# A Preliminary Study into the Effect of Processing on *Eurycoma longifolia* Water Extract Yield

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## Abstract

*Eurycoma longifolia or Tongkat Ali is a traditional Malaysian herb associated with medicinal benefits such as antipyretic, anti-malarial, and aphrodisiac effects. At present it has been identified as a potentially lucrative commercial product to be developed as an international product by the Malaysian government. There is therefore a need to produce Tongkat Ali water extracts of high yield and quality. A key issue in the production of herbal medicines such as Tongkat Ali is the production of extracts standardised to a specific chemical marker. At present Tongkat Ali extracts are standardised to eurycomanone, a quassinoid which is the primary component present in the extract. Due to the varying planting conditions of Tongkat Ali plants, the concentration of the required phytochemicals within the raw material varies in quantity and quality. Thus, phytochemical extract processing methods must be developed which enhance phytochemical extraction to achieve the required phytochemical profile and yield. Varying processing conditions as well as reconstituting mixtures of extracts can be utilised to produce standardised herbal extracts. In this preliminary work, the effects of varying particle size, extraction time, and solvent ratio on the quantity of phytochemicals extracted on a laboratory scale from a single sample of Tongkat Ali was studied. It was found that increasing solvent ratio as well as extraction time increased yield. However, the effect of particle size on yield was inconclusive.*

## Keywords:

Eurycoma longifolia, Phytochemical Processing

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## Introduction

*Eurycoma longifolia*, or Tongkat Ali as it is known locally, is a traditional herb used in Indonesia, Malaysia and Vietnam. Its traditional uses include anti-pyretic, anti-malarial, anti-ulcer, energy boosting, anti-tumour and aphrodisiac applications [1,2]. Traditionally the tap root of the plant is boiled in water and the decoction drunk for these benefits.

Currently, Tongkat Ali has been identified as potential cash crop in Malaysia for production of phyto-medicines for malaria and erectile dysfunction. Studies at Universiti Sains Malaysia have found that Tongkat Ali constituents have stronger anti malarial properties than chloroquinine, an antimalarial drug component [3] and Tongkat Ali extracts can enhance sexual performance of rats [4]. Universiti Kebangsaan Malaysia researchers have discovered the biochemical pathways of Tongkat Ali's aphrodisiac properties. They found that Tongkat Ali inhibits the formation of PDE2 like Viagra as well as PDE3 and PDE5 which increases the amount of cGMP which enhances penile erections similar to Viagra [5, 6].

A key step in producing herbal medicines is effective process technology. Once Tongkat Ali has been harvested, dried and ground, it is needs to be processed to extract the maximum amount of active phytochemicals. A critical issue in herbal medicine production is the production of standardised extracts, extracts which have a specific measurable amount of bioactive component. Optimal process operating conditions and steps need to be known to ensure this. This is especially true as herbal extract are very low yield between 1-5% w/w.

An example of a herbal processing plant is shown in Figure 1 where raw material is first extracted in an extraction vessel, the critical processing step, followed by a spray dryer.


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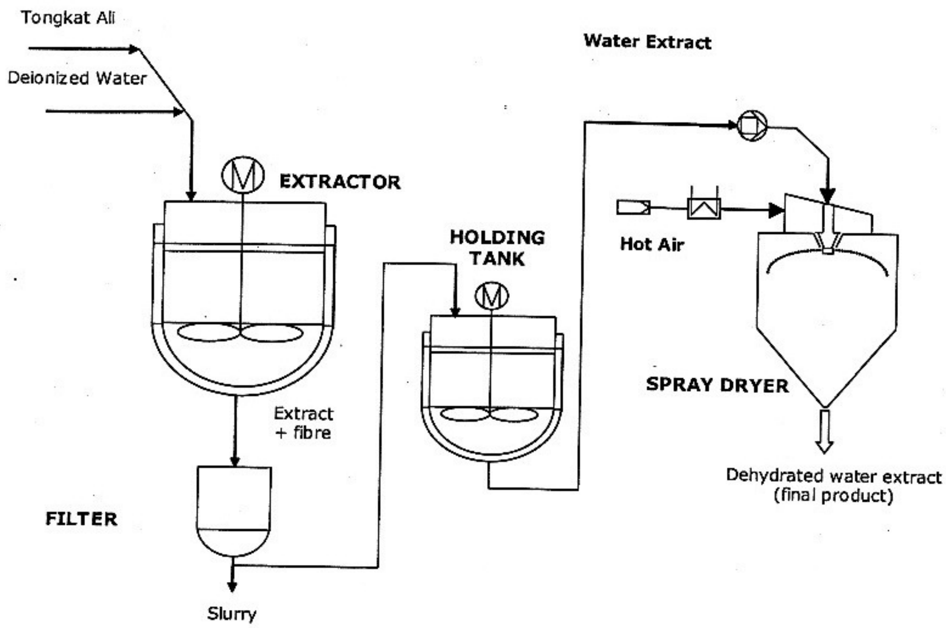


