Application Article
NMR in Traditional Medicinal Plant Study

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Introduction

- *Eurycoma longifolia* (Tongkat Ali, TA) (Fig 1, 2), native to Malaysia and South-East Asia, have been traditionally used as a remedy to boost male sexual desire, libido, energy and fertility[1]. Among the different types of extract, water extract is the most common type of TA extract because consumers usually drink water extracts.

  The study is carried out to look for metabolic discrimination among TA roots from different geographical terrains, especially with respect to the biologically active quassinoids[2]. The analysis results might be useful in that it could suggest the geographical location containing TA plants rich in quassinoids (Fig 4). Quassinoids are the indigenous metabolites of the plants from the Simaroubaceae family and have been proven to be responsible for many of the therapeutic properties of the plant, especially the enhancement of male fertility. NMR-based metabolomics approach is conducted because of its high reproducibility and simple sample preparation.

  Recent advances in NMR spectroscopy, particularly the development of new electronic console, superconducting magnet with external disturbance suppression, better performance probes including the cryogenic-cooled probes and advance data analysis software packages are improving the current perception of low speed and poor sensitivity of NMR analysis. NMR analysis are not limited to single molecule anymore. For the past decade, NMR has been recognized as a powerful technique for discerning the chemical properties of complex mixtures. It has been widely applied to study natural extracts, foods and biological fluids which contain of few hundreds of chemical components. These mixture spectra dereplication can be accomplished by the assistance of reference compounds library or so-called spectra database which have been deposited in either electronic-digital or static-printed form. In fact, this utilizes one of the key advantages of NMR that is highly reproducibility. Because it involves simple sample preparation, NMR provides comprehensive information regarding the chemical components directly from complex mixtures in a fast and simple way. Furthermore, high dynamic range of NMR data allows simultaneous quantification of both, minute and predominant constituents. Universal detection by NMR spectroscopy give it advantages over other detection techniques such as UV, ELSD and MS. Owing to signal intensity is directly proportional to the number of nuclei that give rise to a specific resonance, the signal response is unique to structure properties and not to physical properties. Therefore, any calibrant or certified reference material in one concentration can be employed to quantify multiple components present in mixture simultaneously. This is powerful

approach because obtaining structurally identical standard for quantification calibration demonstrates common challenge to most natural product chemists.

Result and Discussion

Plant Study[2]

Detailed of *E. longifolia* extraction and NMR samples preparation are indicated in Fig 5 and Fig 6, respectively. TA root aqueous extracts from Provinces Perak (n = 30), Selangor, Kedah, Terengganu (n = 5 for each) (Fig 3) were checked for metabolic discrimination via a combined ^{1}H-NMR spectroscopy and Orthogonal Partial Least-Squares Discriminant Analysis (OPLS-DA) as chemometric data analysis technique. Chemometric model (Fig 7) enabled a clear separation of the samples based on their origin and, consequently, choline, lactic acid, succinic acid, eurycomanol, and eurycomanol-2-O-β-D-glucopyranoside were detected as the potential discriminatory metabolites. Terengganu and Perak samples contained higher amounts of eurycomanol and eurycomanol-2-O-β-D-glycopyranoside, respectively.

Subsequently, ^{1}H-NMR spectra of TA root aqueous extracts from Perak were used as plant reference standard for identification of primary metabolites and the quassinoids (Fig 6, 7 and 8). In addition, quantitative NMR approach was also employed towards measuring quassinoid levels in TA roots from different states in Malaysia.

Figures and Tables

Fig 1 *Eurycoma longifolia* (Tongkat Ali)

