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Review Article
Chemical Composition of *Eurycoma longifolia* (Tongkat Ali) and the Quality Control of its Herbal Medicinal Products

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Abstract

Eurycoma longifolia which is known as Tongkat Ali is commonly found in Asian countries such as Malaysia, Indonesia, Thailand, Myanmar and Cambodia. This plant is famously known for its various pharmacological activities. The plant is also reported to consist of various types of important bioactive compounds such as quassinoids, canthine-6-one alkaloids, triterpenes, squalene derivatives, β-carboline alkaloids etc which are mostly found in the root part. The presence of these important phytochemicals contributes to their different types of therapeutic effects more especially in terms of aphrodisiac properties which have resulted in a massive increase in demand and production of their Herbal Medicinal Products (HMP). These situations have resulted in the production of *E. longifolia* HMPs whose quality are questionable, which might be as a result of restricted of sources that might lead to some unethical activities carried out by suppliers and manufacturers in order to gain more profit. Therefore, this review focused on adulteration issues such as contamination and substitution of *E. longifolia* HMP. The review also includes the possible solutions on how to improve the quality of these HMP so as they can be safe for consumption. Embracing pharmacovigilance in the preparation of the HMP, proper implementation of agricultural practices such as Good Agricultural and Collection Practices (GACP) and Good Manufacturing Practices (GMP) together with the establishment of effective regulatory bodies would undoubtedly improve the quality of *E. longifolia* HMP sold in the market. The detailed knowledge about the main composition of the *E. longifolia* HMP will help to ascertain their quality, efficacy and safety as these are very important toward quality control.

Key words: *Eurycoma longifolia*, chemical composition, adulteration, quality control, pharmacological activity, aphrodisiac properties, herbal medicinal product, ethnobotanical uses

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Eurycoma longifolia is a common tropical medicinal plant which belongs to the family Simaroubaceae and is distributed in several parts of Southern Asia including Malaysia. In Malaysia, *Eurycoma longifolia* is known for its aphrodisiacs properties mostly, to improve libido and energy[1]. This plant is also known as Tongkat Ali by the Malays ethnic group due to resemblances of walking stick with twisted roots. The aphrodisiac activity of this plant is mostly contributed by root part[2]. The plant is also used traditionally by local folks for the treatment of other ailments such as bleeding, cough, fever, anti malarial treatment, ulcer, high blood pressure, fevers, etc. and all these unique activities performed by *E. longifolia* is as a result of the presence of very important bioactive compounds known as quassinoid[3,4].

Eurycoma longifolia have been confirmed in several studies to exhibit different types of pharmacological effects such as anti malarial[5-7], aphrodisiac[8-10], anti cancer[11-13], ergogenic[14,15] and toxicity effects[16]. This plant species are also reported to consists of several classes of metabolites and the major among them are the quassinoids, canthin-6-one and beta-carboline alkaloids, squalene derivatives, tirucallane-type triterpenes, biphenylneolignans[17]. Other varieties of secondary metabolites presence in this plants species include tannins, polysaccharides, glycoprotein and mucopolysaccharides[18]. This study tends to highlight the important bioactive compounds present in *E. longifolia* and the risk associated with the HMP consumption and the various possible solutions on how to improve their quality so that they can be safe for consumption.

TAXONOMY, CULTIVATION AND ETHNOBOTANICAL USES

The family of Simaroubaceae consists of about 30 genera and more than 200 species including *Eurycoma longifolia*[19]. Most of the plants in this family generally have bitter taste caused by the presence of quassinoid which contributes most of their pharmaceutical properties[20]. This plant family is generally distributed around tropical Africa, region of Australia, West Africa, Madagascar and Asia (mostly Malaysia)[2]. Only 8 genera and 10 species are found in Malaysia with *Eurycoma longifolia* been the abundant[19]. The scientific classification of *Eurycoma longifolia* plants is shown in Table 1.

There are different species of plants which are referred to as locally Tongkat Ali and these includes; *Entomophthora apiculata*, *Polyalthia bullata*, *Goniothalamus* sp. and *E. longifolia*. Among these four species, *E. longifolia* is the most used[17]. In Asia, due to the richness of biodiversity and suitable climate, *E. longifolia* plant grows naturally. They are also being called by different names depending on the countries such as Tongkat Ali in Malaysia and Singapore, Pasak bumi and Tung saw in Indonesia and Thailand respectively or Cay Ba Binh in Vietnam[21].

Table 1: Systemic classification (taxonomy) of *Eurycoma longifolia*

Classification	Names
Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Sapindales
Family	Simaroubaceae
Genus	*Eurycoma*
Species	*longifolia*

Among all the common names used to describe this plant species worldwide, Tongkat Ali or Long Jack are the most recognized names both locally and internationally. Presently, there are over 200 commercialize HMP available and most of them emphasizing on the aphrodisiac properties[21]. Due to modern lifestyle and health conscious, many Tongkat Ali products have been sold in the market usually formulated into tea, capsule, supplements, coffee and carbonated drinks, which are generally recommended to improve the health condition and libido[21,22].

Eurycoma longifolia is an evergreen slow growing plant which can attain a maximum height of 15-18 m long and start to develop fruit at an approximate age of 2-3 years. This plant species is generally believed that it might take up to 25 years before it attains its complete maturation but for commercial use or purposes, the roots are harvested at the age of four years old. The type of leaves is 10-15 cm pinnate type and consisted of about 10-30 ovate-lanceolate leaflets in spirally arranged (Fig. 1a)[23]. The fruit of *Eurycoma longifolia* is green when unripe and turns red after ripening (Fig. 1b)[24]. The flowers are dioecious in nature (male and female reproductive parts are borne or produce on different trees) and produced large panicles.

Traditionally, almost all the parts of this plant are used for various therapeutic purposes but the root part is the most valuable components that contained the highest concentration of quassinoid[25]. The quassinoid form the major bioactive which are found in this plant species[26]. Apart from the quassinoids, flavonoids, which are present in some medicinal plants such as *Melastoma decemfidum*[27], *Justicia gendarussa*[28], *Orthosiphon stamineus*[29] have also been reported to be present in *Eurycoma longifolia*[30]. Flavonoids are very important phytochemical or secondary metabolites which have strong antioxidant, anti-allergic, anti inflammatory and anti carcinogenic properties[31].

