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PHYTOCHEMICAL ASSESSMENT OF MULTI-LOCATIONAL TONGKAT ALI (*Eurycoma longifolia*) IN PENINSULAR MALAYSIA

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Graphical abstract

Abstract

Phytochemical assessments were conducted on the plant part of Tongkat ali (*Eurycoma longifolia*). The objective of this study was to identify agro-ecological that influence favourably the growth and eurycomanone content of planted Tongkat ali. The samples were collected at throughout Peninsular Malaysia such as from Gua Musang (Kelantan), Jengka (Pahang), Kluang (Johor) and Beseri (Perlis). Traditionally, every part of the plant especially the roots were used as an afterbirth tonic, reducing fevers, curing mouth ulcers and to treat intestinal worms. Organic solvent was used for extraction which followed by fractionation between chloroform and water. The water fraction was fractionated again with *n*-butanol. Eurycomanone content in *n*-butanol fraction was analyzed using HPLC. The highest concentration of eurycomanone content in parts of Tongkat ali (TA) were 6.0568 (leaves), 0.1415 (twigs), 0.0365 (top of stems), 0.0633 (middle of stems), 0.0673 (bottom of stems), 0.3533 (roots) and 5.1137 µg/mL (root barks).

Keywords: Multi-locational, Tongkat ali, eurycomanone

Abstrak

Penilaian fitokimia telah dijalankan ke atas bahagian tumbuhan Tongkat ali (*Eurycoma longifolia*). Objektif kajian ini adalah untuk mengenalpasti agro-ekologi yang mempengaruhi pertumbuhan dan kandungan eurikomanon dalam Tongkat ali yang ditanam. Sampel telah dikumpulkan di seluruh Semenanjung Malaysia seperti dari Gua Musang (Kelantan), Jengka (Pahang), Kluang (Johor) dan Beseri (Perlis). Secara tradisi, setiap bahagian pokok terutamanya akar digunakan sebagai tonik selepas bersalin, mengurangkan demam, mengubati ulser mulut dan untuk merawat cacing usus. Pelarut organik digunakan untuk pengekstrakan yang diikuti dengan pemeringkatan antara kloroform dan air. Fraksi air difraksikan lagi dengan *n*-butanol. Kandungan eurikomanon dalam fraksi *n*-butanol dianalisis menggunakan HPLC. Kepekatan paling tinggi bagi kandungan eurikomanon dikesan dalam bahagian Tongkat ali (TA) iaitu 6.0568 (daun), 0.1415 (ranting), 0.0365 (batang bahagian atas), 0.0633 (batang bahagian tengah), 0.0673 (batang bahagian bawah), 0.3533 (akar) dan 5.1137 µg/mL (kulit akar).

Kata kunci: Lokasi berlainan, Tongkat ali, eurikomanon

1.0 INTRODUCTION

Tongkat ali (*Eurycoma longifolia*) is a small forest tree belongs to the family Simaroubaceae, indigenous to the Southeast Asian rain forest areas. The medicinal properties of Tongkat ali have been known for centuries. Every part of the plant especially the roots are used for among others as an afterbirth tonic, reducing fevers, curing mouth ulcers and to treat intestinal worms [1]. Tongkat ali is more recognized by the public for its aphrodisiac properties. Generally, for healthy people, the recommended daily consumption of freeze-dried water extract of *E. longifolia* root is 50 mg to 200 mg [2].

This plant strives on many soil types, but the well-drained, organic rich soil is more preferred. It requires annual rainfall between 2,000 to 3,000 mm with temperature range of 25 to 30 °C. Natural habitat of this plant is under forest canopy, but with sufficient water and nutrients it grew well in the open area [3]. The root contains active chemical compounds such as quassinoids, canthin, alkaloids, triterpenes, eurycolactone and eurycomanone [4]. Aqueous extract of *E. longifolia* root (30, 60, 90, 150 mg/kg) has been given via oral gavage to Sprague Dawley male rats for 28 days. It enhances sexual activities and sperm quality (sperm count, motility, morphology and viability) of the treated rats [5].

