

Title	High Performance Liquid Chromatography (HPLC) Test
Production Name	AKARALI® Physta® Tongkat Ali (200mg)
Report No	R&D/006/03
Issue No	01
Revision	02
Published Date	5 <sup>th</sup> October 2025
Batch Ref No	AD250606(E)
Production Date	16 <sup>th</sup> June 2025
Expiry Date	15 <sup>th</sup> June 2027
Extract Type	Standardized Hot Water Extract
Species / Part	<i>Eurycoma Longifolia</i> (Root)
Country of Origin	Malaysia

## Test Results

Test Parameter	Specification	Result
<b>Organoleptic</b>		
Color	Light Brown	Complies
Odor	Characteristic	Complies
Flavour	Bitter	Complies
Form / Texture	Fine powder	Complies
Extraneous Material	Free from Foreign matter	Complies
<b>Physical Characteristics</b>		
Moisture Content	< 8.0%	Complies (3.35%)
Average Mesh Size	90% smaller than 120 mesh	Complies
<b>Bioactive Content</b>		
Eurycomanone	0.8% - 1.5%	Complies (1.10%)
Total Protein	≥ 22%	Complies (31.5%)
Total Polysaccharide	≥ 30%	Complies (33.3%)
Glycosaponin	≥ 40%	Complies (50.7%)
<b>Heavy Metal</b>		
Lead (Pb)	< 2.0 ppm	Complies (<1.00* ppm)
Mercury (Hg)	< 0.05 ppm	Complies (<0.05* ppm)
Arsenic (As)	< 1.0 ppm	Complies (<1.00* ppm)
Cadmium (Cd)	< 0.3 ppm	Complies (<0.20* ppm)
<b>Microbial Limit Test:</b>		
Total Bacteria Count	< 10,000 cfu/g	Complies (640 cfu/g)
Yeast & Mould	< 100 cfu/g	Complies (<10* cfu/g)
<i>Salmonella</i>	Absent in 25 g	Complies (Absent)
<i>Escherichia coli</i>	Absent in 1 g	Complies (Absent)
<i>Staphylococcus aureus</i>	Absent in 1 g	Complies (Absent)
Bile-tolerant Gram-negative bacteria	< 100 cfu/g	Complies (<10* cfu/g)

## HPLC Test Results: Profile and Compounds

### Test Methods:

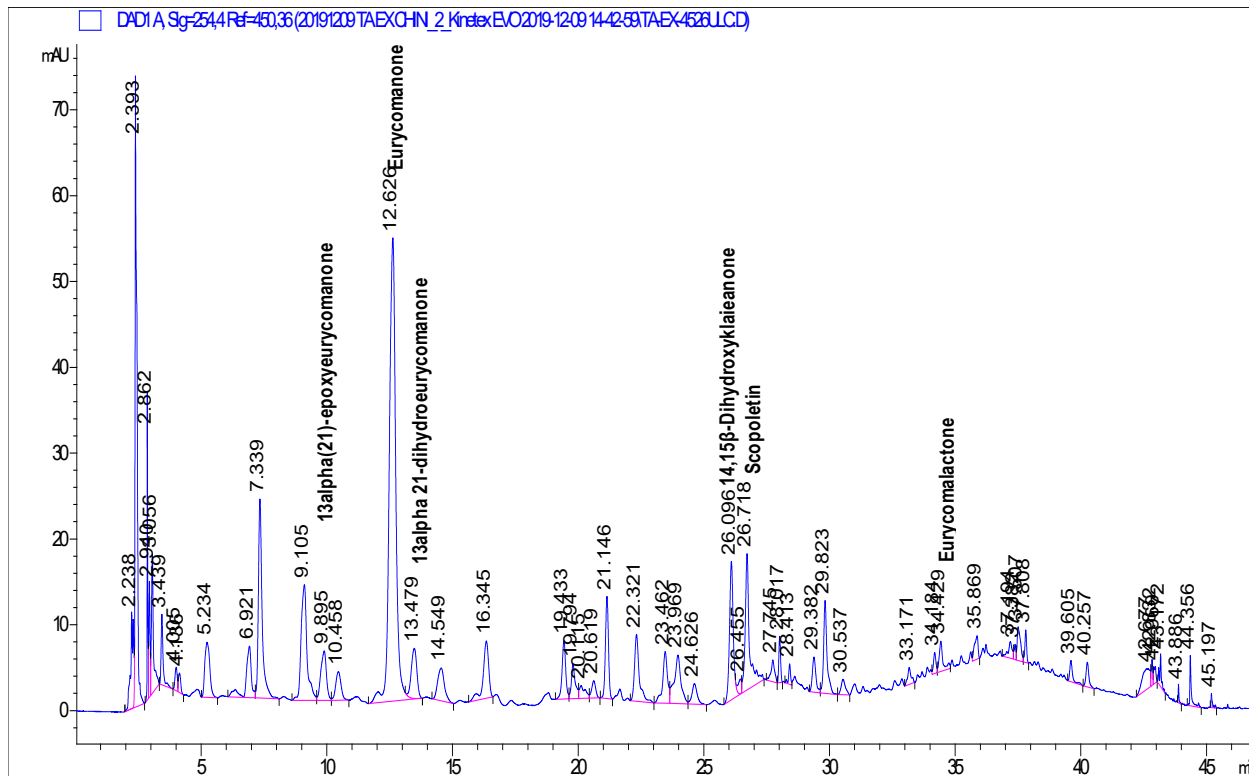
Standard Test Method for TM-R&D-001 ED05 Eurycomanone content in Tongkat Ali (TA) extract by HPLC, TM-R&D-020 ED01, Total Protein in TA extract, TM-R&D-021 ED01 Total Polysaccharides in TA extract and TM-R&D-022 ED04 Total Saponin by Gravimetric in TA extract.