Fig 2 Root of *E. longifolia*

Fig 3. Location of Provinces in Peninsular Malaysia

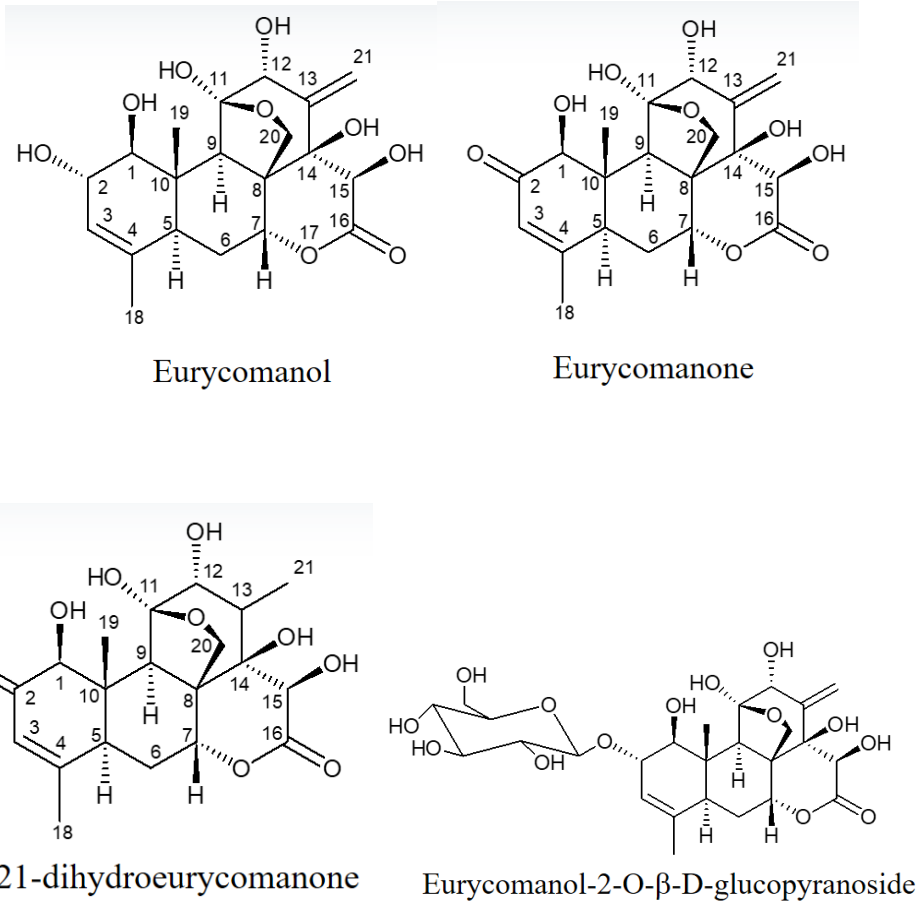

Fig 4. Key secondary metabolites from *Eurycoma longifolia*

Fig 5. Extraction procedures

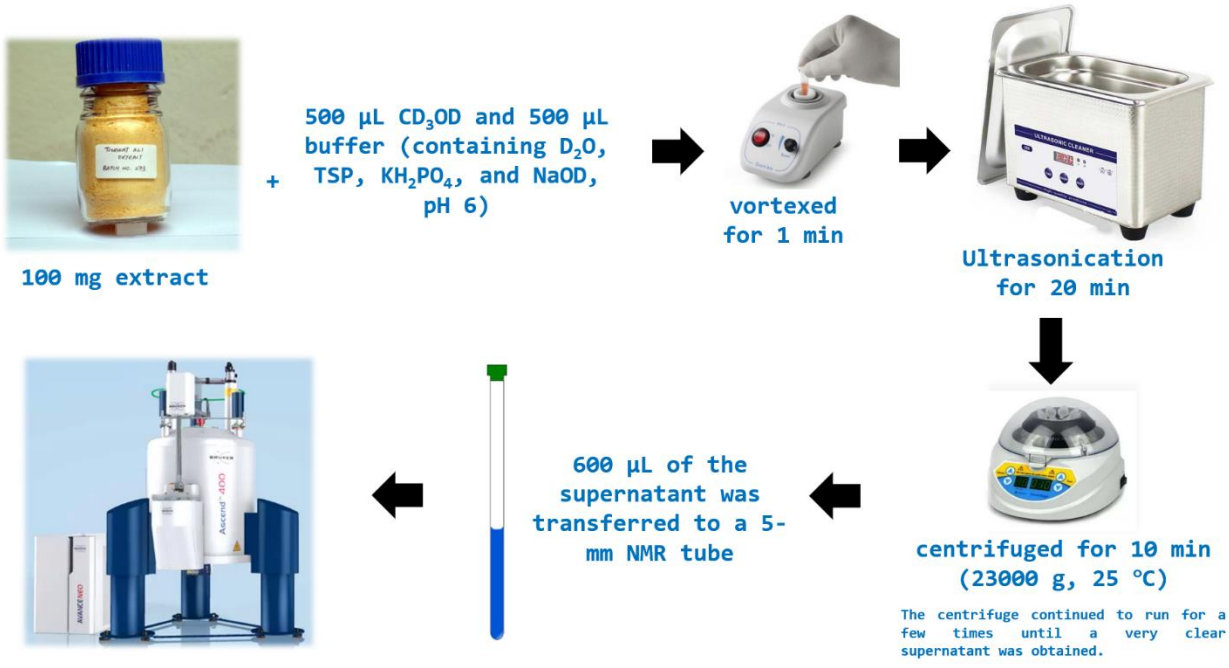

Fig 6. NMR Sample Preparation

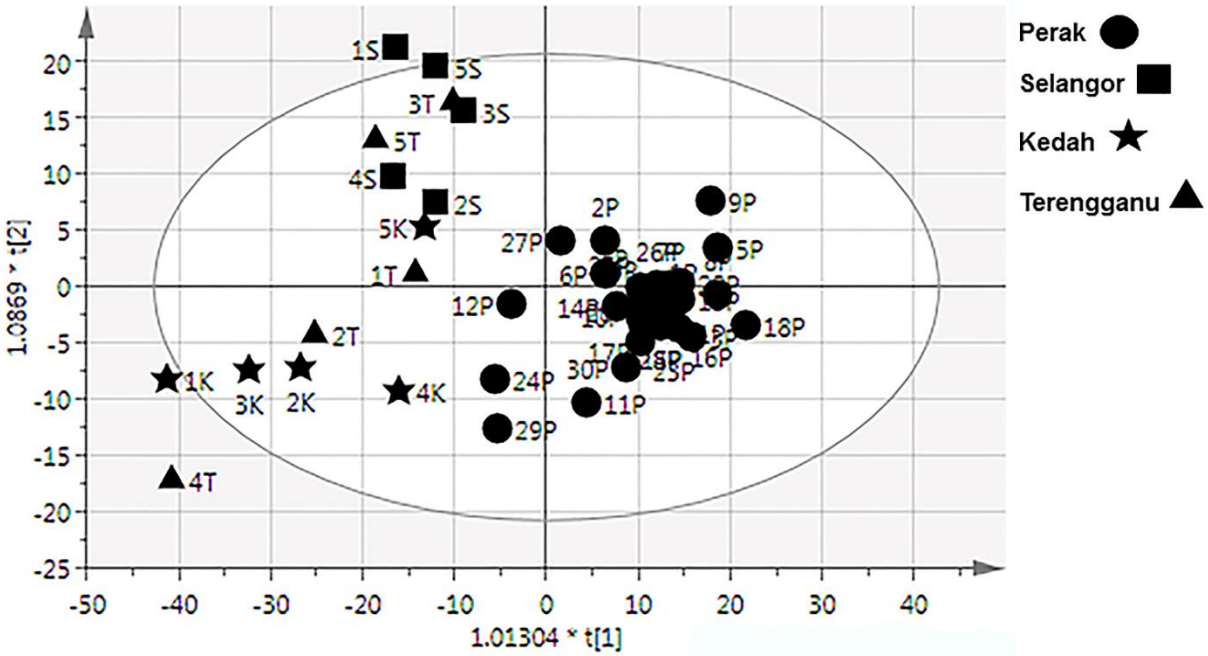

Fig 7. Statistical comparison of *E. longifolia* aqueous extracts from different regions (OPLS-DA Plot of aq extracts) Score plots of OPLS-DA for 45 ^{1}H-NMR profiles of *E. longifolia* root aqueous extracts.
Circles are for the samples from Perak, squares for the samples from Selangor, stars for the samples from Kedah, and triangles for the samples from Terengganu.
The ellipse represents the 95% confidence interval of the model. Acceptable values of goodness of fit ($R^2X = 0.606$) and goodness of prediction ($Q^2 = 0.423$) were obtained from the model

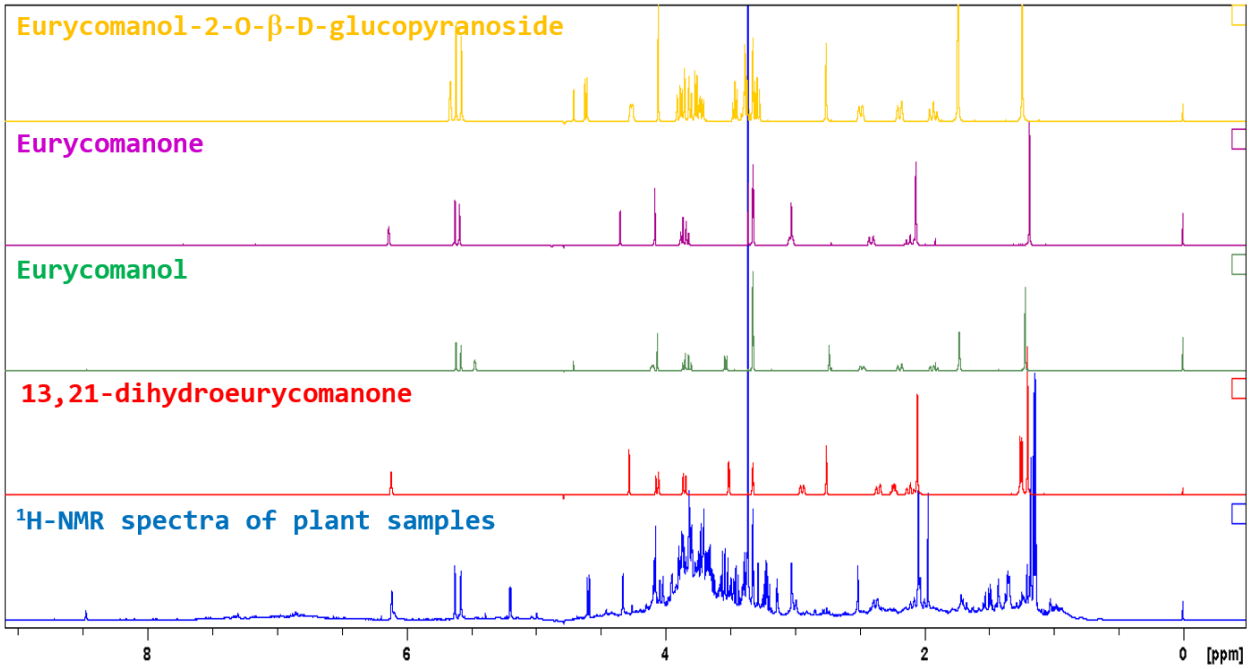

Fig 8. Overlay display of ^{1}H NMR spectra of **Eurycomanol-2-O-β-D-glucopyranoside (orange), Eurycomanone (purple), Eurycomanol (green), 13,21-dihydroeurycomanone (red) and Aqueous TA Extract (blue)**

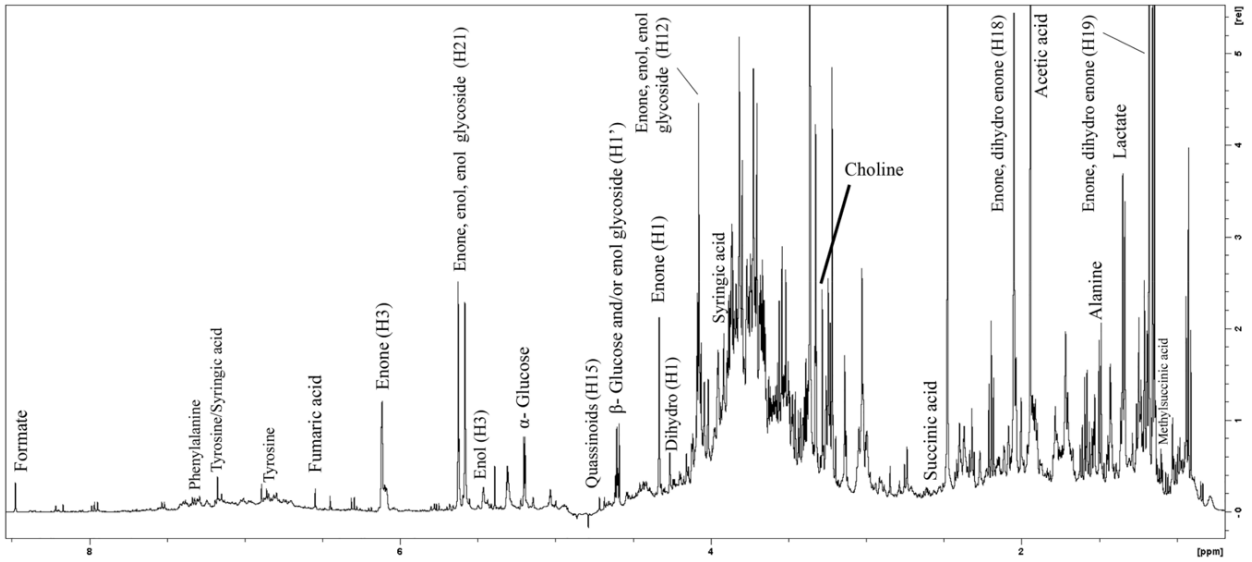

Fig 9. The representative ^{1}H-NMR spectrum of *E. longifolia* root aqueous extract from Perak, together with the identified metabolites
Enone: eurycomanone; **enol:** eurycomanol, **dihydro**: 13,21-dihydroeurycomanone
and **enol glycoside:** eurycomanol-2-*O*-*β*-D-glucopyranoside

References:

1. Forough Ebrahimi, Baharudin Ibrahim, **Chin-Hoe Teh**, Vikneswaran Murugaiyah & Kit-Lam Chan (2017). NMR-based plasma metabolomic discrimination for male fertility assessment of rats treated with *Eurycoma longifolia* extracts. ***Systems Biology in Reproductive Medicine 63 (3) 179–191***.
2. Forough Ebrahimi, Baharudin Ibrahim, **Chin-Hoe Teh**, Vikneswaran Murugaiyah and Kit-Lam Chan (2017). ^{1}H NMR-based discriminatory analysis of *Eurycoma longifolia* from different locations and establishing a profile for primary metabolites identification and quassinoids quantification. ***Planta Medica,* 83:172-182**.