*Figure 1 - Tongkat Ali Process Line*

Among operating conditions that effect extract yield include:

1. Solvent used
2. Solvent to raw material ratio
3. Time of extraction
4. Extraction temperature
5. Extraction pressure
6. Raw material particle size
7. Extraction vessel agitation speed

The selection of the most effective operating conditions as well as operating condition settings will optimise the production of the desired phytochemical.

## Methodology

The objective of this paper is to investigate the effects of processing on the phytochemical yield of the Tongkat Ali water extract. The work was divided into 3 phases:

1. Analytical methods
2. Internal standard preparation
3. Experimental work

### Analytical Methods

The analysis of Tongkat Ali extracts were done with Thin Layer Chromatography (TLC) for quick qualitative analysis as well as High Performance Liquid Chromatography (HPLC) for both qualitative and quantitative analysis.

TLC was done on silica gel prepared plates with a solvent mixture of n-BuOH:$CH_3COOH$:$H_2O$ (6:1:1) based on the Malaysian Herbal Monograph Volume 1 [7]. Extraction samples were tested with TLC to determine if any changes of phytochemical contents occurred with changing operation parameters. The Tongkat Ali water extract TLC showed 4 distinct spots under a UV wavelength of 366nm as illustrated in Figure 2.

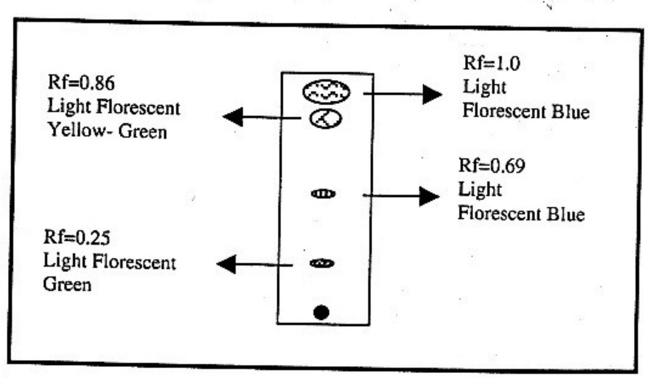


*Figure 2 - TLC profile of Tongkat Ali Water Extract*

The HPLC utilised a C18 reverse phase column (Supelcosil 5 μm, 250mm x 4.6 mm) and a UV detector operated at 238 nm as described by Chan et al [8]. The mobile phase consisted of an isocratic mixture of acetonitrile and water (24:76) at a flow rate of 0.4 ml/min. The low mobile phase flowrate was chosen to allow the peaks to separate more distinctly. An example of a water extract HPLC chromatograph is shown in Figure 3. Note the 4 major peaks present in the HPLC chromatograph.

