A Review on the Exraction Methods of Extracts and Phytochemicals from *Eurycoma longifolia* (Tongkat Ali Jack)

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Abstract— Eurycoma longifolia (Tongkat Ali Jack) is a valuable source of medicinal metabolites such as eurycomanone, which is an important ingredient in food supplement and drugs. The plant extracts and products are known for their aphrodisiac activities beside antimalarial, antiulcer, antitumor and antibacterial activities. Procedures that are involved in extraction of this plant focus on nonconventional methods such as maceration and decoction while nonconventional procedures such as microwave and ultrasonic assisted extraction methods are still to be deeply investigated. Nonconventional methods are highly nominated to increase the extract yield and subsequently the concentration of major metabolites, a concept that is investigated in the field of chemical engineering.

Keywords—Eurycoma longifolia; extraction; yield; conventional; nonconventional; separation; fractionation; elucidation;

1. INTRODUCTION

Malaysia is a global herbal producer with the attention of financial supports for research related focusing on ethnobotany, phytochemicals, processing, biochemistry, pharmacology, and clinical trials of medicinal herbs [1] and exporting products that increase sexual desire and enhance performance and general well-being [2] such as the products of *Eurycoma longifolia*, which known as Tongkat Ali.

E. longifolia is an important source of phytochemicals that are responsible for the various bioactivities of this plant, such activities include aphrodisiac, antimalarial and antitumor activities [3-5]. Studies revealed certain ranges of extract yields and increase in their yield through exhaustive procedures that are considered time and energy consuming, such as maceration, reflux and soxhlet extractions that exceeded 4% total dry yield only in few studies [6, 7], however, the extraction procedures provided increase in phytochemicals concentration by increasing the total yield and reducing the loss in conventional extraction methods [8, 9].

2. BIOLOGICAL BACKGROUND OF *EURYCOMA LONGIFOLIA*

A.Taxonamy and description

Simaroubaceae is a large pantropical family which is known to contain bitter substances called quassinoids [10], it consists of six subfamilies that is distributed into around 200 species [11]. Simarouboideae is the largest subfamily consisting most of the genera such as the genus *Eurycoma* [12, 13]. Genus *Eurycoma* includes two species found in Malaysia, *E. longifolia* and *E. apiculata* [14].

E. longifolia is an evergreen slow growing herbal plant, that reaches a maximum height of 15 to 18 m, completes maturation in up to 25 years, bears 2 to 3 cm long fruits after 2 to 3 years; leaves are pinnate, spirally arranged and reaches 25 to 38 cm long with 10 to 30 leaflets with flowers formed in large panicles [1, 15].

B. Phytochemicals of E. longifolia

As a member of the Simaroubaceae family, *Eurycoma longifolia* is rich in quassinoids, triterpenes, canthin-6-ones, squalene derivatives, biphenylneolignans and β-carboline alkaloids [16], protein, phenolic compounds, terpenoids, flavonoids and cardiac glycosides [17].

Extracts and products of *E. longifolia* are usually standardized by eurycomanone, the major quassinoid and most abundant phytochemical in the plant [18, 19]. Volatiles were proposed in authentication of *E. longifolia* extracts by certain sensors [20]. Peptides and proteins were used in profiling the extracts to determine the geographical origin of the plant [21, 22] illustrated the usefulness as biomarkers for authentication of extracts and commercial products [23-25]. Minerals were also detected in plant tissue and presented two groups of major elements (>1 g/kg) and minor element (<g/kg) accompanied with several contaminant metals such as U, Li and Al [21].

3. EXTRACTION, SEPARATION AND ELUCIDATION METHODS

Several extraction procedures were applied on various parts of *E. longifolia*, but mainly focused on the dried roots [26]. Even though a pioneer study used petroleum ether to extract a 12 g residue from 8 kg of bark [27]; Most studies focused on extracting phytochemicals from dried samples of *E. longifolia* roots, leaves and stems [26] by the usage of methanol, ethanol or water as solvents while using petroleum ether for defatting samples [28, 29]; whereas it has been perceived that methanol extraction procedures documented the highest yield extract percentages and ranged between 1.2% and 15.0% of samples weights [30-32] while ethanol yields ranged between 1.8% and 5.3% [26, 33] and water yields between 2.4% and 4.0% of sample weights [34, 35].

