

PRELIMINARY PHYTOCHEMICAL SCREENING OF EURYCOMANONE FOR SELECTION OF HIGH QUALITY PLANTING MATERIALS: *Eurycoma longifolia*

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ABSTRACT

Eurycoma longifolia has been widely used in traditional herbal preparation and presently there are many commercial products available for general health and libido. However, study on plant breeding of this species is still in its infancy. Thus, Forest Research Institute Malaysia (FRIM) has taken an initiative to conduct provenance trial of this species in order to select the best provenance and eurycomanone is chosen as bioactive marker. The aim of this study was to analyze the percentage of eurycomanone extracted from root, stem and leaves parts of *E. longifolia* from different provenances collected throughout Peninsular Malaysia. The eurycomanone compound is extracted with pure water and the present is detected by Ultra Performance Liquid Chromatography (UPLC). Findings showed that the highest percentage of eurycomanone present in the root extract of Terengganu with 1.46% while the lowest is Melaka with 0.17%. Stem part of the plant showed there was presence of eurycomanone compound relatively in small percentage except for Terengganu, while there was no eurycomanone compound detected in leaves extract from all provenances tested (Johor, Kedah/Perlis, Melaka, Pahang, Perak, Pulau Pinang, Selangor and Terengganu). However, it is still too early to derive any conclusion on the best provenance based on chemical marker itself.

Key words: *Eurycoma longifolia*, provenance trial, eurycomanone, high quality planting materials

INTRODUCTION

Eurycoma longifolia or locally known as “Tongkat Ali” is one of the most popular herbal medicine indigenous to South-East Asian countries like Malaysia, Indonesia and Vietnam. *E. longifolia* is a small tree with maximum height of 15-18 m and it is believed that for complete maturity of this plant might take up to 25 years (Bhat and Karim, 2010). This plant is crowned by an umbrella-like structure of leaves and usually does not have significant branches and its main root is usually unbranched, cylindrical in shape, yellowish white in color and taste very bitter. The fruits are green in color and turn to dark red after ripening and this plant only bears fruits after 2-3 years cultivation (Subramaniam, 2013).

Based on a review of *E. longifolia* on this plant pharmacological importance by Bhat and Karim (2010), root part of the plant species has the potential of antimalarial, aphrodisiac, antiulcer and

antimicrobial properties. Whereas, the stem part has antimalarial and antimicrobial properties, while the leaves part has antihyperglycaemic, anti-tumor and antimicrobial properties. As for pharmacological benefits specifically derived from eurycomanone compound, which also known as pasakbumin-A was reported to have antimalarial (Yusuf *et al.*, 2013; Bhat and Karim, 2010; Kuo *et al.*, 2004; Mohd Azmi *et al.*, 2004), antiulcer and antimicrobial (Bhat and Karim, 2010) and aphrodisiac properties (Subramaniam, 2013; Low *et al.*, 2013; Bhat and Karim, 2010; Mohd Azmi *et al.*, 2004).

Currently, extensive studies have been made on the isolation and characterization of *E. longifolia* especially from the root part. A study conducted by Chua *et al.* (2011) found that quassinoids were significantly present in higher concentration than alkaloids with eurycomanone compound and its derivatives are the major compound. It was also reported that there are more than 85 compounds have been isolated from *E. longifolia*. These compounds are majority from the classes of canthin-6-one

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alkaloids, carboline alkaloids, squalene derivatives, tirucallane-type triterpenes and biphenylneolignans, instead of quassinoids (Chua *et al.*, 2011).

This study aims to evaluate the percentage of eurycomanone compound from the root, stem and leaves part of *E. longifolia* from different provenances (Johor, Kedah/Perlis, Melaka, Pahang, Perak, Pulau Pinang, Selangor and Terengganu) in evaluation of which provenance could be declared as high quality planting materials. Eurycomanone compound is chosen as the bioactive marker as eurycomanone is from the quassinoid class which present in the highest chemical compound of *E. longifolia* (Chua *et al.*, 2011). Chan (2004) reported that several bioactive compounds that have been identified such as 9-methoxycanthine 6-one, 14, 15- β -dihydroxyklaineaneone, 13, 21-epoxyeurycomanone including eurycomanone, are commonly used as standard markers for standardization of *E. longifolia* products. Thus, this study will implement eurycomanone as one of the standard markers in the assessment of quality declared planting materials of *E. longifolia* which extensively being carried out by Plant Improvement Programme, FRIM.

MATERIALS AND METHODS

Plant Materials and Samples Preparation

Plant materials of *E. longifolia* were harvested from natural forest reserve areas of eight different provenances which were Johor, Kedah/Perlis, Melaka, Pahang, Perak, Pulau Pinang, Selangor and Terengganu. The harvested plants were washed, cleaned and dried in an oven for samples preparation.

Extraction by Sonicator and Evaporation by Rotary Evaporator

The dried root, stem and leaves parts were extracted with pure water by bath sonicator (Branson, 2510) for two hours and concentrated using rotary evaporator. The concentrated plants segment extract were diluted with pure water.

Phytochemical Analysis by Ultra Performance Liquid Chromatography (UPLC)

The extracts were analyzed by Ultra Performance Liquid Chromatography (UPLC), Waters. The size of the column used was 5 cm long with a diameter of 2.1 cm. As for the binary solvent manager, 0.05% phosphoric acid and acetonitrile were used. The run time for each samples was about 8 minutes with 5 injections per sample (there were total of 3 replicates per samples of each root, stem and leaves part). Eurycomanone compound is detected at 244 nm wavelength with retention time (RT) approximately at 1.59 minutes.

Data Analysis

Quantity calculations were made according to the linear calibration curves of standard ($y = mx+c$). Percentage of eurycomanone compounds were calculated based on the peak area produced by UPLC profiles.

RESULTS AND DISCUSSION

Fig. 1 shows the UPLC profile for detected eurycomanone compound in the extract of root and stem, while Fig. 3 shows the mean percentage of eurycomanone compound detected in root and stem from Johor, Kedah/Perlis, Melaka, Pahang, Perak, Pulau Pinang, Selangor and Terengganu. The highest percentage of eurycomanone present in root extract of Terengganu provenance with 1.46% while Melaka gave the lowest with 0.17%. On the other hand, the highest percentage of eurycomanone in stem, present in Kedah/Perlis provenance with 0.59% and the lowest is Pulau Pinang with 0.1%.

Eurycomanone compound (relatively in low percentage) present in the extract of stem samples from all provenances except for Terengganu (Fig. 1 and 3). Traditionally, only the root of *E. longifolia* is recognized as tonic and used in herbal preparation, however, current studies have shown that the stem of *E. longifolia* also content eurycomanone compound that could give therapeutic effects. There was no eurycomanone compound detected in leaves extract from all provenances analyzed (Fig. 2). In this study, young wildings of *E. longifolia* were used as samples, thus eurycomanone compound might not be present in the leaves yet at early age of the plant. However, Bhat and Karim (2010) reported that there are seven types of quassinoids were successfully isolated from the leaves part of *E. longifolia* which are; lonilactone, 6-dehydrolonilactone, 11-dehydroklaineaneone, 12-epi-dehydroklaineaneone, 15 β - hydroxyklaineaneone, 14,15 β -dihydroklaineaneone and 15- β -O-acetyl-14-hydroxyklaineaneone.

Findings showed that concentration of eurycomanone compound found in root part is higher than stem part of the plant for all provenances analyzed except for Pahang which showed that stem part (0.38%) content higher percentage of eurycomanone than root part (0.18%). Previous studies also suggested that the concentrations of secondary metabolites for different plant species are varied from different parts of the plant from the same species (Abdul Kabir *et al.*, 2009). Differences of eurycomanone content had also been studied on tissue culture plantlets compared with matured root of *E. longifolia*. The study showed yield of eurycomanone in tissue culture plantlets was higher (120.76 ppm/mg) than matured root of *E. longifolia* (101.26 ppm/mg) (Hassan *et al.*, 2012).

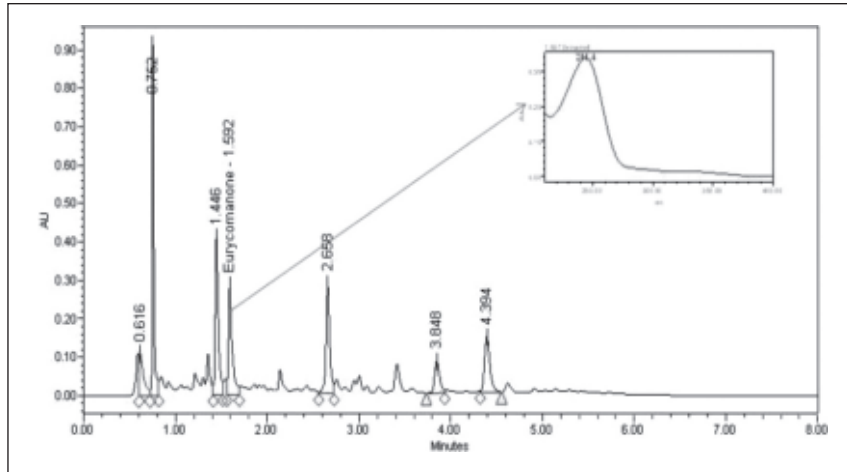


Fig. 1. UPLC profiles of detected eurycomanone compound from root and stem extract

