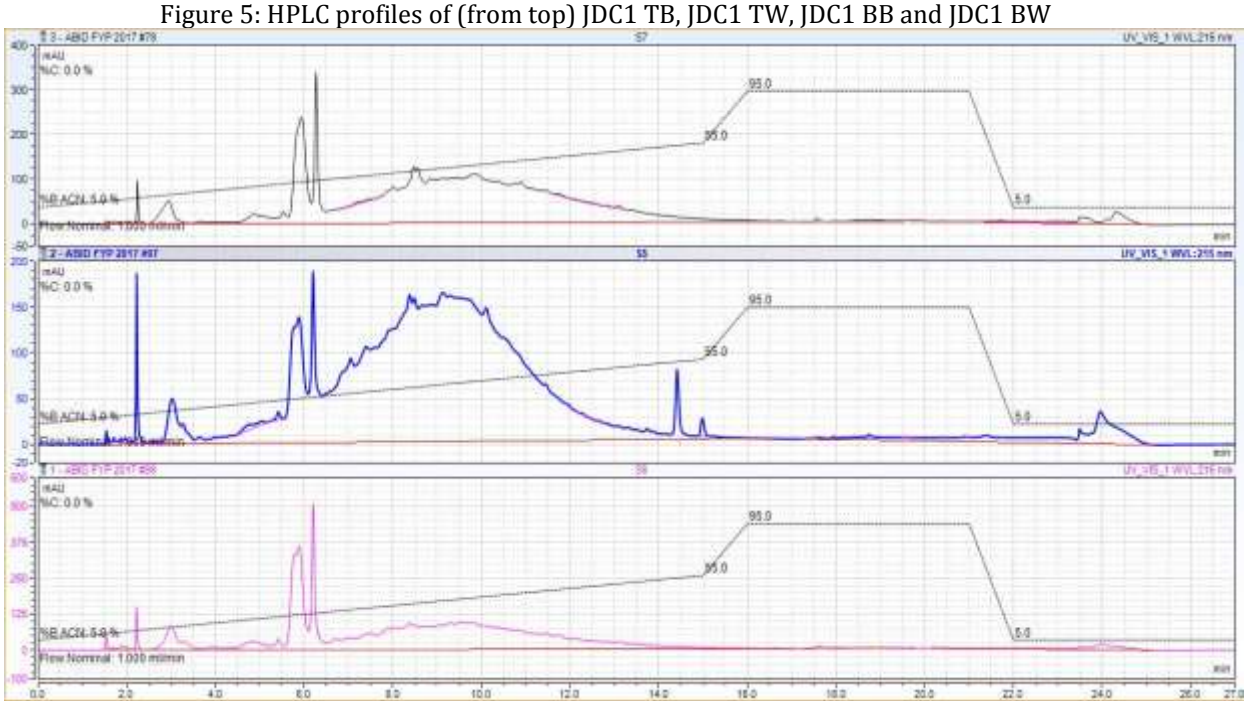


Figure 6: HPLC profiles of (from top) JDC2 TB, JDC2 TW and JDC2 BW

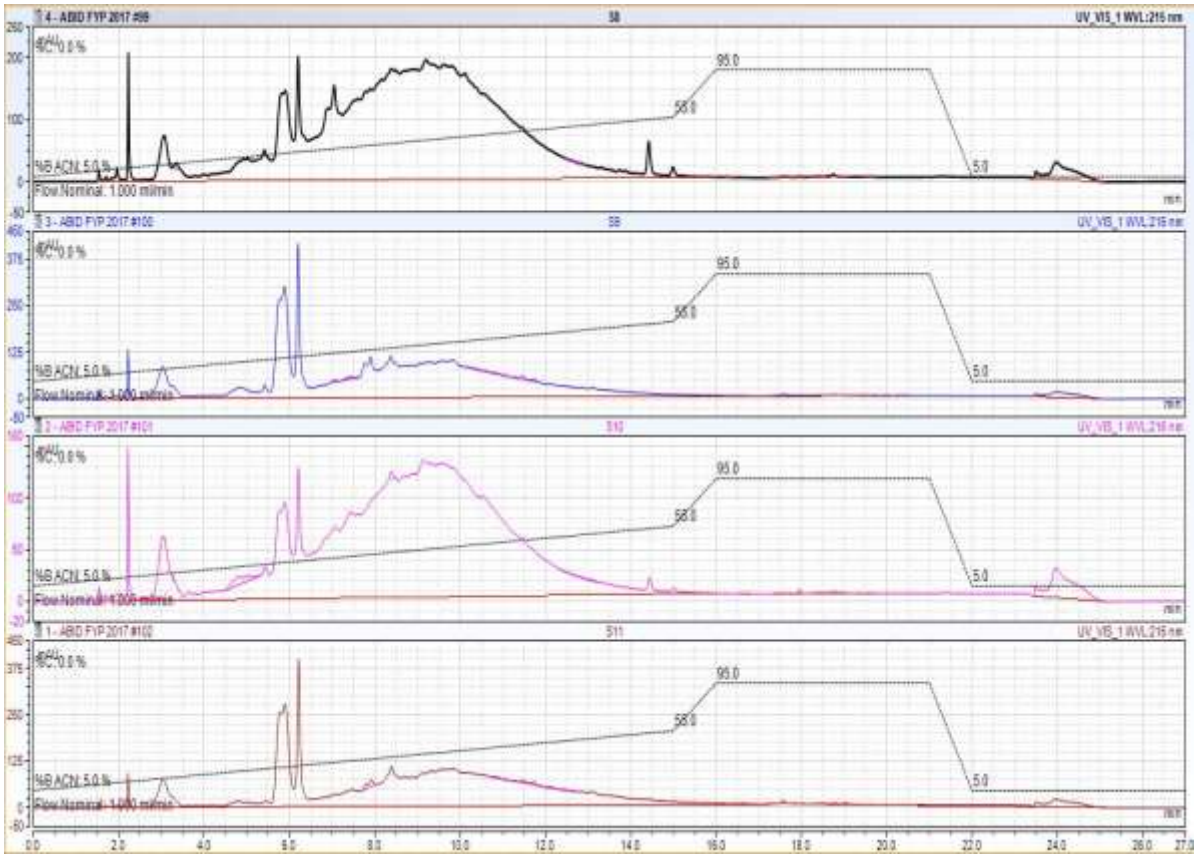


Figure 7: HPLC profiles of (from top) JDC3 TB, JDC3 TW, JDC3 BB and JDC3 BW

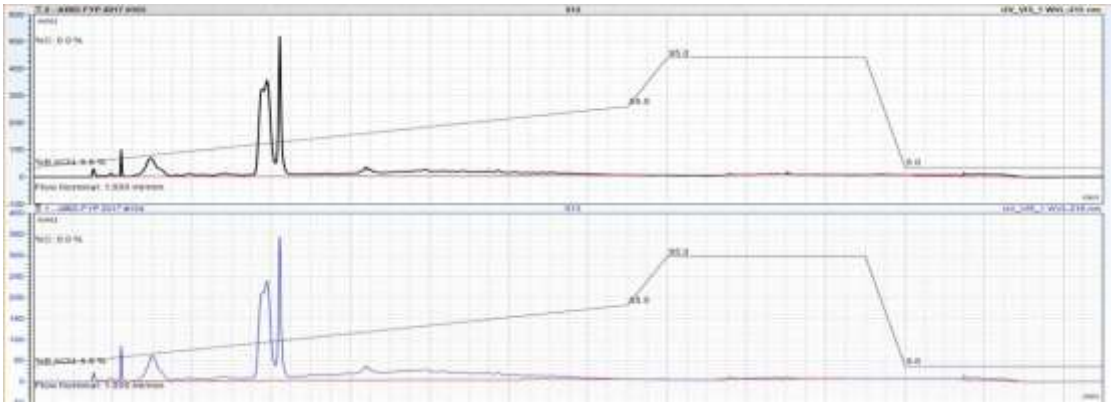
Crinitus from Keniam

*D. crinitus*collected in Keniam represented by two replicates and each replicate only represented by two samples. Replicate 1 is represented by buttress wood (BW) and trunk wood (TW) whereas replicate 2 is by buttress bark (BB) and buttress wood (BW). Table 4 (excerpt from table 1) shows the eight samples of *D. crinitus* from Keniam.