### HPLC Profiling Parameters:

<b>Mobile Phase Preparation</b>	Channel A - 0.02% Trifluoroacetic Acid is added into 1000 mL of deionised water. Channel B - Acetonitrile.
<b>Chromatographic System</b>	Agilent 1290 Infinity
<b>Column</b>	Kinetex EVO C18 100 Å (150 x 4.6mm; 2.6µm particle size)
<b>Temperature</b>	30 °C
<b>Flow rate</b>	0.60 mL/min for t=0-40min & 1.00 mL/min for t=45-47min (Gradient)
<b>Wavelength</b>	UV at 254 nm, reference at 450 nm
<b>Run time:</b>	47 minutes (with 7.0 min post run)
<b>Injection Volume</b>	5.0 µL

HPLC-diode array detection analyses was performed using an Agilent 1290 Infinity instrument equipped with photo diode array, autosampler and column thermostat. As the stationary phase, a Kinetex EVO C18 100 Å (150 x 4.6mm; 2.6µm particle size) column was used. Water containing 0.02% of trifluoroacetic acid (A) and acetonitrile (B) as the mobile phases. The solvent composition was set to t=0 min 5% B; t=9 min 7% B; t=12 min 7.4% B; t=15min 8% B followed by isocratic of 12% B between t=17-23 min and 18% B from t=24-28 min. A gradual gradient was followed from t=30 min 20% B; t=35 min 30% B, t=40 min 35% B before final t=45-47 min at 95% B. A post-time of 7 min was applied. The flow rate was 0.6 ml/min from t=0-40 min and 1.0 ml/min from t=45-47 min and temperature was set to 30 °C. The injection volume was 5.0 ul and chromatogram was recorded at 254nm.

### HPLC Test Result: AKARALI Physta® Chromatogram Profile



#### Identification of Bioactive Peaks

No	Compound Name	Retention Time (RT) in min
1	13 $\alpha$ (21)-Epoxyeurycomanone	9.105
2	Eurycomanone	12.626
3	13 $\alpha$ , 12-Dihydroeurycomanone	13.479
4	14, 15 $\beta$ -Dihydroxyklaianone	26.096
5	Scopoletin	26.718
6	Eurycomalactone	34.429