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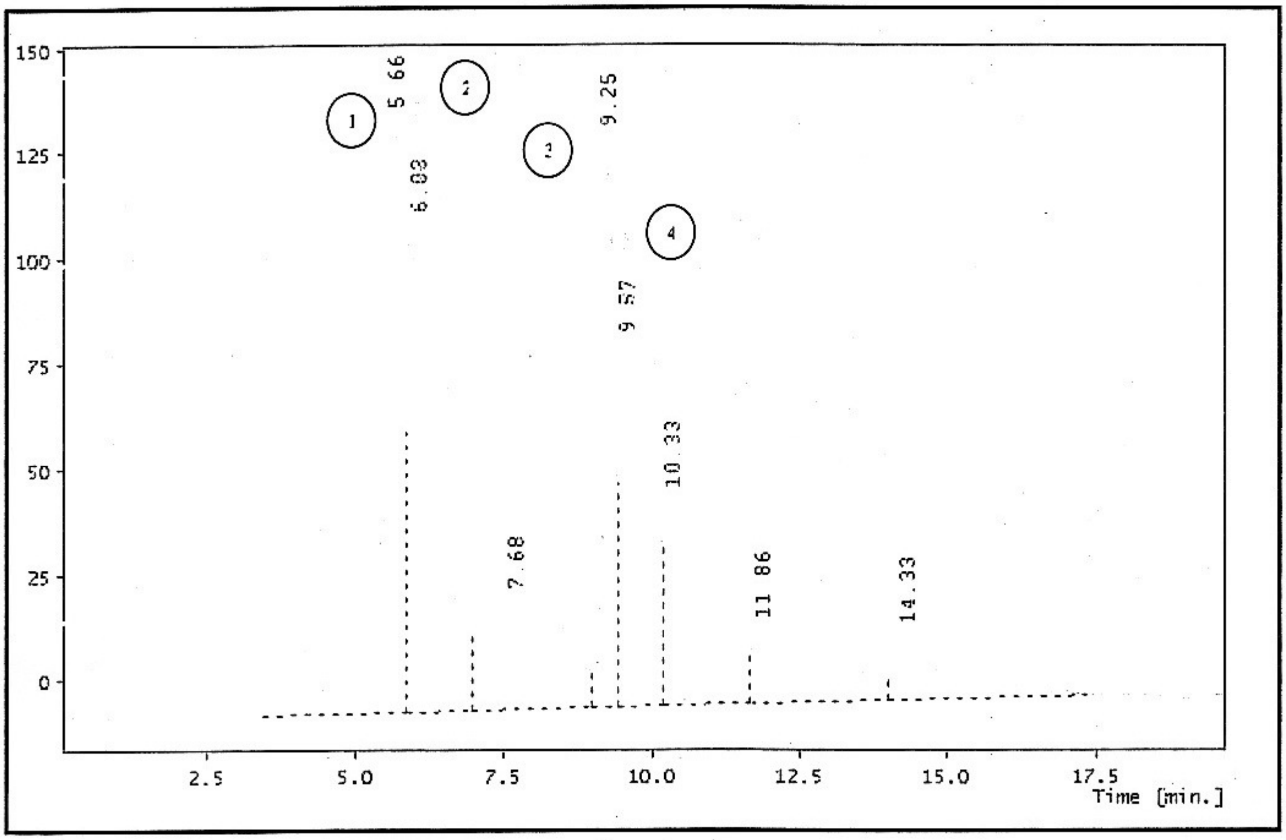


*Figure 3 - Tongkat Ali Water Extract HPLC Chromatograph*

## Internal Standard Preparation

At present there are no commercially available reference standards for Tongkat Ali phytochemicals. Among the major phytochemical components of Tongkat Ali include eurycomanone, longilactone, eurycomalactone, 6α-hydroxyeurycomalactone, 14,15-β hydroklaineanone, and eurycomanol [8]. The current component used for standardisation is eurycomanone, the largest phytochemical component in the mixture [8].

However, as the objective of the investigation was to investigate the effect of processing parameters on phytochemical yield, a simplified standard was made based on Tongkat Ali water extract powder produced at the Chemical Engineering Pilot Plant. The extract powder was dissolved to various concentrations and injected into the HPLC. The area under peak 4 was measured and calibrated to the extract powder concentration. Table 1 shows the calibration data while Figure 4 displays the calibration curve. The calibration equation was found to be:

$$Concentration\ (g/ml) = 7E\text{-}07 * Peak\ 4\ Area \qquad (1)$$

*Table 1 - Extract Powder Calibration Data*

| Sample | Peak 4 Area | Concentration (g/ml) |
|---|---|---|
| CAL1 | 6819 | 0.005 |
| CAL2 | 3093 | 0.0025 |
| CAL3 | 1525 | 0.00125 |
| CAL4 | 810 | 0.000625 |
| CAL5 | 410 | 0.000313 |