Table 4: Numbers and labels for samples from Keniam

Location	Sample replicate	Plant part	Label	Sample No.
Keniam	*D. crinitus* 1	Buttress wood	KDC1 BW	20
		Trunk wood	KDC1 TW	21
	D. crinitus 2	Buttress bark	KDC2 BB	22
		Buttress wood	KDC2 BW	23

The limitation of the variety of samples restricts our ability to compare and contrast the profiles. The available chromatogram, however shows an interesting profile, which is of the buttress bark from replicate 2. While the other chromatograms are similar, this profile shows bigger hump and two additional peaks at the end of the chromatographic run. This demontrate the richness of secondary metabolites in bark as compared to wood, as can be seen in three other chromatogram, which samples are from woods.



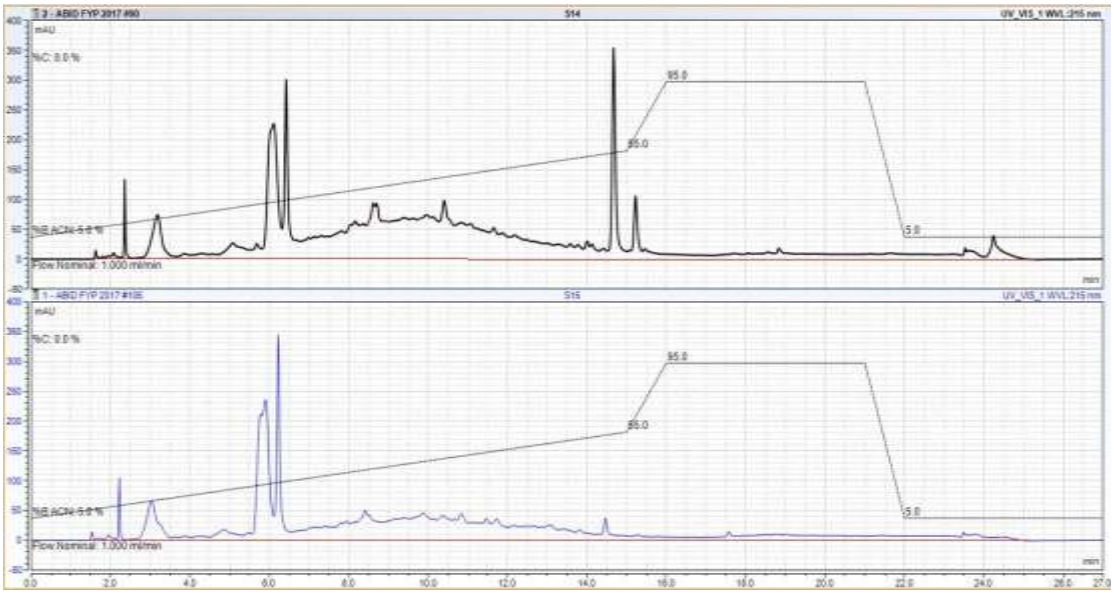


Figure 8: HPLC profiles of (from top) KDC1 BW, KDC1 TW, KDC2 BB and KDC2 BW

CONCLUSIONS

This study showed that the use of different HPLC techniques is an effective method to determine the differences and similarities of samples containing closely related chemical compositions. The ability of the systems to develop a suitable chromatographic condition prior to the actual recording of chromatographic profiles ensures good resolution for better observation.

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