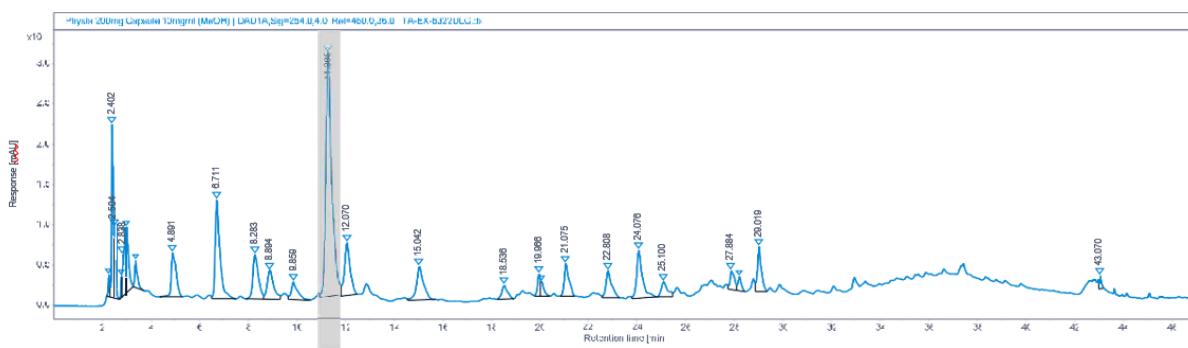
### Observations:

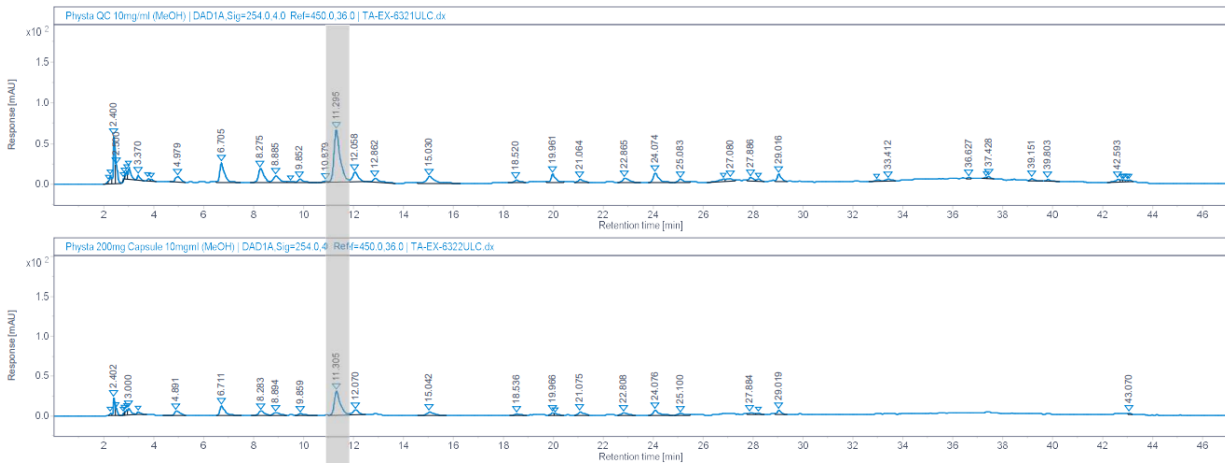
The chromatogram profile from the HPLC test results along with bioactive compounds taken from AKARALI Physta® 200mg showed compliance to the MS2409 Tongkat Ali standards, suggesting high level of authenticity and purity of the extract (without the presence of adulteration from externally-induced food agents, chemicals, illegal substances or quassinoid-induced solvents from other plants or species).

This is further supported by similar peak characteristics that match the reference HPLC profile of a MS2409-compliant standardized hot water Tongkat Ali extract, indicated by the following:

- A dominant eurycomanone peak at the RT of 12.6 minutes.
- Additional quassinoid peaks from eurycomanone derivatives (e.g., eurycomanol, eurycomalactone, epoxyeurycomanone, etc.) at RT of 9.1, 13.4, 26.0 and 34.4 minutes
- Stronger smaller peaks after RT 12 minute to RT 45 minute corresponding to other bioactive compounds (and minerals) that naturally exist in Tongkat Ali root extract.
- Clean baseline (with low noise) and absence of unexpected late-eluting/solvent-induced peaks in the UV 254–260 nm trace

Further test analysis on various production batches showed that HPLC chromatogram profile of AKARALI Physta® is consistent with similar peaks and features that match closely with other standardized Tongkat Ali (*Eurycoma Longifolia*) extracts, with distinct peaks across RT 12 to 38 range.



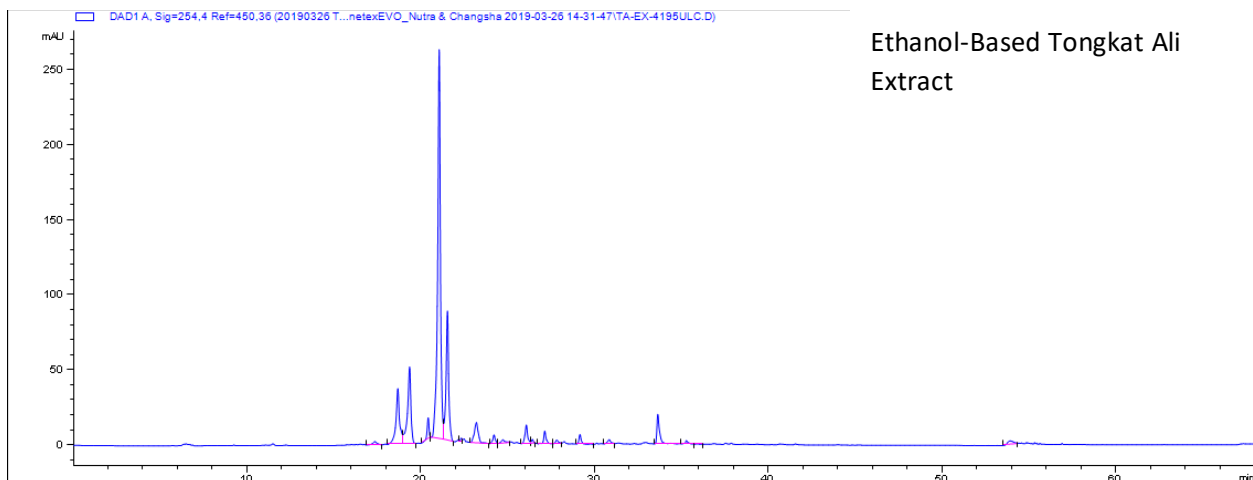
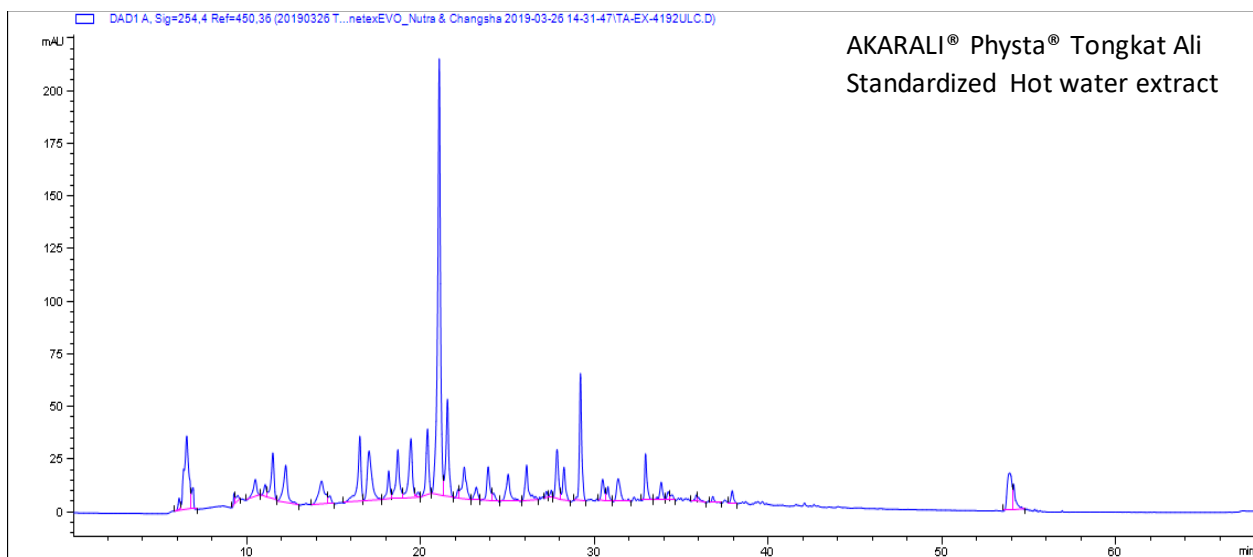


Additionally, AKARALI Physta® Tongkat Ali extract is unique due to the hot water standardization process with more than 20 peaks found along the RT range up until 45 minutes. This suggests that standardized hot water extract produce a consistent high concentration of eurycomanone (and its derivatives), eurypeptides, crude protein, glycosaponin and polysacharrides that meets the globally-recognized Tongkat Ali industry standard MS2409.

A standardized MS2409-compliant HPLC profile such as the one found in AKARALI® Physta® may be used as a blueprint or as a fingerprint reference for manufacturers. These significant-yet-consistent peaks could serve as quality and potency biomarkers used in human clinical studies, in-vitro, in-vivo or in efficacy research tests.

## HPLC Comparison Test: AKARALI Physta Standardized Hot Water Extract Vs Ethanol Tongkat Ali Extract

Comparison HPLC test showed that standardized hot water extract used in AKARALI Physta® has a distinctive HPLC chromatogram profile compared to ethanol-based Tongkat Ali extract found in other Tongkat Ali supplement brands in the US, UK, Canada and Australia as shown below.



**Key differences:**

1. Chromatogram profile analysis on ethanol-based Tongkat Ali extract in this test showed no visible or dominant eurycomanone peaks at RT 12 min - 13 min and the number of eurycomanone derivative peaks were significantly less (and almost non-existence) throughout the RT 2 min to RT 26m min range.
2. Using various composition of ethanol (i.e.g 70%, 80%, 90% and 100%) as a solvent during the Tongkat Ali extraction process may result in a significantly different HPLC profile compared to the reference standard HPLC profile that is compliant to the MS2409 Tongkat Ali Standards.
3. The lower number of peaks found in ethanol-based extract indicate weak presence of bioactive compounds in Tongkat Ali, and the peak profile was found to be similar to the chromatogram profiles found in other non-standardized or non MS2409-compliant Tongkat Ali extracts.
4. Furthermore, there were only a few significant / prominent peaks observed at RT 21 - 22 min, but these were not identified as eurycomanone. In fact, there were lesser and weaker peaks observed throughout the RT range.

**Conclusions:**

1. AKARALI® Tongkat Ali Physta® standardized hot water extract meets the specifications of Tongkat Ali MS2409 Standards based on HPLC test results, bioactive compounds, microbial and heavy metal limits. This information is regularly published in the Certificate of Analysis (COA) reports available on AKARALI website.
2. Multiple HPLC tests on production batches confirm that the HPLC chromatogram profiles match the standardized MS2409-complaint HPLC reference profile indicated by dominant eurycomanone peak, followed by other prominent peaks throughout the RT range from 2.00 minute to 45.08 minute.
3. HPLC test results suggest high level of purity found in AKARALI 200mg capsules, with no indication of adulteration from external quassinoid-induced plants, solvents or food agents.

**Disclaimer:**

This HPLC Lab Test is independently conducted, analyzed, verified and evaluated by the Biotropics research team. It is not sponsored by any companies and have not received any form of incentives, funds, grants or subsidies from any organizations. We remain independent in our evaluation and not liable for any inaccuracies resulting from the assessment or test results.

**Copyright & Terms of Use:**

Information published in this document is only for personal use. No part of this document may be reproduced, stored in a retrieval system, transmitted in any means (electronic, mechanical, photocopying, recording, online or on social media platforms) without the written permission of the copyright owner. This includes screenshots of charts, texts, figures and the use of logo or images.