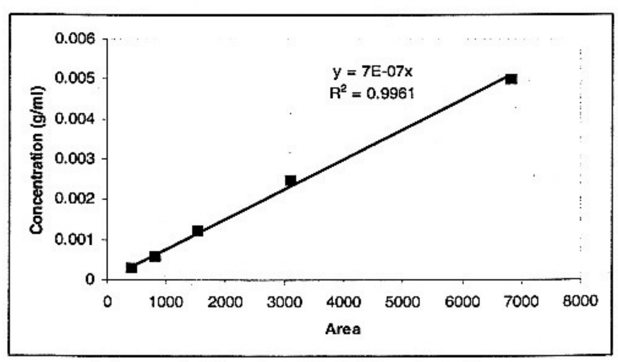


*Figure 4 - Tongkat Ali Calibration Curve*


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**Experimental work**

*Reflux extraction was chosen as the experimental apparatus to simulate the extraction vessel on a laboratory scale. Of the 7 main operating parameters identified in the introduction section 3 main parameters were chosen to investigate the effects of processing: particle size, time of extraction, and ratio of solvent. A full statistical experimental design of $3^n$, where 3 is the number of variables and n is the number of treatments that can be applied, was chosen in this preliminary investigation.*

Tongkat Ali shavings were poured through with a sieve to separate into rough shavings (up to 2 cm in length) and powder (less than 1mm diameter). The time of extraction was chosen to be 1 hour and 2 hours while the volume ratio of water to Tongkat Ali volume was 6:1 and 4:1. In addition an exhaustive extraction of Tongkat Ali power at 3 hours and 6:1 was carried out for comparison. Table 1 shows the parameter values for each sample.

*Table 2 - Sample Parameter Values*

| Sample | Particle size | Time | Ratio |
|---|---|---|---|
| 1 | Fine | 1 hour | 4:1 |
| 2 | Fine | 1 hour | 6:1 |
| 3 | Rough | 1 hour | 4:1 |
| 4 | Rough | 1 hour | 6:1 |
| 5 | Fine | 2 hours | 4:1 |
| 6 | Fine | 2 hours | 6:1 |
| 7 | Rough | 2 hours | 4:1 |
| 8 | Rough | 2 hours | 6:1 |
| 9 | Fine | 3 hours | 6:1 |

After extraction, the liquid extract was filtered to remove the gross solids followed by centrifugation and filtration to remove fine suspended solids. The filtered extract was rediluted to 300 ml before injecting into the HPLC. The HPLC was washed with acetonitrile between every 5 samples to ensure a clean column.

## Results and Discussion

The results of the extraction are shown on Table 3, Figure 5, and Figure 6.

*Table 3 - Extraction Results*

| Sample | Concentration (g/ml) | Yield (g) | Sample weight (g) | % yield (w/w) |
|---|---|---|---|---|
| 1 | 0.0025 | 0.74 | 11.69 | 6.36 |
| 2 | 0.0036 | 1.07 | 11.36 | 9.43 |
| 3 | 0.0028 | 0.85 | 11.56 | 7.32 |
| 4 | 0.0033 | 1.00 | 11.56 | 8.67 |
| 5 | 0.0032 | 0.96 | 11.41 | 8.44 |
| 6 | 0.0037 | 1.12 | 11.43 | 9.78 |
| 7 | 0.0035 | 1.05 | 11.56 | 9.07 |
| 8 | 0.0033 | 0.98 | 11.53 | 8.51 |
| 9 | 0.0039 | 1.16 | 11.43 | 10.18 |

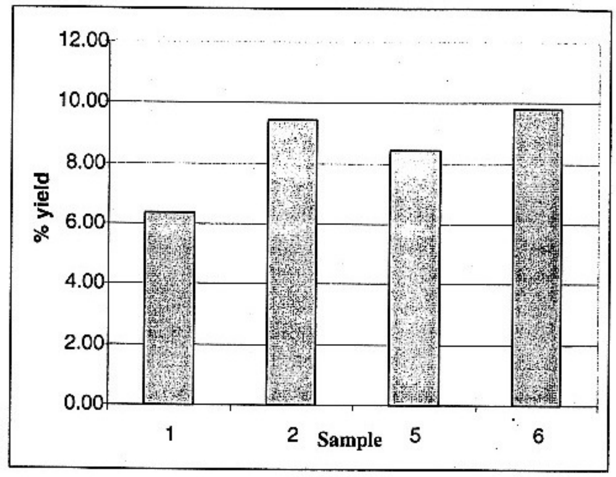


*Figure 5 - Fine Tongkat Ali Sample Percentage Yield at Varying Time and Solvent Ratio*