Industrial production of *E. longifolia* extracts depends on water extraction; but it is challenged by quantity and quality losses that could reach 35% [8, 36], this situation called for various studies on water extraction parameters and their effects on the total yield [9, 37].

Extraction is followed by fractionation of crude extracts to separate the main classes of phytochemicals with different polarities [38]; crude extracts of *E. longifolia* were partitioned by various mixtures of solvents such as dichloromethane, *n*- butanol and water [39], ethyl acetate and water [26], Chloroform and water [40], *n*-butanol and chloroform [41], *n*-butanol and water [42], diethyl ether and *n*-butanol [4], diethyl ether , *n*-butanol and water [43], ethyl acetate , *n*-butanol and water [44], *n*-butanol , chloroform and water [45] and *n*-hexane, diethyl ether , ethyl acetate, *n*-butanol [31]. Chromatographic techniques usually follow fractionation to obtain the most purified fractions of metabolites that leads to the structural elucidation stage to identify the compounds (Table 1).

Abbreviations:
MeOH: methanol; EtOH: ethanol; BuOH: butanol; CH_2Cl_2: dichloromethane: Et_2O: diethyl ether; EtOAc: ethyl acetate; MeCN: acetonitrile; CC: Column Chromatography; HPLC: High Pressure Liquid Chromatography; RP-HPLC: Reverse Phase High Pressure Liquid Chromatography; Semipreparative HPLC: Semi-preparative High Pressure Liquid Chromatography; MPLC: Medium Pressure Liquid Chromatography; UPLC-Q Trap MS: Ultra-Performance Liquid Chromatography Quadrupole trap mass spectroscopy; RP- MPLC: Reverse Phase Medium Pressure Liquid Chromatography; IR: Infra-red spectroscopy; UV: Ultra-violet spectroscopy; MS: Mass spectroscopy; LC-QTOF MS: liquid chromatography quadrupole time of flight mass spectroscopy; H-NMR: Proton nuclear magnetic resonance; C-NMR: Carbon nuclear magnetic resonance; HMBC: heteronuclear multiple bond coherence; COSY: 2D NMR correlation spectroscopy; NOESY: 2D NMR nuclear Overhauser effect; HRESIMS: High-resolution electrospray ionization mass spectrometry.

Table (1): Phytochemicals of *E. longifolia,* from plant parts to extraction, fractionation, separation and structural elucidation techniques

Plant part	Extraction solvent	Total yield (g/g) %	Fractionation	Chromatographic technique *Stationary phase **Mobile phase	Elucidation techniques	Phytochemicals	Reference
Roots	MeOH	4.9	• Et_2O • BuOH • H_2O	CC *silica gel **EtOAc-EtOH-H_2O (100: 10: 1)	IR UV MS ^{1}H NMR ^{13}C NMR	Eurycomanone	Darise et al., 1982[46]
Stems	MeOH	4.3	• CH_2Cl_2 • BuOH • H_2O	CC * silca gel **CH_2Cl_2- MeOH grad. system	^{13}C NMR	Eurycomalactone 6α-hydroxyeurycomalactone 14,15β-dihydroxyklaineanone 11-dehydroklaineanone	Itokawa et al., 1992[47]
Leaves	MeOH	4.3	• CH_2Cl_2 • MeOH	CC *silica gel *RP-MPLC **CH_2Cl_2-MeOH ** MeOH- MeCN	X ray analysis ^{1}H-NMR HMBC HR-FABMS	6-Dehydoxylongilactone 7α-hydroxyeurycomalactone Epoxyeurycomanones 12-Acetyl-13,21-dihydroeurycomanone Hydroxyklaineanone	Morita et al., 1993[48]
Leaves	EtOH	1.8	• EtOAc • H_2O	Preparative HPLC **35% MeOH	MS	Longilactone 6-dehydrolongilactone 11-dehydroklaineanone Hydroxyklaineanones	Jiwajinda et al., 2001[26]
	EtOH	2.8	• Et_2O • BuOH	Semipreparative HPLC C18 **MeCN: H_2O	^{13}C NMR	Eurycomanone Eurycomalactone Eurycomanol 9-methoxycanthin-6-one	Chan et al., 2004[4]
Stems	EtOH	5.3	• EtOAc • BuOH • H_2O	CC *silica gel **MeOH-CH_2CL_2 grad. system *MPLC **EtOAc-hexane grad. system **MeOH-benzene grad. system **MeOH-$CHCl_3$ grad. system ** MeOH-CH_2Cl_2 grad. system	^{1}H-NMR ^{13}C NMR HRESIMS NOESY	Longilactone 14-*epi*-13, 21-dihydroeurycomanone 5α-hydroxyeurycomalactone 6α-hydroxyeurycolactone E 6α,14,15 β-trihydroxyklaineanone 3α,4α-epoxyeurycomalide B	Miyake et al., 2009[33]
Roots	MeOH	4.1	• EtOAc • BuOH • H_2O	CC *Diaion HP **H_2O-MeOH grad. system	HRESIMS	2,3-dehydro-4α-hydroxylangilactone Scopolin	Teh et al., 2010 [44]
Roots	H2O	2.3 -2.6	LC–MS/MS	LC QTOF MS **H_2O-MeCN grad. System	UPLC-Q Trap MS	Eurycomanone Eurycomanol Eurycolactone A, B, C, D and E	Chua et al., 2011[18]

Plant part	Extraction solvent	Total yield (g/g) %	Fractionation	Chromatographic technique *Stationary phase **Mobile phase	Elucidation techniques	Phytochemicals	Reference
						Eurycomalactone Eurycomalides A and B Eurycomanol-2-o- β-D-glycopyranoside β -Carboline-1-propionic acid 11-Dehydroklaineanone Ailanthone Canthin-6-one Canthin-6-one-3*N*-oxide Eurylene Klaineanolide B Laurycolactones A and B	
Roots	MeOH	1.0	• EtOAc • BuOH • H_2O	CC *Silica gel **CH_2CL_2-MeOH grad. system	X ray diffraction analysis ^{1}H NMR ^{13}C NMR ^{1}H^{13}C NMR NOESY ^{1}H^{1}H COSY	Eurycomanone Eurycomanol Eurycomadilactones $\Delta^{4,5}$, 14-hydroxyglaucarubol	Meng et al., 2014[49]
Roots	MeOH	2.2	• Chloroform • BuOH • H_2O	CC *Silica gel **hexane-acetone grad. system **$CHCL_2$-acetone **$CHCL_2$-MeOH HPLC **MeCN		Eurycomanone Eurycomalactone Longilactone Eurylactone A, E, F and G Eurycomalide D and E	Park et al., 2014[45]
Roots	MeOH	8.0	• *n*-hexane • Et_2O • EtOAc • BuOH	HPLC *C18 **0.02% TFA-MeOH	UV FTIR HRESIMS	Eurycomanone Eurycomalactone Eurycolactone E Eurycomalide C 9-hydroxycanthin-6-one 9-methoxycanthin-6-one Canthin-6-one 9-O-β-D-glucoside Eeurylene fraxidin Isoaloeresin D Laurycolactone A Laurycolactone B Longilactone β-carboline	Tran et al., 2014[31]

4. CONCLUSIONS AND PROSPECT

E. longifolia is continuously proving to be a strong candidate for further therapeutic research to expand commercial purposes [50] with various products [51, 52]; here further photochemical studies of this plant are needed for explanation of the found results [53], beside further clarification on the toxicity of *E. longifolia* [54] and development of methods for standardization and quality assessment of *E. longifolia* in dietary supplements [55, 56] by using reference products or standards for direct comparison in investigating adulterated food and drug detection is needed [57].

As clinical data in support of *E. longifolia* are debated between approved and conflicted scientific views, further biochemical and clinical studies are required [58, 59]; *E. longifolia* needs more studies for its' quality and safety especially for the long term usage to fulfill the terms of the Drug Control Authority (DCA) of Malaysia [60-62].

The field of chemical engineering needs to address the processed knowledge that targets standardization and increase the phyto-medicinal value of *E. longifolia* extracts, innovation is also required to fulfill the application of nonconventional extraction methods with deeper detailed besides those which were previously investigated [36, 63] with the regard to optimization of final products. It is necessary to investigate the ability of reducing utility usage, extraction solvents and processing time with the increase of phytochemical yield.

Chemical engineering also needs to focus on the development of previously applied analytical and purification methods, and enrich this field with physical and chemical data of phytochemicals in herbs extracts by exploring novel and alternative methods for extraction, downstream process, finalization and standardization, not only on laboratory levels but also on industrial scales [64].

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REFERENCES

[1] R. Bhat and A. A. Karim. "Tongkat Ali (*Eurycoma longifolia* Jack): a review on its ethnobotany and pharmacological importance." Fitoterapia. 81(7) pp. 669-79. 2010.
[2] D. Cyranoski. "Malaysian researchers bet big on home-grown Viagra." Nat Med. 11(9) pp. 912. 2005.
[3] B.-S. Low, P. K. Das and K.-L. Chan. "Standardized quassinoid-rich Eurycoma longifolia extract improved spermatogenesis and fertility in male rats via the hypothalamic-pituitary-gonadal axis." Journal of Ethnopharmacology. 145(3) pp. 706-714. 2013.
[4] K. L. Chan, C. Y. Choo, N. R. Abdullah and Z. Ismail. "Antiplasmodial studies of *Eurycoma longifolia* Jack using the lactate dehydrogenase assay of *Plasmodium falciparum*." J Ethnopharmacol. 92(2-3) pp. 223-7. 2004.
[5] P. F. Wong, W. F. Cheong, M. H. Shu, C. H. Teh, K. L. Chan and S. AbuBakar. "Eurycomanone suppresses expression of lung cancer cell tumor markers, prohibitin, annexin 1 and endoplasmic reticulum protein 28." Phytomedicine. 19(2) pp. 138-44. 2012.
[6] P. C. Kuo, A. G. Damu, K. H. Lee and T. S. Wu. "Cytotoxic and antimalarial constituents from the roots of *Eurycoma longifolia*." Bioorg Med Chem. 12(3) pp. 537-44. 2004.
[7] C. H. Teh, V. Murugaiyah and K. L. Chan. "Developing a validated liquid chromatography-mass spectrometric method for the simultaneous analysis of five bioactive quassinoid markers for the standardization of manufactured batches of *Eurycoma longifolia* Jack extract as antimalarial medicaments." J Chromatogr A. 1218(14) pp. 1861-77. 2011.
[8] M. Mohamad, M. Ali and A. Ahmad. "Modelling the extraction of major phytochemical components from *Eurycoma longifolia*." J. Appl. Sci. 10(21) pp. 2572-2577. 2010.
[9] M. Mohamad, M. W. Ali, A. Ripin and A. Ahmad. "Effect of extraction process parameters on the yield of bioactive compounds from the roots of *Eurycoma longifolia*." Jurnal Teknologi. 60(1) pp. 51–57. 2013.
[10] J. Clayton, *Simaroubaceae*, in *Flowering Plants. Eudicots*. 2011, Springer. p. 408-423.
[11] I. A. Alves, H. M. Miranda, L. A. Soares and K. P. Randau. "Simaroubaceae family: botany, chemical composition and biological activities." Revista Brasileira de Farmacognosia. 24(4) pp. 481-501. 2014.
[12] H. P. Nooteboom. "Generic delimitation in Simaroubaceae tribus Simaroubeae and a conspectus of the genus Quassia L." Blumea-Biodiversity, Evolution and Biogeography of Plants. 11(2) pp. 509-528. 1962.
[13] H. P. Nooteboom. "Flavonols, leuco-anthocyanins, cinnamic acids, and alkaloids in dried leaves of some Asiatic and Malesian Simaroubaceae." Blumea-Biodiversity, Evolution and Biogeography of Plants. 14(2) pp. 309-315. 1966.
[14] M. Nordin. "Distribution of the population of Tongkat Ali (*Eurycoma spp.*) in Malaysia based on data taken from herbarium records." Med. Aromat. Plants. 3(2) pp. 155. 2014.

[15] H. Keng, *Orders and families of Malayan seed plants: synopsis of orders and families of Malayan gymnosperms, dicotyledons, and monocotyledons*. 1978: NUS Press. 443.
[16] S. Hajjouli, S. Chateauvieux, M. H. Teiten, B. Orlikova, M. Schumacher, M. Dicato, C. Y. Choo and M. Diederich. "Eurycomanone and eurycomanol from *Eurycoma longifolia* Jack as regulators of signaling pathways involved in proliferation, cell death and inflammation." Molecules. 19(9) pp. 14649-66. 2014.
[17] Z. Khanam, C. S. Wen and I. U. H. Bhat. "Phytochemical screening and antimicrobial activity of root and stem extracts of wild *Eurycoma longifolia* Jack (Tongkat Ali)." J King Saud Univ Sci. 27(1) pp. 23-30. 2015.
[18] L. S. Chua, N. A. Amin, J. C. Neo, T. H. Lee, C. T. Lee, M. R. Sarmidi and R. A. Aziz. "LC-MS/MS-based metabolites of *Eurycoma longifolia* (Tongkat Ali) in Malaysia (Perak and Pahang)." J, Chromatogr, B, Analy,t Technol, Biomed, Life Sci,. 879(32) pp. 3909-19. 2011.
[19] Y. Pan, K. H. Tiong, B. A. Abd-Rashid, Z. Ismail, R. Ismail, J. W. Mak and C. E. Ong. "Effect of eurycomanone on cytochrome P450 isoforms CYP1A2, CYP2A6, CYP2C8, CYP2C9, CYP2C19, CYP2E1 and CYP3A4 in vitro." J. Nat. Med. 68(2) pp. 402-406. 2014.
[20] A. K. M. Shafiqul Islam, Z. Ismail, B. Saad, A. R. Othman, M. N. Ahmad and A. Y. M. Shakaff. "Correlation studies between electronic nose response and headspace volatiles of *Eurycoma longifolia* extracts." Sensors and Actuators B: Chemical. 120(1) pp. 245-251. 2006.
[21] L. S. Chua, N. Abdul-Rahman, B. Rosidi and C. T. Lee. "Plant proteins, minerals and trace elements of *Eurycoma longifolia* (Tongkat Ali)." Nat. Prod. Res. 27(4-5) pp. 314-8. 2013.
[22] L. S. Chua, N. Abd Rahman and M. R. Sarmidi. "Plant Protein Extraction and Identification from Eurycoma longifolia by Gel Electrophoresis and Mass Spectrometry." Curr. Proteomics. 11(3) pp. 161-170. 2014.
[23] O. Asiah, M. Nurhanan and A. Mohd Ilham. "Determination of bioactive peptide (4.3 kDa) as an aphrodisiac marker in six Malaysian plants." Journal of Tropical Forest Science. 19(1) pp. 61. 2007.
[24] J. Vejayan, V. Iman, S. Foong and H. Ibrahim. "Protein markers useful in authenticating eurycoma longifolia contained herbal aphrodisiac products." Malaysian Journal of Science. 32(1) pp. 15-23. 2013.
[25] N. Bolong, S. Kumaresan, I. Saad, T. Thasan and R. Ramli. "Tongkat Ali extraction using hollow fiber membranes modified by negatively charged-modifying macromolecules." Jurnal Teknologi. 70(2) pp. 7-10. 2014.
[26] S. Jiwajinda, V. Santisopasri, A. Murakami, N. Hirai and H. Ohigashi. "Quassinoids from *Eurycoma longifolia* as plant growth inhibitors." Phytochemistry. 58(6) pp. 959-62. 2001.
[27] T. Le Van and S. Nguyen Ngoc. "Constituents of *Eurycoma longifolia* Jack." J Org Chem. 35(4) pp. 1104-9. 1970.
[28] H. H. Ang and H. S. Cheang. "Studies on the anxiolytic activity of Eurycoma longifolia Jack roots in mice." Jpn J Pharmacol. 79(4) pp. 497-500. 1999.
[29] H. H. Ang, T. H. Ngai and T. H. Tan. "Effects of *Eurycoma longifolia* Jack on sexual qualities in middle aged male rats." Phytomedicine. 10(6-7) pp. 590-3. 2003.
[30] L. B. Kardono, C. K. Angerhofer, S. Tsauri, K. Padmawinata, J. M. Pezzuto and A. D. Kinghorn. "Cytotoxic and antimalarial constituents of the roots of *Eurycoma longifolia.*" J. Nat. Prod. 54(5) pp. 1360-7. 1991.
[31] T. V. Tran, C. Malainer, S. Schwaiger, A. G. Atanasov, E. H. Heiss, V. M. Dirsch and H. Stuppner. "NF-$_{K}$B inhibitors from *Eurycoma longifolia.*" J Nat Prod. 77(3) pp. 483-8. 2014.
[32] P. B. Ngoc, T. B. Pham, H. D. Nguyen, T. T. Tran, H. H. Chu, V. M. Chau, J.-H. Lee and T. D. Nguyen. "A new anti-inflammatory β-carboline alkaloid from the hairy-root cultures of Eurycoma longifolia." Nat. Prod. Res., pp. 1-6. 2015.
[33] K. Miyake, Y. Tezuka, S. Awale, F. Li and S. Kadota. "Quassinoids from Eurycoma longifolia." J. Nat. Prod. 72(12) pp. 2135-40. 2009.
[34] T. T. Tee and H. L. Azimahtol. "Induction of apoptosis by *Eurycoma longifolia* Jack extracts." Anticancer Res. 25(3B) pp. 2205-13. 2005.
[35] R. H. Mokhtar, N. Abdullah and A. Ayob. "Effects of *Eurycoma Longifolia* Extract on the Isolated Rat Heart." IMJM. 13(1). 2014.
[36] A. Athimulam, S. Kumaresan, D. C. Y. Foo, M. R. Sarmidi and R. Aziz. "Modelling and optimization of Eurycoma longifolia water extract production." Food Bioprod. Process. 84(2) pp. 139-149. 2006.
[37] C. Foong, M. J. Kamaruddin, A. Johari, T. Abdullah, T. Amran, M. H. Hassim, K. Kidam, Z. Zakaria and W. R. W. Sulaiman. *Effect of Processing Parameters and Heating Techniques on the Extraction Yield of Eurycoma longifolia (Tongkat Ali)*. in *Advanced Materials Research*. 2015. Trans Tech Publ.
[38] J. B. Harborne *Phytochemical methods - A guide to modern techniques of plant analysis, 3rd edition*. 1998. 302.
[39] H. Itokawa, X.-R. Qin, H. Morita and K. Takeya. "C18 and C19 quassinoids from *Eurycoma longifolia.*" Journal of Natural Products. 56(10) pp. 1766-1771. 1993.
[40] H. H. Ang, Y. Hitotsuyanagi and K. Takeya. "Eurycolactones A–C, novel quassinoids from *Eurycoma longifolia.*" Tetrahedron Letters. 41(35) pp. 6849-6853. 2000.
[41] P. C. Kuo, L. S. Shi, A. G. Damu, C. R. Su, C. H. Huang, C. H. Ke, J. B. Wu, A. J. Lin, K. F. Bastow, K. H. Lee and T. S. Wu. "Cytotoxic and antimalarial beta-carboline alkaloids from the roots of *Eurycoma longifolia.*" J Nat Prod. 66(10) pp. 1324-7. 2003.
[42] E. Bedir, H. Abou-Gazar, J. N. Ngwendson and I. A. Khan. "Eurycomaoside: a new quassinoid-type glycoside from the roots of *Eurycoma longifolia.*" Chem Pharm Bull (Tokyo). 51(11) pp. 1301-3. 2003.

[43] K. L. Chan and C. Y. Choo. "The toxicity of some quassinoids from *Eurycoma longifolia.*" Planta Med. 68(7) pp. 662-4. 2002.
[44] C. H. Teh, H. Morita, O. Shirota and K. L. Chan. "2,3-Dehydro-4α-hydroxylongilactone, a novel quassinoid and two known phenyl propanoids from *Eurycoma longifolia* Jack." Food Chemistry. 120(3) pp. 794-798. 2010.
[45] S. Park, N. Nguyen Xuan, K. Phan Van, M. Chau Van, T. Bui Huu, N. Kim, H. H. Yoo, J.-H. Song, H.-J. Ko and S. H. Kim. "Five new quassinoids and cytotoxic constituents from the roots of Eurycoma longifolia." Bioorganic & Medicinal Chemistry Letters. 24(16) pp. 3835-3840. 2014.
[46] M. Darise, H. Kohda, K. Mizutani and O. Tanaka. "Eurycomanone and eurycomanol, quassinoids from the roots of *Eurycoma longifolia.*" Phytochemistry. 21(8) pp. 2091-2093. 1982.
[47] H. Itokawa, E. Kishi, H. Morita and K. Takeya. "Cytotoxic quassinoids and tirucallane-type triterpenes from the woods of *Eurycoma longifolia.*" Chem. Pharm. Bull. 40(4) pp. 1053-1055. 1992.
[48] H. Morita, E. Kishi, K. Takeya, H. Itokawa and Y. Iitaka. "Highly oxygenated quassinoids from Eurycoma longifolia." Phytochemistry. 33(3) pp. 691-696. 1993.
[49] D. Meng, X. Li, L. Han, L. Zhang, W. An and X. Li. "Four new quassinoids from the roots of *Eurycoma longifolia* Jack." Fitoterapia. 92 pp. 105-10. 2014.
[50] R. A. Aziz, M. R. Sarmidi, S. Kumaresan and Z. M. Taher. "Phytochemical processing: The next emerging field in chemical engineering–aspects and opportunities." Jurnal Kejuruteraan Kimia Malaysia 3pp. 45–60. 2003.
[51] N. Qinna, H. Taha, K. Z. Matalka and A. A. Badwan. "A new herbal combination, Etana, for enhancing erectile function: an efficacy and safety study in animals." Int J Impot Res. 21(5) pp. 315-20. 2009.
[52] S. B. Ismail, W. M. Wan Mohammad, A. George, N. H. Nik Hussain, Z. M. Musthapa Kamal and E. Liske. "Randomized clinical trial on the use of PHYSTA freeze-dried water extract of *Eurycoma longifolia* for the improvement of quality of life and sexual well-being in men." Evid Based Complement Alternat Med. 2012 pp. 429268. 2012.
[53] A. Adimoelja. "Phytochemicals and the breakthrough of traditional herbs in the management of sexual dysfunctions." Int J Androl. 23 Suppl 2 pp. 82-4. 2000.
[54] C. Wiart. "A note on the relevance of *Eurycoma longifolia* Jack to food and food chemistry." Food Chem. 134(3) pp. 1712. 2012.
[55] K. L. Chan, C. Y. Choo, H. Morita and H. Itokawa. "High performance liquid chromatography in phytochemical analysis of *Eurycoma longifolia.*" Planta Med. 64(8) pp. 741-5. 1998.
[56] Y. M. Han, M. Jang, I. S. Kim, S. H. Kim and H. H. Yoo. "Simultaneous quantitation of six major quassinoids in Tongkat Ali dietary supplements by liquid chromatography with tandem mass spectrometry." J Sep Sci. 38(13) pp. 2260-6. 2015.
[57] M. M. Said, S. Gibbons, A. C. Moffat and M. Zloh. "Rapid detection of sildenafil analogue in *Eurycoma longifolia* products using a new two-tier procedure of the near infrared (NIR) spectra database." Food Chemistry. 158 pp. 296-301. 2014.
[58] K. L. Chan. "A response to the comments on the relevance of *Eurycoma longifolia* Jack to food and food chemistry." Food Chem. 134(3) pp. 1713. 2012.
[59] C. Ulbricht, J. Conquer, K. Flanagan, R. Isaac, E. Rusie and R. C. Windsor. "An evidence-based systematic review of Tongkat Ali (*Eurycoma longifolia*) by the Natural Standard Research Collaboration." J Diet Suppl. 10(1) pp. 54-83. 2013.
[60] H. H. Ang, E. L. Lee and K. Matsumoto. "Analysis of lead content in herbal preparations in Malaysia." Hum Exp Toxicol. 22(8) pp. 445-51. 2003.
[61] H. H. Ang. "An insight into Malaysian herbal medicines." Trends in pharmacological sciences. 25(6) pp. 297-298. 2004.
[62] H. H. Ang, E. L. Lee and H. S. Cheang. "Determination of mercury by cold vapor atomic absorption spectrophotometer in Tongkat Ali preparations obtained in Malaysia." Int J Toxicol. 23(1) pp. 65-71. 2004.
[63] C. Sim, S. Kumaresan and M. R. Sarmidi. "Mass transfer coefficients of *Eurycoma longifolia* batch extraction process." Proceedings of the 18th symposium of Malaysian chemical engineers, pp. 362-367. 2004.
[64] R. Abdul Aziz, M. R. Sarmidi, S. Kumaresan and D. C. Y. Foo. "Engineering aspects of herbal and phytochemical processing: a Malaysian perspective." Jurutera, pp. 10-19. 2005.