2.0 EXPERIMENTAL

2.1 Plant Materials

Tongkat Ali (TA) samples were collected at four different places i.e. Gua Musang in Kelantan, Jengka in Pahang, Kluang in Johor and Beseri in Perlis. It were harvested for the whole plant and were separated according plant parts including leaves, twigs, top of stems, middle of stems, bottom of stems, roots and root barks. The fresh samples were solar dried for 1 week and dried samples were ground for eurycomanone analysis.

2.2 Analysis of Eurycomanone in Every Plant Parts of Tongkat Ali

10 g of dried and powdered leaves, twigs, top of stems, middle of stems, bottom of stems, roots and root barks were extracted using methanol. The methanol solvent was evaporated using rotary evaporator. Crude extract of methanol were fractionated using chloroform:water (1:1). Then the water fraction was fractionated with *n*-butanol also with ratio (1:1). *n*-Butanol fraction was evaporated and diluted with 1 mL methanol HPLC. The diluted samples were filtered through syringe filter 0.45 µm and then analyzed using HPLC. Each extraction was carried out in duplicate [6].

2.3 High Performance Liquid Chromatography (HPLC)

The samples were separated using gradient. Column C8 (150 mm x 4.6) Purospher STAR RP-8e (5 µm) Lichrocart (Merck). Flow 0.8 mL/min with run time 20 min. The peak of eurycomanone was detected at 254 nm. The mobile phase were prepared which comprised of 0.05% H_3PO_4 in water (A) and 0.05% H_3PO_4 in ACN (B). The program of mobile phase as follows :

Time (minutes)	Flow	A (%)	B (%)
	0.8	85	15
15.0	0.8	60	40
20.0	0.8	85	15

The eurycomanone standard (Figure 2) was found at retention time, R_t = 2.833 min. Calibration curve of standard eurycomanone at concentration (0.0325, 0.0650, 0.0975, 0.1300 mg/mL) was $Y = 1.12 \times 10^7 X + 4.02 \times 10^5$ with $R^2 = 0.99$.

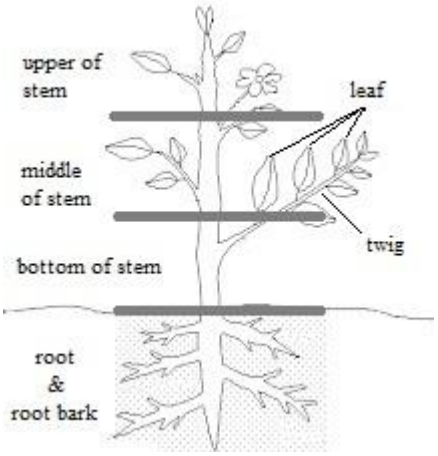

Figure 1 The parts of Tongkat ali separated to seven parts

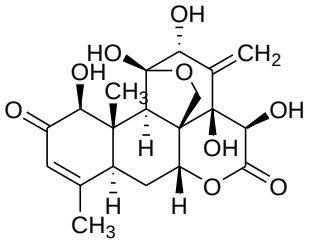

Figure 2 Chemical structure of TA major compound

3.0 RESULTS AND DISCUSSION

Different parts of Tongkat ali planted at Gua Musang, Jengka, Kluang and Beseri have been analysed using HPLC and it showed a good data of eurycomanone content. Eurycomanone is well known as a chemical marker in Tongkat ali (*E. longifolia*). The parts of TA

were separated as Figure 1 (leaf, twig, top of stem, middle of stem, bottom of stem, root and root bark). The parts that have been showed a significant eurycomanone content were leaves, bottom of stems, roots and root barks (as illustrated in bar graph in Figure 3).

At Beseri and Gua Musang, TA were planted at open and shaded area. At Beseri, the eurycomanone content of whole part plant of TA that planted at open area was higher than shaded area with the value of 1.8699 and 0.3146 μg/mL, respectively. Whereas whole part plant of TA planted at Gua Musang, at the shaded area gave the higher value of eurycomanone content than the open area with the value of 7.0579 and 6.4603 μg/mL, respectively.

At Kluang and Jengka, TA were planted on plots. The samplings were divided into 4 plots (1-4) for locations of Kluang and 3 plots (A-C) for Jengka. Between 4 plots at Kluang, Plot 2 gave the highest data of whole part plant at 6.1033 μg/mL for Kluang while plot A gave the highest data 1.3273 μg/mL for Jengka. Hence, among all four location, the highest value of eurycomanone content for whole part plant was under shaded area which at Gua Musang, Kelantan. The data were shown in Table 1 and Table 2.

The parts of TA that synonym to the TA and eurycomanone are roots and root barks. For open and shaded area at Beseri and Gua Musang, the highest eurycomanone content in total roots (roots plus root barks) was at Gua Musang with the value 1.2152 and 0.8264 μg/mL, respectively. While at Kluang and Jengka, the highest eurycomanone content in total roots at Kluang with the value 5.1259 μg/mL. Table 3 shown a detection of eurycomanone compound in TA planted at four locations.

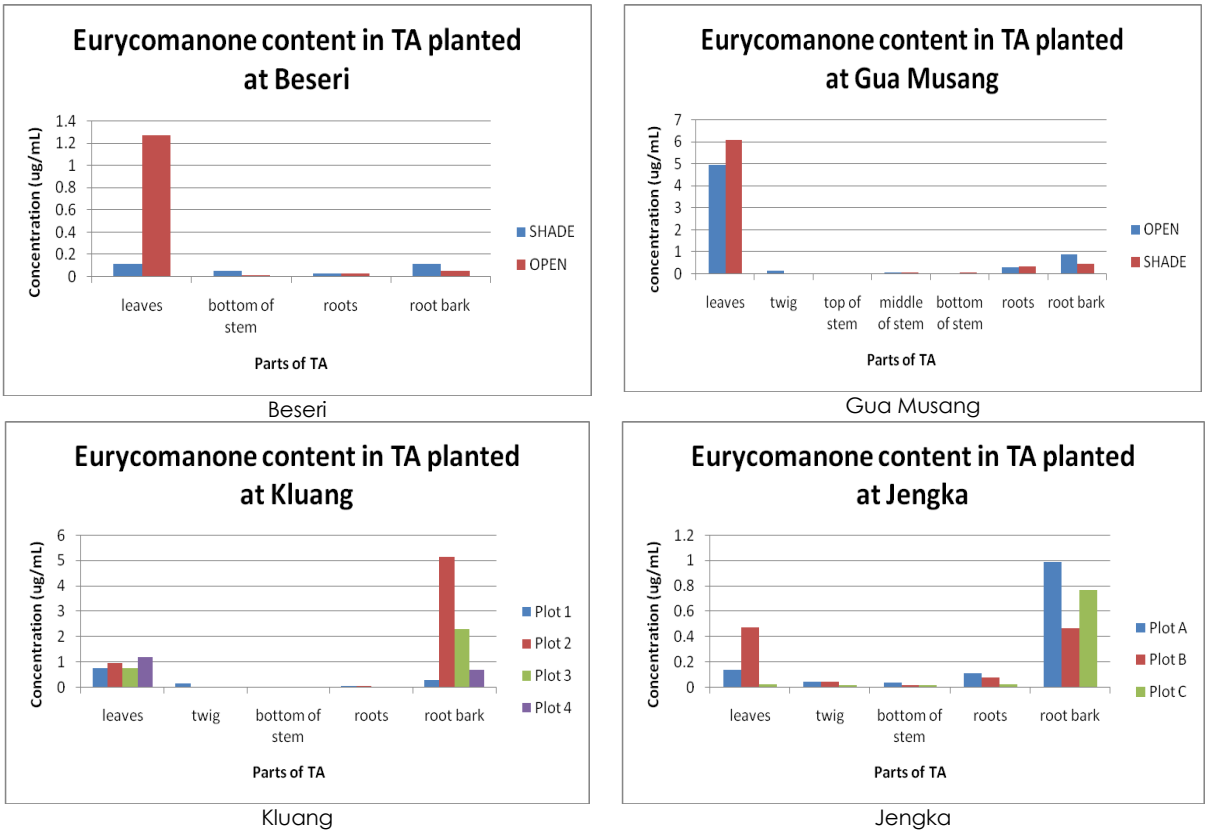

Figure 3 Bar graph of eurycomanone content in different part of TA planted at different locations

Table 1 Eurycomanone content in parts of TA planted at Beseri and Gua Musang

Location	Beseri				Gua Musang			
Parts of TA	Shade	Std deviation	Open	Std deviation	Shade	Std deviation	Open	Std deviation
Leaves	0.1146	0.0627	1.7697	0.7081	6.0568	9.3660	4.9321	2.9634
Twigs	n.d	0	n.d	0	0.0436	0.0439	0.1655	0.1800
Top of stems	n.d	0	n.d	0	0.0005	0.0003	0.0365	0.0155
Middle of stems	n.d	0	n.d	0	0.0633	0.0564	0.0597	0.0195
Bottom of stems	0.0531	0.0265	0.0134	0.0094	0.0673	0.0089	0.0513	0.0143
Roots	0.0308	0.0159	0.0311	0.0219	0.3533	0.2690	0.3123	0.2574
Root barks	0.1162	0.0883	0.0556	0.0363	0.4731	0.2543	0.9030	0.2998
Whole part plant	0.3146	0.1713	1.8699	0.7759	7.0579	9.3060	6.4603	3.4565

n.d = not detected, (unit µg/mL)

Table 2 Eurycomanone content in parts of TA planted at Kluang and Jengka

Location	Kluang				Jengka		
Parts of TA	Plot 1	Plot 2	Plot 3	Plot 4	Plot A	Plot B	Plot C
Leaves	0.7476	0.9385	0.7402	1.1929	0.1429	0.4737	0.0279
Twigs	0.1415	n.d	n.d	n.d	0.0461	0.0441	0.0210
Top of stems	0.0116	n.d	n.d	n.d	n.d	n.d	n.d
Middle of stems	0.0123	0.0380	0.0273	n.d	n.d	n.d	n.d
Bottom of stems	0.0113	0.0120	0.0142	0.0244	0.0421	0.0181	0.0199
Roots	0.0333	0.0392	0.0278	0.0394	0.1119	0.0827	0.0295
Root barks	0.2675	5.1137	2.2736	0.6845	0.9842	0.4658	0.7691
Whole part plant	1.2012	6.1033	3.0557	1.9411	1.3273	1.0844	0.8675

n.d = not detected, (unit µg/mL)

Table 3 Detection of eurycomanone content in parts of TA planted at Beseri, Gua Musang, Kluang and Jengka

Location	Parts						
	Leaves	Twigs	Top of stems	Middle of stems	Bottom of stems	Roots	Root barks
Gua Musang	✓	l.d	l.d	l.d	l.d	✓	✓
Jengka	✓	✓	n.d	n.d	l.d	✓	✓
Kluang	✓	l.d	l.d	l.d	l.d	l.d	✓
Beseri	✓	n.d	n.d	n.d	✓	✓	✓

✓ = high detected, l.d = low detected, n.d = not detected

4.0 CONCLUSION

In conclusion, all parts of Tongkat ali planted at Gua Musang, Jengka, Kluang and Beseri have been analyzed. Only leaves, bottom of stems, roots and root barks showed contain eurycomanone. From all locations data, the highest eurycomanone content in roots, bottom of stems, middle of stems and leaves were in shaded area at Gua Musang while top of stems in open area at Gua Musang. The other parts, i.e. twigs was at the highest value of eurycomanone in Plot 1 at Kluang while root barks was in plot 2 at Kluang. This is the first report on the eurycomanone compound in several part plant of TA and multi-location.

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