Fig. 1(a-b): (a) Ovate-lanceolate leaflets spirally arranged and (b) Foliage and cluster of ripe fruits[24]

The valuable components are used for the treatment of various ailments such as sexual insufficiency, dysentery, aches, glandular swelling, persistent fever[3]. Traditionally, the Tongkat Ali root can be consumed as a tea by boiling the root. This practice is not always easy due to the bitter taste, therefore it is suggested to mix with additives such as honey, sugar crystal and dates in order to make more palatable. According to experience traditional healers, the bitter taste of this plant species is the one responsible for their efficacy and this due to the presence of secondary metabolite[3].

CHEMICAL COMPOSITION

Numerous types of compounds have been isolated from *E. longifolia* roots such as quassinoid, coumarins, anthraquinone, triterpenes, alkanoids, flavonoid, steroids and other types of secondary metabolites[2]. Eurycomanone and eyrycomanol, two highly oxygenated quassinoid are the most abundant bioactive compounds present in root and were first isolated by Darise *et al.*[32] (Fig. 2). Other than that, three types of squalene-type triterpenes which are eurylene, 14-deacetyl eurylene and longilene peroxide were isolated from the root and determined by spectroscopic, X-ray and chemical analysis[33].

Kuo *et al.*[34] demonstrated the isolation of 3 new compounds (n-pentyl â-carboline- 1-propionate, 1-hydroxy-9-methoxycanthin-6-one and 5-hydroxymethyl-9-methoxycanthin-6-one) and 19 known β-caroline alkaloids from the roots of *E. longifolia* and characterized them by 1D and 2D NMR and by mass spectral data. Out of the three β-caroline lkaloids compound isolated, two of them (1-hydroxy-9-methoxycanthin-6-one and 1-hydroxy-9-methoxycanthin-6-one) were found to exhibit a significant cytotoxity effect against human lungs and breast cancer (MCF-7). Morita *et al.*[35] also isolated two types of Biphenylneoligans (2-hydroxy-3,2′,6′-trimethoxy-4′-(2,3-epoxy-1-hydroxypropyl)-5-(3-hydroxy-l-propenyl)-biphenyl and 2-hydroxy-3,2′-dimethoxy-4′-(2,3-epoxy-1-hydroxypropyl)-5-(3-hydroxy-1-propenyl)biphenyl) from roots part.

Three form of quassinoid (Eurycolactone D, E and F) were also isolated from the root part as reported by Ang *et al.*[36]. The isolated chemicals were determined by spectroscopic method and further confirmed using X-ray crystallography. Miyake *et al.*[37] also isolated 24 types of quassinoid from the stem part and discover 10 new compounds. The new types of quassinoid compounds found include two eurycomanone-type C_{20}, one C_{19}, one klaineanone-type C_{20} and six eurycomalactone-type C_{19} quassinoids. Epoxy group type of eurycomalactone quassinoid was first observed in this study. Evaluation of all the compounds isolated have shown to contain cytotoxicity properties against HT-1080 human fibrosarcoma cell line and the result showed that the entire compound isolated possesses cytotoxicity activity.

The use of various types of chromatographic separation, different forms of quassinoid (pasakbumin-C, 13α, 21-epoxyeurycomanone and eurylactone A) have been detected in roots of *E. longifolia* as reported by Huyen *et al.*[38]. Following the characterization by 1D-NMR and 2D-NMR, a new C_{19}-quassinoid-type glycoside was also encountered for the first time as reported by Bedir *et al.*[39]. This new C_{19}-quassinoid type (Eurycomaoside) which possessed the C (1)-glycosidation site was isolated from the roots part. The structure of this

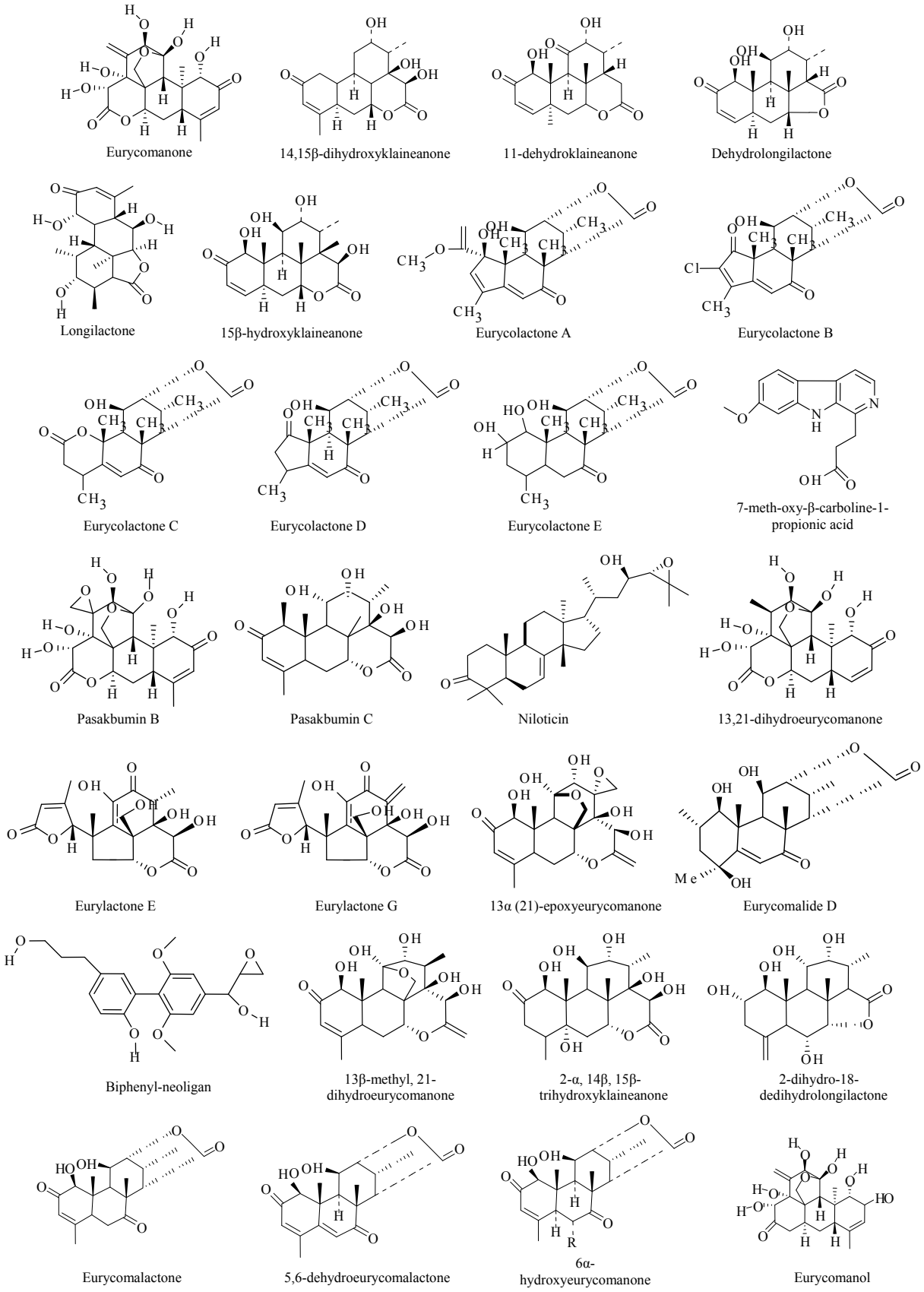

Fig. 2: Chemical structures of some of biological active compounds of *E. longifolia*

compound was determined using a combination of 1 and 2 dimensional NMR technique which includes 1H-13C-heteronuclear correlation spectroscopy (HMQC), 1H-1H-correlation spectroscopy (COSY) and 1H-13C-heteronuclear multiple-bond correlation spectroscopy (HMBC) as well as HR-ESI-FT-MS (High Resolution Electro Spray Ionization Fourier Transformation Mass Spectrometry) data.

The GC-MC analysis of methanol and water extract using solid-phase extraction showed a total number of 13 different types of volatile compounds present in 12 *E. longifolia* samples which were either in methanol and water-freeze or spray dried[40]. The results showed that higher number of 83 volatiles were extracted using a freeze dried extract technique compared to 28 volatiles using spray dried methods[40]. The quantification of volatile compounds detected were analysed by correlating the sensor response with the volatile compounds. The result showed that a total number of 8 volatiles are common in all the samples and these were used as predictable variables. The correlation between the GC-MS and QCM showed the presence of different types of compounds, including 3-phenoxy-1-propanol, 2-phenoxy-ethanol, 4-ethynyl-4-hydroxy-3, 5, 5-trimethyl-2-cyclohex-1-enone, benzoic acid, acetic acid and octanoic acid. They concluded that this novel technique (Nose approach) is very simple when compared to the GC-MS traditional method which is expensive. This approach has been shown to be very sensitive to the extent that it can detect small changes in the volatiles; therefore it can be useful for rapid detection and evaluation of herbal volatiles. The summary of the most important chemical constituent found in *E. longifolia* plant with their various activities is shown in Table 2 while their chemical structures are shown in Fig. 2.

QUALITY CONTROL OF *E. longifolia* HMP

The numerous therapeutic effects of *E. longifolia* have resulted in the development of various types of it HMP in the market, thus becoming a lucrative business. Among the different types of pharmacological properties exhibited by *E. longifolia*, the aphrodisiac properties such as the ability to improve Erectile Dysfunction (ED), male libido and sexual prowess[21,69] are among the driving force that made their HMP to be highly demanding and patronized by consumers. Different types of clinical[10,70,71] and animal studies[68,72,73] have shown that *E. longifolia* extract plays a very vital role in enhancing sexual reproductive activities. Still on the aspect of clinical studies, it was recently reported that *E. longifolia* can be used to restore testosterone levels, therefore making it to be a natural alternative for the treatment of Testosterone Deficiency Syndrome (TDS)[74]. The TDS is a deficiency which arises as a result of decline in serum testosterone level leading to fatigue, low libido, osteoporosis or erectile dysfunction and more than 80% of people suffering from this deficiency are as a result of old age.

The HMP are generally made up of varieties of self-prescribed preparation of plant origin which can be categorised as either food and beverages or dietary supplements[75,76]. As such, they existed in a form of either mono herbal component or polyherbal (mixed with other components such as in coffee and beverages).

In Malaysia, it is reported that there are more than 140 licensed manufacturers of *E. longifolia* products and these produces over 200 products together with it neighbouring countries, Indonesia and Thailand[77]. Due to the massive and rapid increased in the consumption of *E. longifolia* HMP, there are over 300 popular coffees, tonic, candies and beverages premixed with root extract commercially available in Malaysia alone to improve health and libido[24]. The popularity and consumption of *E. longifolia* HMP are increasing tremendously therefore, various safety-related issues to the public is of major concern and needs to be addressed. The common perception by people which is sometimes portrayed by mass media in marketing and health advertisement that natural or plant based product are completely safe are untrue and misleading. It is a complete error in the sense that besides their adverse effects, they can sometimes produce life-threatening consequences. This common assumption of "natural" is "harmless" has resulted in a massive increase in the patronizing of these HMPs in developed countries in recent decades[75]. The safety information about most of the constituents of medicinal plants responsible for their pharmacological and possible toxic effects is mostly scanty. This is due to the fact that out the humongous number of medicinal plants that existed as herbal remedies, only few numbers of them have been subjected to in-depth analytical, pharmacological and clinical studies.

The misconception of tagging everything natural as being safe or harmless has resulted in less regulation control in the market of such products[78]. Consequently, quality control issues such as contamination by pathogenic microbials or natural toxins, misidentification, adulteration with toxic compounds, standardization of dose, methods of processing and product uniformity are becoming major problems for medicinal plants and their HMP[78,79].

Several literatures[80-82] have shown that contamination of HMP with certain toxic substances can result to a wide range of adverse or unexpected side effects and this can result in serious injuries and even death. The contamination or

Table 2: Summary of chemical compounds and pharmacological activities of different parts of *E. longifolia*

Isolated chemical compounds	Plant parts	Pharmacological activity	References
Eurycomanone	Roots	Cytotoxicity towards human lung cancer (A549) Cytotoxic effect only in MCF-7. Cytotoxicity towards Hela cell lines Strong anti-malarial activities Cytotoxicity towards MGC-803 Cytotoxicity towards KB-V1 cell lines Antiangiogenic activity *In vitro* antimalarial activity against *P. falciparum* strain TD7	Darise *et al.*[32], Chan *et al.*[41], Kardono *et al.*[42], Wong *et al.*[43], Yusuf *et al.*[44], Park *et al.*[45], Kuo *et al.*[46], Al-Salahi *et al.*[47] and Meng *et al.*[48]
Eurycomanol	Roots	Cytotoxicity towards human gastric cancer BGC-823 cell Cytotoxicity towards human lung cancer (A549) *In vitro* antimalarial activity against *P. falciparum* strain TD7 Cytotoxicity towards duman gastric cancer MGC-803 Cytotoxicity towards cytotoxic effect only in MCF-7 Anti-malarial activity	Darise *et al.*[32], Ang *et al.*[49], Meng *et al.*[48] and Yusuf *et al.*[44]
14,15p-dihydroxyklaineanone	Roots	Not confirmed	Chan *et al.*[50]
13α(21)-epoxyeurycomanone	Roots	Cytotoxicity towards Hela cell lines Anti-leukaemic activity against P388 cell lines Antiangiogenic activity	Morita *et al.*[51], Al-Salahi *et al.*[47], Huyen *et al.*[38] and Park *et al.*[45]
13β,18-dihydroeurycomanol	Roots	Anti-malarial activity	Chan *et al.*[50] and Ang *et al.*[49]
Triperpenes 14-deaacteyl eurylene, longilene peroxide Eurylene Teurilene	Stem	Cytotoxicity against lymphocytes leukemia	Morita *et al.*[33], Itokawa *et al.*[52] and Sheikh[53]
Eurylene	Root	Not confirm	Itokawa *et al.*[52]
9-methoxycanthin-6-one 9-methoxycanthin-6-one-N-oxide 9-hydroxycanthin-6-one 9-hydroxycanthin-6-one-N-oxide	Roots	Cytotoxicity towards human lung cancer (A549) Cytotoxicity towards human breast cancer (MCF-7) Cytotoxicity towards KB and murine lymphocytic leukemia (P-388) line cells Cytotoxicity towards colon, fibrosarcoma and melanoma cell lines Anti-malarial activity NF-κB inhibitors	Kardono *et al.*[42], Kuo *et al.*[34] and Tran *et al.*[54]
Laurycolactones A and B (C_{18})	Root	NF-κB inhibitors	Itokawa *et al.*[55], Miyake *et al.*[37] and Tran *et al.*[54]
Laurycolactone B	Root	Antiprotozoal activity	Girish *et al.*[56]
18-dehydro-6α-hydroxyeurycomalactone	Root	*In-vitro* antimalarial activity against *P. falciparum* strain TD7	Yusuf *et al.*[44]
6-dehydroxylongilactone	Root and leaves	Cytotoxicity towards human lung cancer (A549) Cytotoxicity towards human breast cancer (MCF-7) Anti-leukaemic activity against P388 cell lines	Morita *et al.*[51] and Kuo *et al.*[46]
5-iso-eurycomadilactone	Root	Cytotoxicity towards MGC-803 Cytotoxicity towards human breast cancer (MCF-7)	Meng *et al.*[48]

Table 2: Continue

Isolated chemical compounds	Plant parts	Pharmacological activity	References
Eurycomalactone	Root	Cytotoxicity towards human lung cancer (A549) Cytotoxicity towards human breast cancer (MCF-7) Cytotoxicity towards Hela cell lines Cytotoxic activity against HT-1080 cell line Anti-protozoal activity Potent activity against murine colon carcinoma colon (26-L5), Lewis lung carcinoma (LLC) and human lung adenocarcinoma (A549)	Itokawa *et al.*[57], Kuo *et al.*[34], Park *et al.*[45], Miyake *et al.*[37,58] and Girish *et al.*[56]
Eurycolactone D Eurycolactone E Eurycolactone F	Root and stem	NF-κB inhibitors	Ang *et al.*[36], Miyake *et al.*[37] and Tran *et al.*[54]
7-methoxy-β-carboline- 1-propionic acid	Root	Anti-malarial activities	Kardono *et al.*[42]
7α-hydroxyeurycomalactone	Leaves	Anti-leukaemic activity against P388 cell lines	Morita *et al.*[51]
6α-acetoxy-14,15β-dihydroxyklaineanone	Leaves	Anti-leukaemic activity against P388 cell lines	Morita *et al.*[51]
Biphenylneolignans	Stem	Not confirm	Morita *et al.*[35]
Eurylactone A	Root	Not confirm	Huyen *et al.*[38]
Eurycomaoside	Root	Not confirm	Bedir *et al.*[39]
Pasakbumin A	Roots	Anti-ulcer activity	Tada *et al.*[59]
Pasakbumin C	Roots	Cytotoxic activities against A549 Cytotoxicity towards human breast cancer (MCF-7)	Tada *et al.*[59], Kuo *et al.*[46] and Huyen *et al.*[38]
Pasakbumin B	Roots	Strong anti-malarial activities Anti-ulcer activity	Kuo *et al.*[46] and Tada *et al.*[59]
Pasakbumin D	Root	Not confirm	Tada *et al.*[59]
12-acetyl-13,21-dihydroeurycomanone	Leaves	Anti-leukaemic activity against P388 cell lines	Morita *et al.*[51]
Canthin-6-one 9-O-β-glucopyranoside	Roots	Cytotoxic activities against A549 Cytotoxicity towards Human breast cancer (MCF-7)	Kuo *et al.*[46]
12-epi-11-dehydroklaineanone	Leaves	Not confirm	Jiwajinda *et al.*[60] and Miyake *et al.*[37]
6α-hydroxyeurycomalactone	Roots	Cytotoxic activity against leukaemia P388 Cytotoxic activity against human KB cancer cells	Chan *et al.*[61] and Itokawa *et al.*[57]
Eurylactone E Eurylactone F Eurylactone G Eurycomalide D Eurycomalide E	Roots	Not confirm	Park *et al.*[45]
11-dehydroklaineanone	Leaves	Potent plasmodicidal activity	Jiwajinda *et al.*[62], Tran *et al.*[54]
Eurycomalide A Eurycomalide B	Roots	Cytotoxic activities against lung human cancer (A549) Cytotoxic activities against human breast cancer (MCF-7)	Kuo *et al.*[63] and Miyake *et al.*[37]
Longilactone		Cytotoxic activities against A549 Cytotoxic activities against MCF-7 Cytotoxicity towards Hela cell lines	Itokawa *et al.*[57], Muhamad *et al.*[64], Park *et al.*[45], and Kuo *et al.*[46]

Table 2: Continue

Isolated chemical compounds	Plant parts	Pharmacological activity	References
15β-O-acetyl-14-hydroxyklaineanone	Leaves	Potent plasmodicidal activity	Jiwajinda *et al.*[62]
14,15β-dihydroxyklaineanone	Roots and leaves	Cytotoxic activities against A549 Cytotoxic activities against MCF-7 Cytotoxicity towards Hela cell lines NF-κB inhibitors Cytotoxic activity against HT-1080 cell line Anti-tumour activity	Itokawa *et al.*[57], Park *et al.*[45], Tran *et al.*[54], Miyake *et al.*[37], Kuo *et al.*[46] and Jiwajinda *et al.*[62]
13,21-dihydroeurycomanone	Roots	NF-κB inhibitors Anti-protozoal activity Cytotoxic activity against HT-1080 cell line	Morita *et al.*[65], Tran *et al.*[54], Girish *et al.*[56], and Miyake *et al.*[37]
13,21-dehydroeurycomanone	Roots	Cytotoxicity towards Hela cell lines	Park *et al.*[45] and Tran *et al.*[54]
Niloticin Dihydroniloticin Piscidinol Bourjotinolone 3-episapeline Melianone Hispidone	Root	Cytotoxic activity against leukaemia P388 Cytotoxic activity against human KB cancer cells	Itokawa *et al.*[57]
14-epi-13,21-dihydroeurycomanone	Stems	Not confirm	Miyake *et al.*[37]
6α,14,15β-trihydroxyklaineanone	Stems	Not confirm	Miyake *et al.*[37]
5,6-dehydroeurycomalactone	Roots	Cytotoxic activity against human KB cancer cells Cytotoxic activity against P388 cells	Itokawa *et al.*[57]
7-methoxy-β-carboline-1-propionic acid	Root	Anti-malarial activities	Tada *et al.*[59] and Kardono *et al.*[42]
β-carboline-1-propionic acid	Roots	Anti-malarial activities	Tada *et al.*[59], Kardono *et al.*[42] and Yusuf *et al.*[44]
9-methoxycanthin-6-one 9,10-dimethoxycanthin-6-one	Roots	NF-κB inhibitors	Tran *et al.*[54]
13β, 21-dihydroxyeurycomanone	Root	Not confirm	Morita *et al.*[65], Park *et al.*[45] and Meng *et al.*[48]
Canthin-6-one	Roots	Anti-malarial activities Cytotoxic activities against lung Human cancer (A549) Cytotoxicity towards Human breast cancer (MCF-7) Anti-protozoal activity	Kuo *et al.*[46] and Girish *et al.*[56]
15β-hydroxyklaineanone	Leaves	Anti-tumour activity	Jiwajinda *et al.*[62]
Dehydrolongilactone	Leaves	Anti-tumor promoting activities	Jiwajinda *et al.*[62]
2,3-dehydro-4a-hydroxylongilactone 2,3-dihydroxy-1-(40-hydroxy-30-methoxyphenyl)-propan-1-one Scopolin	Root	Not confirm	Teh *et al.*[66]
7-methoxyinfractin	Root	Not confirm	Han *et al.*[67]
9-hydroxycanthin-6-one	Root	NF-κB inhibitors Induces penile erection and delays ejaculation	Tran *et al.*[54] and Chan *et al.*[68]

adulteration of HMP with heavy metals such as lead, arsenic, mercury, cadmium and copper are always of great concern. However, some of the *E. longifolia* HMP which are sold in the market have been reported to contain some of these common heavy metals.

In the recent years, there has been a big rise in herbal-based product consumptions due to their claimed health benefits both locally and globally. However, due to the challenging financial climate, adulteration of HMP with closely related species can be risky as this can result to unexpected side effects[82]. The HMP available to consumers in the marketplace substituted with these undeclared ingredients on the labels might pose a major threat to consumer safety. Adulteration in some cases can be lethal if it is substituted with toxic adulterants.

Thus, consumers have become increasingly concerned about the HMP authenticity and the quality of the purchased. High incidence of mislabelling reports had also increased the customer awareness towards this problem. The consumer may have the misconception that natural or plant base products are safe or harmless has resulted in less regulation control in the market sample of such products[73]. Consequently, quality control such as contamination by pathogenic microbes or natural toxins, incorrect species used, adulteration with toxic compounds/adulterants, standardization of dose, methods of processing and product uniformity are becoming major problems for medicinal for HMP[73, 74]. The HMP contamination with heavy metals is often common, especially in Asian countries[83]. Analytical investigation has shown that contamination of HMP with heavy metals can occur because of (1) Environmental condition where the plant are grown, (2) During drying and processing step, (3) Improper storage and transportation condition and finally manufacturing process which the HMP are produced. Several literatures[75,77] have shown that consumption HMP with certain toxic substance can result to a wide range of adverse or unexpected side effects and this can result in serious health problems, even death.

Ang *et al*.[84] reported the presence of lead in a large number of *E. longifolia* HMP consisting of both Malaysian Registered Products (MRP) and Malaysian Unregistered Products (MUP) obtained from the various pharmaceuticals store, health store using atomic absorption spectrophotometer. Among of the 100 HMP analyzed, 8% of sample contained lead between 12.24-20.72 ppm and this does not complied with the quality requirement for traditional medicine in Malaysia which should not exceed 5 ppm as stipulated by the Drug Control Agency (DCA). The further disturbing issue, none of these HMP was registered with DCA. The high amount of lead is toxic to the human body as it may affect some vital tissues such as kidney, nervous system, liver, bone, heart and reproductive system[85].

One year later, Ang *et al*.[86] reported another heavy metal contamination, mercury in a number of *E. longifolia* HMP. From their reports, 36% of the over-the-counter HMP sold possess higher (0.52-5.30 ppm) mercury content and this was not in conformity with the standard quality requirement of Malaysia which should not exceed 0.5 ppm. The report of the study also shows that 5% out of the 36% HMPs were not registered with DCA. High concentration of mercury in human body is detrimental as it can cause many damages such as nervous tissue and brain damage, damages of chromosomes and damages of the reproductive functionality[85].

Therefore, the quality of *E. longifolia* HMP sold in the market needs to be regulated from manufacturing processes until production; hence protecting the consumer. This can be effectively conducted by firstly regulating the correct use of raw material used in the production[87]. This will ensure that the customer gets the best of their purchased. From plant cultivation to the point of production of the HMP, there are several factors which can influence the quality of these HMP. Because of the high demand of *E. longifolia* HMP in the market, unscrupulous suppliers may perform some unethical activities. Adulteration is the addition of inferior, improper or extraneous ingredient to make an impure HMP and this has generated a lot of questions about their efficacy and safety. This is due to the fact that the quality assurances of any HMP solely relied on the proper authentication and correct use of raw plant materials (ingredients). The breakage of this essential rule as a result of adulteration can lead to undesirable or adverse effect after consumption. According to the WHO, this activity is not only a fraud but a threat to the consumer health[88].

The different types of adulteration which are commonly observed in *E. longifolia* HMP include; (1) Use of stem instead of the roots as most of the active compounds are found in roots part, (2) Addition of the high amount of fillers such as rice and wheat and finally and (3) Include less concentration of *E. longifolia* extract/raw material to gain more profit[89,90] . In some cases, *E. longifolia* HMP sold in the market may not even contained any bioactive compounds which are responsible for the therapeutic properties of the plant[91]. Therefore, authentication of *E. longifolia* HMP is the most basic quality step to ensure that they have met the required pharmacopeia standard as this is very crucial towards quality control.

In a study conducted by Norhidayah *et al*.[90], investigation of the authenticity from 41 *E. longifolia* HMP showed that 12 were classified as international products (IR), 16 are Malaysian

Registered Product (MRP) and 13 Malaysian Unregistered Product (MUP) using HPLC analysis. The results from Norhidayah *et al*.[90] study demonstrated that eurycomanone was only detected in 58.9% of the total HMP. The Malaysian standard (2011) stated that the level of eurycomanone in *E. longifolia* should be approximately 0.8-1.5 % (w/v)[76,90]. Out of the 24 HMP which contained the standard, only 11 (0.84-8.48% w/v) fulfilled the Malaysian standard criteria while 9 (1.6-8.48 % w/v) of the HMP is above the set criteria. Norhidayah *et al*.[90] also proved that out of the eight premixed coffee samples allegedly contain *E. longifolia* in their label, eurycomanone was not detected.

In the same year, Han *et al*.[92] developed liquid chromatography with tandem mass spectrometry method to validate or confirmed the presence of bioactive compound in *E. longifolia* HMP (capsule and tablets) using six major quassinoids (eurycomanone, 13, 21-dihydroeurycomanone, 13α (21)-epoxyeurycomanone 14,15β-dihydroxyklaineanone, eurycomalactone and longilactone). It is interesting to note that out of the seven HMP sample tested in their study, the target bioactive compounds in two of the samples (TB2 and TB3) were either present below the detection or detected with only trace amount while other contain great amount of the quassinoids. This inconsistency or variation in the amount of bioactive compounds present among the product is alarming due to the fact that *E. longifolia* extract was reported to be toxic in male rats[93]. Therefore, for standardization purpose, consistency of the bioactive compounds needs to be ensured. The absent or present of poor quassinoid content in some of the *E. longifolia* HMP have resulted in the addition of other unlisted illegal drugs compounds. The detection of synthetic phosphodiesterase-5 inhibitors (PDE-5i) such as sildenafil,vardenafil and tadalafil in HMP that are tagged or labelled as natural products that enhances sexual libido and increase testosterone level is becoming interestingly common[94-96]. A recent survey by Gilard *et al*.[97] confirmed that 61% from 150 HMP advertised to enhance sexual libido were found to be adulterated with synthetic PDE-5i, this finding represents an alarming risk to the consumers health.

However, even though the uses of some synthetic PDE-5i were approved in the treatment of erectile dysfunction but their usage must be under medical supervision. This is due to the fact that their interaction with certain drugs can cause negative effects. Hyperlipidemia, hypertension and ischemic heart diseases are mostly treated with drugs containing nitrates, therefore patient suffering from such ailment should not take synthetic PDE-5i as this may drastically lower their blood pressure[98]. Therefore, the unknowingly consumption of such HMP that contain undeclared synthetic PDE-5i may pose a serious health threat to the patient.

As a result of the growing trend towards intentional adulteration nowadays, some studies have revealed the adulteration of *E. longifolia* HMP with certain type's synthetic drugs. Said *et al*.[99] reported detection of sildenafil-like compound in some of the *E. longifolia* HMP purchased online. Champagne and Emmel[100] also reported such kind of deliberate adulteration and this type of situation is not only a fraud but a threat to public health.

Therefore the misconception among the consumers regarding natural terms that are safe should be the utmost priority. The reality is that "safety" and "natural" are not synonymous[101]. However, to overcome these hurdles despite the facts that each country has its own unique regulatory body, the government regulatory authorities involved should enact new policies in addition to the existing ones so as to ensure that *Eurycoma longifolia* HMP, which are introduced into the market are safe for public consumption. As quality control or quality assurance relied on the use of authentic raw materials, the effort of government in addition to research institutes and herbal industries should voluntarily establish the use of advance techniques as quality control standard. A reliable molecular approach which utilizes the use of DNA barcoding in addition to other techniques can also be used for the authentication of raw materials used in the manufacturing of *Eurycoma longifolia* HMP as most of them are sourced from the wild[102].

As suggested by Sahoo *et al*.[103], quality issue of the HMP arises from cultivation until to the manufacturing and finally circulation. The quality assurance of the HMP can also be controlled through the implementation of the various agricultural practices recommended by WHO to ensure their efficacy and safety[103]. Agricultural practice such as Good Agricultural and Collection Practices (GACP) is a very critical in the collection of herbal materials and inadequate of quality control can be overcome if properly adopted. External issues which involved misidentification of plant species, contamination by heavy metals and other toxic substances can be effectively minimized through the adoption of GACP practice by ensuring that the product collected are safe for consumption. Another great advantage of implementing GACP is that it will help to assist in ensuring that the quality of the starting herbal materials is reproducible[104].

Since after the issue of GACP guideline by WHO in 2003 for medicinal plants, other countries such as Japan, China and

European unions have also developed their national and regional GACP in order to effectively improve that quality of HMP which are disseminated to the public[105]. Their developed guidelines ensures that the soil and water which they will use for the cultivation of the medicinal plants are within the standard limit or free from toxic substances such as heavy metals, pesticides, microbes or mycotoxins. Implementation of Good Manufacturing Practices (GMP) together with post marketing quality assurance surveillance can also improve the quality of the *E. longifolia* HMP sold in the market. The initiation of pharmacovigilance practice which is a very essential and common tool used for assessing the safety of synthetic drugs can also be developed for HMP with little modifications. The pharmacovigilance will assist in providing reliable information about the safety or risk of using *E. longifolia* HMP. This will promote safe use of *E. longifolia* HMP and protect the public from the adverse effect of the HMP. The establishment of precise systemic methodologies and clinical trials by researchers, manufacturers and various regulating bodies as reported by Mukherjee *et al.*[78] can assist in the production of high quality *E. longifolia* HMP which will be are introduced to the market. This will help to increase the consumers confident in the consumption of *E. longifolia* HMP.

CONCLUSION

The proper tightening and implementation of the existing regulation in the various countries must be the way forward by ensuring that *E. longifolia* HMP that circulates in the market are of good quality, safe, efficacious and sustainable. Furthermore, the use of pharmacovigilance to provide reliable information about the safety and risk of using *E. longifolia* HMP coupled with the proper implementation of various agricultural practices, DNA-based and metabolite-based method will help to minimize problems associated with adulteration, contamination and substitution. This will also make the *E. longifolia* HMP more credible, reliable and acceptable, therefore increasing consumer's confidence.

SIGNIFICANCE STATEMENT

This study highlighted the importance of different bioactive compounds present in *Eurycoma longifolia* and the consequences that might arise as a result of compromising the quality of their HMP sold in the market. This study proposed the need to have stringent regulatory measures on such HMPs, implementation of various agricultural practices and the use of multidisciplinary approaches to their quality assurance and quality control. This will tremendously help to reduce the exposure of consumers to various health risks.

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REFERENCES

1. Talbott, S.M., J.A. Talbott, A. George and M. Pugh, 2013. Effect of Tongkat Ali on stress hormones and psychological mood state in moderately stressed subjects. J. Int. Soc. Sports Nutr., Vol. 10. 10.1186/1550-2783-10-28.
2. Alves, I.A., H.M. Miranda, L.A. Soares and K.P. Randau, 2014. Simaroubaceae family: Botany, chemical composition and biological activities. Rev. Brasil. Farmacogn., 24: 481-501.
3. Bhat, R. and A.A. Karim, 2010. Tongkat Ali (*Eurycoma longifolia* Jack): A review on its ethnobotany and pharmacological importance. Fitoterapia, 7: 669-679.
4. Ayob, Z., W. Alina and A.S. Azman, 2013. Potential of tissue cultured medicinal plants in Malaysia. J. Teknologi, 62: 111-117.
5. Al-Adhroey, A.H., Z.M. Nor, H.M. Al-Mekhlafi and R. Mahmud, 2010. Ethnobotanical study on some Malaysian anti-malarial plants: A community based survey. J. Ethnopharmacol., 132: 362-364.
6. Wernsdorfer, W.H., S. Ismail, K.L. Chan, K. Congpuong and G. Wernsdorfer, 2009. Activity of *Eurycoma longifolia* root extract against *Plasmodium falciparum in vitro*. Wiener Klinische Wochenschrift, 121: 23-26.
7. Sholikhah, E.N., M.A. Wijayanti and L.H. Nurani, 2008. Stage specificity of pasak bumi root (*Eurycoma longifolia* Jack) isolate on *Plasmodium falciparum* cycles. Med. J. Malaysia, 63: 98-99.
8. Wahab, N.A., M.M. Norfilza, W.N.H.A. Halim and S. Das, 2010. The effect of *Eurycoma longifolia* Jack on spermatogenesis in estrogen-treated rats. Clinics, 65: 93-98.
9. Tambi, M.I.B.M. and M.K. Imran, 2010. *Eurycoma longifolia* Jack in managing idiopathic male infertility. Asian J. Androl., 12: 376-380.
10. Ismail, S.B., W.M.Z.W. Mohammad, A. George, N.H.N. Hussain, Z.M.M. Kamal and E. Liske, 2012. 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. Evidence-Based Complement. Alternat. Med., Vol. 2012.

11. Tee, T.T., Y.H. Cheah and L.P.A. Hawariah, 2007. F16, a fraction from *Eurycoma longifolia* jack extract, induces apoptosis via a caspase-9-independent manner in MCF-7 cells. Anticancer Res., 27: 3425-3430.
12. Nurhanan, M., H. Azimahtol, A. Azizol, C. YuShyun, M. Mohtar, V. Subramaniam and N. Yunos, 2002. Cytotoxicity studies of *Eurycoma longifolia* extracts against a panel of human cancer cell lines. Proceedings of the Seminar on Medicinal and Aromatic Plants, Towards Modernisation of Research and Technology in Herbal Industries, July 24-25, 2001, Forest Research Institute Malaysia (FRIM), pp: 124-127.
13. Tee, T.T. and H.L.P. Azimahtol, 2005. Induction of apoptosis by *Eurycoma longifolia* Jack extracts. Anticancer Res., 25: 2205-2213.
14. Muhamad, A.S., C.C. Keong, O.F. Kiew, M.R. Abdullah and C.K. Lam, 2010. Effects of *Eurycoma longifolia* jack supplementation on recreational athlete's endurance running capacity and physiological responses in the heat. Int. J. Applied Sports Sci., 22: 1-19.
15. Tambi, M.I. and A.A. Kadir, 2006. *Eurycoma longifolia* Jack: A potent adaptogen in the form of water-soluble extract with the effect of maintaining men's health. Asian J. Androl., 8: 49-50.
16. Razak, M.F.A. and K.E. Aidoo, 2011. Toxicity studies of *Eurycoma longifolia* (Jack)-Based remedial products. Asian J. Pharm. Clin. Res., 4: 23-27.
17. Mohamed, A.N., J. Vejayan and M.M. Yusoff, 2015. Review on *Eurycoma longifolia* pharmacological and phytochemical properties. J. Appl. Sci., 15: 831-844.
18. Varghese, C.P., C. Ambrose, S.C. Jin, Y.J. Lim and T. Keisaban, 2013. Antioxidant and anti-inflammatory activity of *Eurycoma longifolia* Jack, a traditional medicinal plant in Malaysia. Int. J. Pharmaceut. Sci. Nanotechnol., 5: 1875-1878.
19. Ang, H.H., Y. Hitotsuyanagi and K. Takeya, 2000. Eurycolactones A-C, novel quassinoids from *Eurycoma longifolia*. Tetrahedron Lett., 41: 6849-6853.
20. Muhammad, I., E. Bedir, S.I. Khan, B.L. Tekwani and I.A. Khan *et al*., 2004. A new antimalarial quassinoid from *Simaba orinocensis*. J. Natural Prod., 67: 772-777.
21. Low, B.S., S.B. Choi, H.A. Wahab, P.K. Das and K.L. Chan, 2013. Eurycomanone, the major quassinoid in *Eurycoma longifolia* root extract increases spermatogenesis by inhibiting the activity of phosphodiesterase and aromatase in steroidogenesis. J. Ethnopharmacol., 149: 201-207.
22. Vejayan, J., V. Iman, F. Siew-Liang and H. Ibrahim, 2013. Protein markers useful in authenticating *Eurycoma longifolia* contained herbal aphrodisiac products. Malaysian J. Sci., 32: 15-23.
23. Zanoli, P., M. Zavatti, C. Montanari and M. Baraldi, 2009. Influence of *Eurycoma longifolia* on the copulatory activity of sexually sluggish and impotent male rats. J. Ethnopharmacol., 126: 308-313.
24. Lim, T., 2016. *Eurycoma longifolia*. In: Edible Medicinal and Non-Medicinal Plants, Lim, T. (Eds.). Springer, Germany, pp: 250-276.
25. Alfaqeh, H.H., 2014. Effect of long-term use of *Eurycoma longifolia* Jack on histopathological changes in the liver in rats. Int. Med. J. Malaysia, 13: 29-33.
26. Ariff, A.S.T., I.N. Soelaiman, J. Pramanik and A.N. Shuid, 2012. Effects of *Eurycoma longifolia* on testosterone level and bone structure in an aged orchidectomised rat model. Evidence-Based Complement. Altern. Med. 10.1155/2012/818072.
27. Jamalnasir, H., A. Wagiran, N.A. Shaharuddin and A.A. Samad, 2013. Isolation of high quality RNA from plant rich in flavonoids, *Melastoma decemfidum* Roxb ex. Jack. Aust. J. Crop Sci., 7: 911-916.
28. Amani, B.A. and M.S. Faezah, 2013. High quality cDNA synthesis and amplification of chalcone synthase gene (CHS) from *Justicia gendarussa* burm. F. J. Teknol., 64: 1-4.
29. Almatar, M., Z. Rahmat and F.M. Salleh, 2013. Preliminary morphological and anatomical study of *Orthosiphon stamineus*. Indian J. Pharmaceut. Biol. Res., 1: 1-6.
30. Khanam, Z., C.S. Wen and I.U.H. Bhat, 2015. Phytochemical screening and antimicrobial activity of root and stem extracts of wild *Eurycoma longifolia* Jack (Tongkat Ali). J. King Saud Univ.-Sci., 27: 23-30.
31. Sulaiman, M., H.I. Tijani, B.M. Abubakar, S. Haruna, Y. Hindatu, J.N. Mohammed and A. Idris, 2013. An overview of natural plant antioxidants: Analysis and evaluation. Adv. Biochem., 1: 64-72.
32. Darise, M., H. Kohda, K. Mizutani and O. Tanaka, 1982. Eurycomanone and eurycomanol, quassinoids from the roots of *Eurycoma longifolia*. Phytochemistry, 21: 2091-2093.
33. Morita, H., E. Kishi, K. Takeya, H. Itokawa and Y. Iitaka, 1993. Squalene derivatives from *Eurycoma longifolia*. Phytochemistry, 34: 765-771.
34. Kuo, P.C., A.G. Damu, K.H. Lee and T.S. Wu, 2004. Cytotoxic and antimalarial constituents from the roots of *Eurycoma longifolia*. Bioorg. Med. Chem., 12: 537-544.
35. Morita, H., E. