



Yesterday's traditional wisdom
Tomorrow's innovative health solutions

**HPTLC: Simple and Effective Tool to Detect
Adulterated Mixtures of *Eurycoma longifolia*
Root**

7 July 2011

- **At a Glance**
- Global Brands
- Clinical Research



- Incorporated in 2007 as a government link corporation
- Involves in research & development, extraction, manufacturing and marketing of identified biological active compounds from herbs and plants for the development of high-value herbal products.
- Products includes herbal standardized extracts; dietary supplements; personal care; ingredients for nutraceuticals; functional foods and cosmeceuticals; with on-going research to develop herbal medicines and botanical drugs.
- Develops high-value herbal products based from Malaysian bio-resources and 'Ramuan' health tradition through modern science.

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Global Brands

HERBAL SUPPLEMENTS & COSMETICS

- Malaysia – NuPrep, Nu Femme, Acnaed, Scalpro
- USA – Tenaga, Asmara, Super Chews, Ramuan Health
- Canada
- Hong Kong – Tenaga XX



PROPRIETARY STANDARDIZED EXTRACTS

- Physta
- Phyllanthus
- AVCO



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CLINICAL RESEARCH ON *Eurycoma Longifolia* Water Soluble Extract

• EFFICACY

Sexual Intercourse Attempts (Satisfaction)	Outcome	P-value
Sexual Experience and Erection	Significantly higher than placebo	0.001
Hardness of Erection	Significantly higher than placebo	0.028
Erection Hardness Scale	Outcome	P-value
Sexual Performance and Erection	Significantly harder at 12 weeks	0.012
Aging Males Symptoms Score (AMS)	Outcome	P-value
Health Related QOL (Dissatisfaction)	Significantly lower than placebo	0.037
Sexual Health Inventory for Men (SHIM)	Outcome	P-value
Overall emotional and physical wellbeing	Significantly higher for active group by 12 weeks	0.003

- Improved Sexual Performance
- Improved Erectile Hardness
- Improvement in AMS
- Improved Emotional and Physical Wellbeing

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CLINICAL RESEARCH ON *Eurycoma Longifolia*
Water Soluble Extract

- **SAFETY PROFILE**

- Comparison at Baseline and After Treatment
- Variables
 - Complete blood count
 - Comprehensive Metabolic Panel

- **Summary**

- Clinically proven with positive effects on sexual performance and well being in men with mild erectile dysfunction.
- Water soluble extract of *Eurycoma longifolia* roots are well tolerated with excellent safety profiles.

Eurycoma longifolia

- **At A Glance**
- Challenge
- Objectives
- Integrated Approach
 - FT-NIR
 - HPLC-DAD
 - Microscopy
 - HPTLC



- *Eurycoma longifolia* Jack (Family: Simaroubaceae) is a traditional Malaysian herb originated from South East Asia.
- Also popularly known as ‘Tongkat Ali’ or ‘Malaysian Ginseng’, which is used as energy booster, enhancing male libido and improving male fertility.
- Traditionally used for its antimalaria, aphrodisiac, anti-diabetic, antimicrobial and anti-pyretic activities, proven scientifically.
- Rich in alkaloids, bitter principles, quassinoids and saponins (e.g. Eurycomaoside, Eurycolactone, Eurycomalactone, Eurycomanone and other Pasakbumin).

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- Medical benefits are found primarily in the root
- Authenticated supply for commercial extraction becomes a challenge with unscrupulous mixing of other plant parts along with the root.

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- To develop a simple and effective tool to screen and isolate adulterated supply of incoming raw materials.
- To develop method with high sensitivity to detect a very low amount of adulterant.

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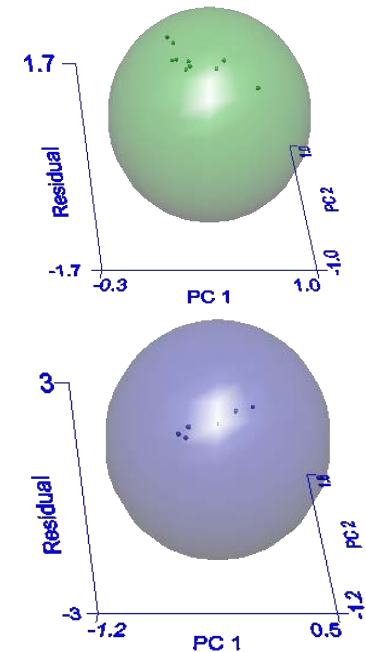
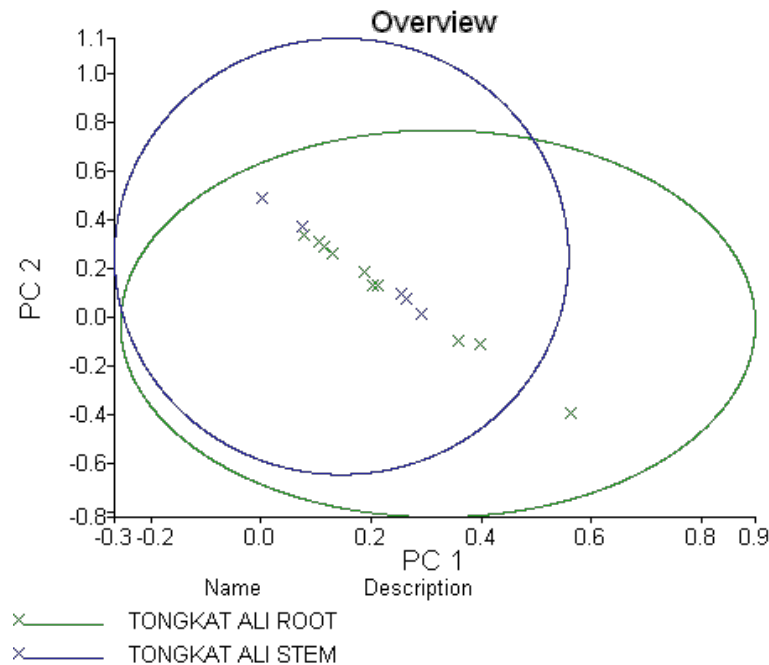
- FT-NIR
 - Near Infrared – molecular vibrations
 - Soft Independent Modeling of Class Analogy (SIMCA)
- HPLC-DAD
 - Chromatographic separation
 - Wavelength: UV to Visible
- Optical Microscopy
 - Plant anatomy and microstructure
- HPTLC
 - Planar chromatography / separation
 - Wavelength: UV to Visible
 - Post Chromatographic Derivatization

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- SIMCA
 - Root and stem cluster overlapped leads to inconsistent result
 - ineffective to discriminate between root and stem

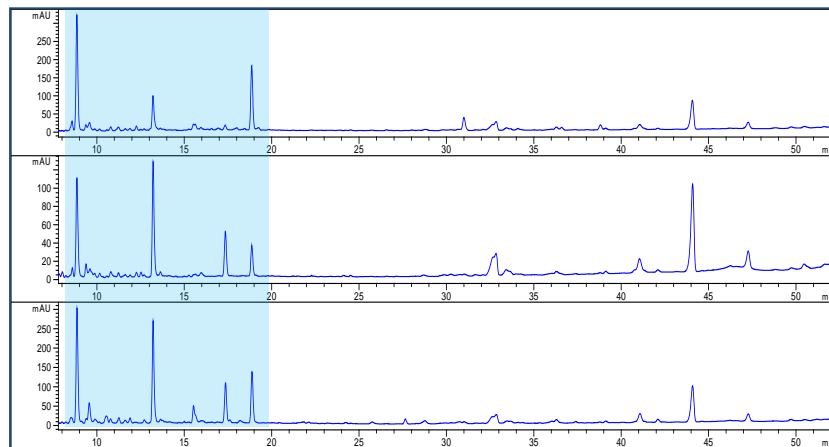


Eurycoma longifolia

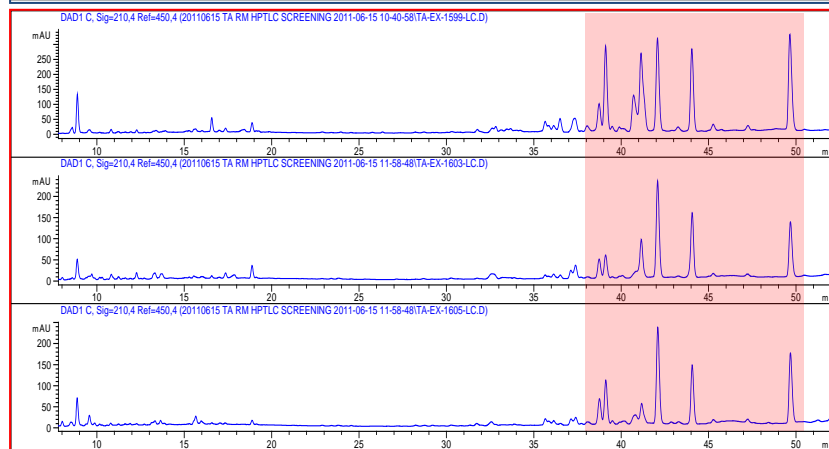
- At A Glance
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 - **HPLC-DAD**
 - Microscopy
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- Fingerprinting Profile for UV Active Compounds



ROOT

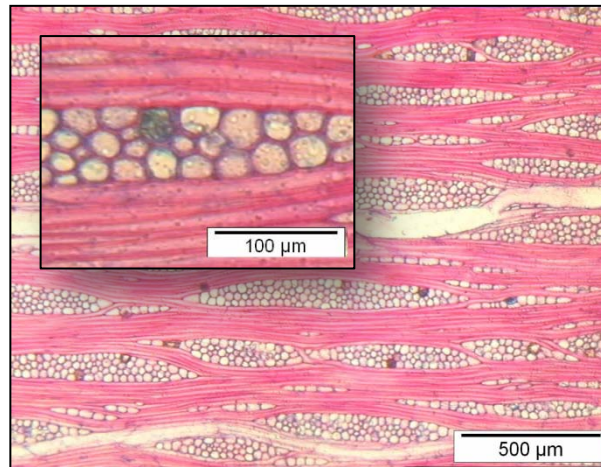


STEM

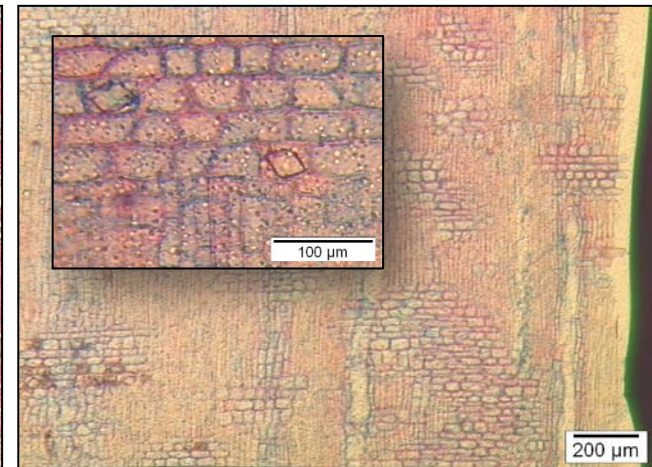
- At A Glance
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- Anatomy and micro structure of *E. longifolia*
 - Herbal powder analysis
 - Sled microtome and sectioning technique (TS, TLS, RLS)
- Only minor variations are detected between the root and stem of *E. longifolia*
- Tedious and long lead time to obtain result