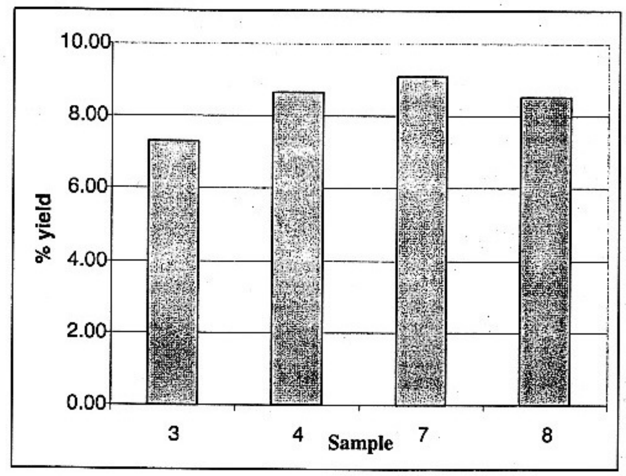


*Figure 6 - Rough Tongkat Ali Sample Percentage Yield at Varying Time and Solvent Ratio*

Several initial conclusions can be drawn from the data. With the exception of sample 8, higher solvent ratio tends to increase yield. Also, with the exception of sample 8, longer extraction tends to increase yield. However, it is not conclusive from the data if the size of the Tongkat Ali particles will affect the yield as in comparison between Sample 1 and 3, indicates that a smaller particle size decreases yield but a comparison between Sample 2 and 4 indicates that a smaller particle increases yield.

A point to note is that the yield percentage calculated is extremely high, in the 5 to 10 percent range. A possible cause for this measurement is the possibility that the

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extract powder had absorbed some water, thus increasing its weigh which was taken into account.

## Conclusion

It can be safely concluded that increasing extraction time and solvent ratio increases extraction yield. However, more investigation is needed to find the effect of particle size.

Further work will need to be done to get rate and yield data for the extraction of Tongkat Ali. The number of treatments as well as the number of processing variables need to increased to enrich the data. In addition, in future work, the yield needs to be calibrated to a standard such as eurycomanone to ensure a path to standardisation.

The result of this study will be utilised to determine the critical parameters as well as range for optimal extraction to ensure the standardization of the herbal extract. The processing data will also be used to build a herb leaching model to enable process optimisation and scale up studies to be carried out.

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## References

[1] Jiwajinda, S, Santisopasri, V, Murakami, A, Kawanaka, M, Kawanaka, H, Gasquet, M, Eilas, R, Balansard, G, and Ohigashi, H. 2002. *In vitro anti-tumor promoting and anti-parasitic activities of the quassinoids from Eurycoma longifolia, a medicinal plant in Southeast Asia.* Journal of Ethnopharmacology. 82(1): 55-58.

[2] Ang H H, Ikeda S, and Gan E K 2001. *Evaluation of the potency activity of aphrodisiac in Eurycoma longifolia Jack.* Phytotherapy Research. 15(5): 435-6.

[3] Ang H H; Chan K L; Mak J W. 1995. *Effect of 7-day daily replacement of culture medium containing Eurycoma longifolia Jack constituents on the Malaysian Plasmodium falciparum isolates.* Journal of Ethnopharmacology. 49(3): 171-5

[4] Ang, H. H and Ngai, T. H. 2001. *Aphrodisiac evaluation in non-copulator male rats after chronic administration of Eurycoma longifolia Jack.* Fundamental Clinical Pharmacology. 15(4), 265-268.

[5] Pihie, A.H.L. 2003. *Phytopharmaceutical product targeting erectile dysfunction and energy boosting effect from Eurycoma Longifolia Jack.* NatPro 2003 Brochure. Natural Products 2003, Kuala Lumpur, April 10-12, 2003

[6] Pihie, A.H.L. 2003. *Mechanism of action of E.longifolia extract in sexual stimulant study.* NatPro 2003 Brochure. Natural Products 2003, Kuala Lumpur, April 10-12, 2003

[7] Ismail, Z, Ismail, N., and Lassa, J. 1999. Malaysian Herbal Monograph. Kuala Lumpur: Malaysian Monograph Committee.

[8] Chan, K.L, Choo, C.Y, Morita, and H, Itokawa, H. 1998. *High performance liquid chromatography in phytochemical analysis of Eurycoma longifolia.* Planta Medica 64(8): 741-745.



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