# LC-MS/MS APPROACHES TO CHARACTERIZE OLIGOSTILBENES DIRECTLY FROM COMPLEX CRUDE EXTRACT MIXTURES

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**KELULUSAN SKIM GERAN PENYELIDIKAN FRGS FASA 01/2010**

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| Tajuk Projek | : | LC-MS/MS Approaches To Characterize Oligostilbenes Directly From Complex Crude Extract Mixtures |
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Dengan hormatnya perkara di atas adalah dirujuk.

Sukacita dimaklumkan pihak Kementerian Pengajian Tinggi melalui surat JPT.S(BPKI) 2000/011/010 Jilid. 2 (19) telah meluluskan cadangan penyelidikan Prof/Prof. Madya/Dr./Tuan/Puan untuk di biayai di bawah Skim Geran Penyelidikan Fundamental (FRGS) Fasa 1/2010.

Bagi pihak Universiti kami mengucapkan tahniah kepada Prof/Prof. Madya/Dr./Tuan/Puan kerana kejayaan ini dan seterusnya diharapkan berjaya menyiapkan projek ini dengan cemerlang.

Untuk tujuan mengemas-ini, pihak Prof/Prof. Madya/Dr./Tuan/Puan adalah di minta untuk menyusun perancangan semula bajet yang baru seperti yang diluluskan. Sila lihat lampiran bagi tatacara tambahan untuk pengurusan projek.

Sekian, harap maklum.

**"SELAMAT MENJALANKAN PENYELIDIKAN DENGAN JAYANYA"**

Yang benar

**MUSTAFAR KAMAL HAMZAH**
Ketua INFOREC
Merangkap Ketua Penyelidikan (Sains & Teknologi)

# 5. Report

## 5.1 Proposed Executive Summary

LC-MS analysis has become a valuable tool in drug discovery studies from natural products. The technique was used in various studies, such as discrimination of known estrogenic compounds in pomegranate, detection of high molecular weight proanthocyanidins, or differentiation of isomeric of glycosidic flavonoids. Some 400 oligostilbenoids are already known. There are found abundantly in local dipterocarp timber trees, mostly in the form of resveratrol dimers, trimers and tetramers. This project aims at dereplicating dipterocarp extracts. For this purpose, we need to be able to discriminate between compounds from long series of isomers, including closely related diastereoisomers. A standardized LC-MS gradient system will be used to quickly identify known compounds and for initial characterization of unknowns. The detection of chemical constituents from a crude extract of Neobalanocarpus heimii will be performed on a triple quadrupole and/or ion trap liquid chromatography-mass spectrometry (LC-MS) with an electrospray-ionization (ESI) interface. For the efficient use of this technique, an efficient chromatographic analysis will be achieved on a UPLC system similar to the LC component on the LC-MS system. Parameters to be used to generate informative MS/MS spectra will be optimized for the recording of fragmentation pattern in standardized conditions. The collision-induces dissociation (CID) MS/MS spectra of the compounds in the crude plant extract will be recorded and the data will be compared with those of previously obtained. The isolation of chemical constituents will be accomplished on a fully-automated semi-preparative HPLC system, by the same chromatographic condition as in the LC-MS. Their structures and configurations will be established by spectroscopic methods. The identified compounds will be compared to those stored in the library to further confirm the identification of compounds in the crude extract. We expect positive identification of known compounds in mixtures solely from their fragmentation pattern, independently from retention time or other data, thus saving very significant amount of time and resources.

## 5.2 Enhanced Executive Summary

This work was undertaken in order to check on the possibility of dereplication closely related compounds in a complex mixtures by means of LC-MS/MS. In order to fulfil the objectives of this work, a polyphenolic extract from *Neobalanocarpus heimii* known to be rich in oligostilbenes of various degrees of condensation was used as test material. Seventeen oligostilbenes were isolated from this extract on a fully automated semi-preparative HPLC system. Their structures and configurations were established by spectroscopic methods. All isolated compounds then subjected to an LC-MS/MS to study their fragmentation patterns and the spectra were kept as reference data. The experiments were performed on a triple quadrupole liquid chromatography-mass spectrometry (LC-MS) with electrospray-ionization (ESI) interface in positive mode. MS/MS spectra of each pure compound were recorded by direct infusion in identical conditions and their product ion spectra were analysed. Some subtle yet significant differences were observed between the spectra of oligostilbenes from the various diastereoisomeric series. In order to demonstrate the potential of this approach, the polyphenolic crude mixture was analysed with the LC-MS/MS system and the MS/MS spectra extracted for each peak of interest. The fragmentation patterns were compared with those of anticipated pure compounds that were previously recorded. All compounds were successfully identified. It is therefore believed that the LC-$MS^n$ potential for dereplication of structurally similar compounds in a crude mixture was thus firmly established. The dereplication technique is still a work in progress. The potential of $MS^n$ as a future dereplication tool is positively recognized. The oligostilbenes from cengal were shown to be a good model to test the idea. When the above-mentioned improvements would be feasible, the dereplication by of $MS^n$ will be one of the most reliable dereplication techniques as it would be fast, accurate and hopefully portable.

## 5.3 Introduction

This project presents a study on the characterization of compounds in crude plant extracts by a dereplication process using LC-MS/MS. The model compounds chosen for this study were oligostilbenes, extracted from dipterocarpaceous plant *Neobalanocarpus heimii*.

The Dictionary of Natural Product (2009) includes 170 000 natural compounds, out of which some 140 000 are fully characterized. With the rising number of characterized compounds, the possibility of re-isolating known compounds from natural resources has increased. The complexity of naturally occurring compounds requires high-end spectrometric techniques, highly skilled operator and time-consuming elucidation works.

A dereplication technique is very useful to avoid spending excessive time on the re-isolation and re-identification of known natural products. Dereplication refers to a process of testing samples of mixtures to recognize and eliminate from consideration those substances already studied. It is a stage subsequent to the preliminary screening in the process of discovery of new substances in mixtures of natural products.

With the development of fully automated dereplication technology, the tedious isolation of known compounds could be avoided, allowing to focus on the targeted novel constituents. As a modern screening technology, a dereplication method is expected to be fast, sensitive, reproducible, robust and portable. Further, they must include effective data processing and information retrieval systems to permit comparison with internal and external sources of information on known compounds.

The main objective of the study was to develop a technique to distinguish chemical constituents from a compound-mixture. This project aims to show that the LC-MS/MS method efficiently identifies known compounds. Using the technique, samples can be characterized and compounds identified at a very low concentration, in semi-purified broths or in complex mixtures.

Mass data including MS/MS spectra for ion fragmentation are additional data to help in elucidation. The fragmentation pathways are proposed based on the MS/MS data to suggest the preferred mechanism of the compounds. Many of the ion structures presented in the fragmentation schemes were necessarily hypothetical.