Druse



Rhombus Crystal

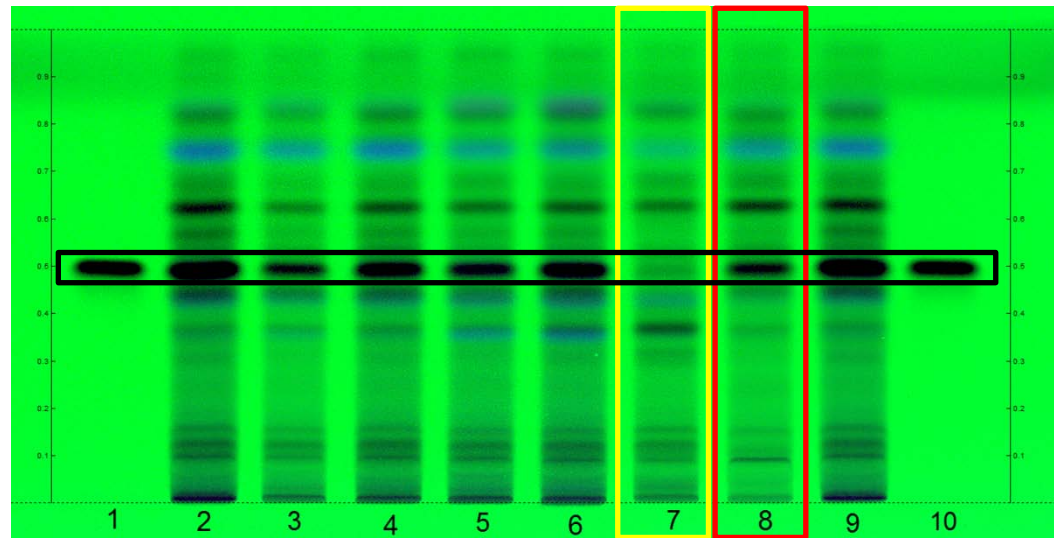
Eurycoma longifolia

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HPTLC - Identification

- Identification of *Eurycoma longifolia* Roots by HPTLC Fingerprinting
 - Raw Materials, Extracts, Finished Products (Single Herb)
 - Identification based on Eurycomanone
 - Fingerprinting based on Botanical Reference Material



CHCl₃:MeOH:H₂O (70:30:4)

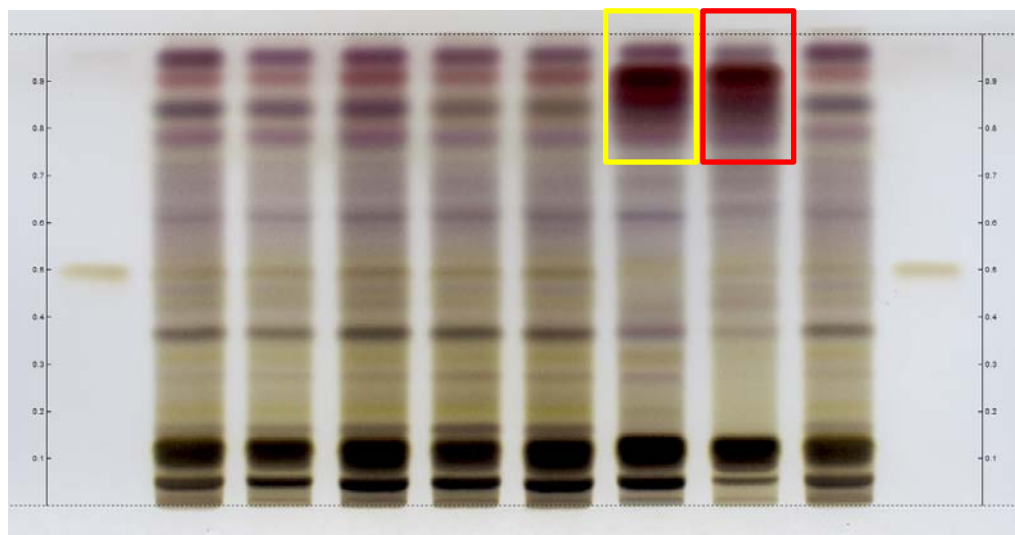
Eurycoma longifolia

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HPTLC - Identification

- Derivatization by Sulfuric Acid Reagent
 - Clear differences between root and stem (mid polar region)
 - A second method is used to analyze the non polar region



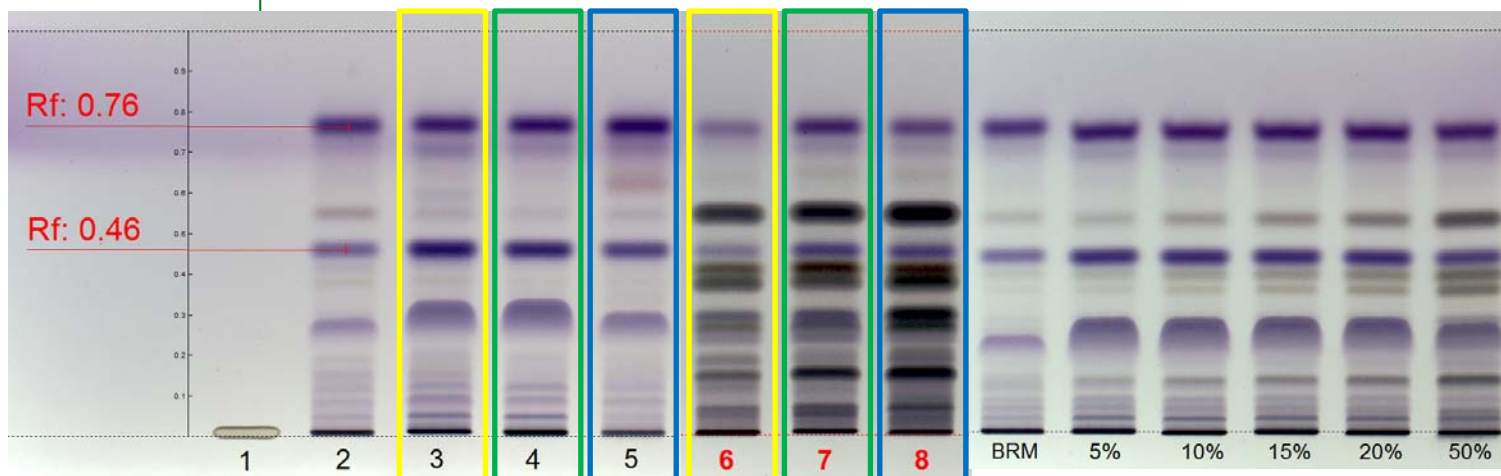
CHCl₃:MeOH:H₂O (70:30:4), Sulfuric Acid Reagent

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HPTLC – Qualification

- Raw Material Qualification
 - Second method – perform fingerprinting on mid-polar region (bitter principles and saponins)
 - Major differences between roots and stem of *E. longifolia*
 - Detection Limit – min of 5% adulterant (w/w)



CHCl₃:EtOAc (60:40), Anisaldehyde – Sulfuric Acid Reagent

- **Scope of Validation**
- Results
- Summary



Scope of Validation

- **Stability**
 - Analytes (during chromatography, on plate, in solution and derivatization)
- **Specificity and Selectivity**
 - Authenticated and commercial samples, adulterants, processed materials (extract) and finished products
- **Precision and Repeatability**
 - Precision on plate and intra-assay precision
- **Intermediate Precision**
 - Different day, sample preparation, analyst
- **Robustness**
 - Chamber saturation, developing distance, relative humidity, composition of the M/P and sample preparation

- Scope of Validation
- **Results**
- Summary



- **Stability**
 - Reference standard and test samples are stable during chromatograph, on plate and in solution (7 days)
 - Derivatization result stable up to 1 hour
- **Specificity and Selectivity**
 - Specific and selective for the identification of Eurycomanone in *E. longifolia* roots. Sufficient to distinguish adulterants with the absence of Eurycomanone.
- **Precision and Repeatability**
 - No interferences – repeatable with min deviation (ΔR_F 0.01)
- **Intermediate Precision**
 - No interferences – sufficiently precise with min deviation (ΔR_F 0.01)
- **Robustness**
 - Developing distance (60 – 80 mm)
 - Relative humidity (40 – 65% rH)
 - Sample prep (Sonication Time of 5 to 15 min)
 - Chamber saturation (at least 10 minutes)

- Scope of Validation
- Results
- **Summary**



Summary

- Application of HPTLC
 - **Method 1 - Qualitative analysis**
 - Identification of Eurycomanone
 - Fingerprint with reference to the BRM
 - Raw materials, processed materials, finished products
 - **Method 2 - Qualification analysis**
 - Raw Materials – extended method
 - Identification with reference to the BRM
 - Discrimination of adulterants (as low as 5%)
 - Rapid, selective and distinctive
- Extensively used in qualification of raw material supply and Identification of processed materials and finished products.



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