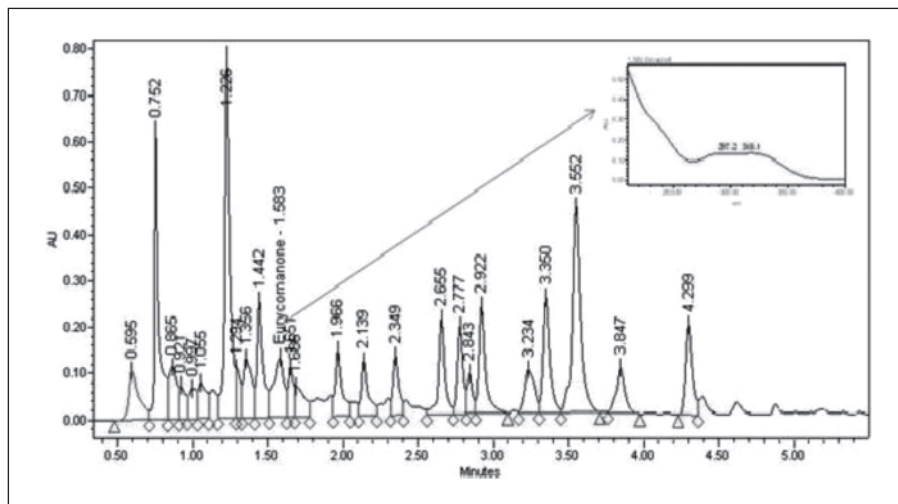


Fig. 2. UPLC profiles of non-detected eurycomanone compound from leaves extract

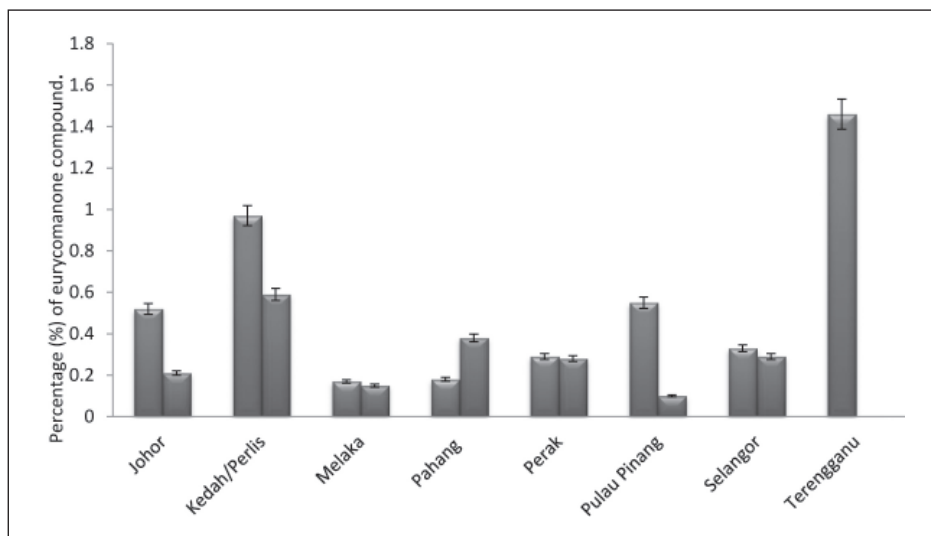


Fig. 3. Mean percentage (%) of eurycomanone compound present in root and stem from 8 provenances

Eurycomanone compound is selected as phytochemical marker due to the uniqueness of the compound which confirms the correct botanical plant. Even though eurycomanone is a secondary metabolites compound which could be affected by abiotic factors, the interior quality of medicinal plants usually determined by its secondary metabolites as the compound gave the pharmacological importance of the plant species (Dong *et al.*, 2011).

There are several factors that might affect the differences in percentage of eurycomanone present in different localities of *E. longifolia*. First, different geographical locations of the plant species as sampling locations of the plants were varied from island population, coastal population and hill forest population. This is supported by previous study conducted on *Mentha spicata* by Ullah *et al.* (2012) confirms that variations in phytochemical content are related to geographical location. According to the report, the impact of different altitudes, moisture and temperature of different locations are the factors contributing to changes in secondary metabolites. Another study by Dong *et al.* (2011) on secondary metabolites content in the leaves extract of *Eucommia ulmoides* from different province showed that growing locations had significant impacts.

However, it is still too early to conclude whether the highest or the lowest content of eurycomanone would be the best indicator for high quality planting materials of *E. longifolia*. Further and extensive studies are needed in order to improve the plants propagation especially from the aspects of phytochemistry, breeding, and genetic.

CONCLUSION

Provenance trial of *E. longifolia* is still at initial phase, there are many aspects to take into consideration before selection of the best provenance could be made and declared as high quality planting materials. As of now, four trial plots are being established at FRIM's Research Station (SPF) Mata Ayer, Perlis, SPF Maran, Pahang, SPF Jeli, Kelantan and SPF Selandar, Melaka in evaluation of the growth performance of each provenance consequently catered the plant breeding information.

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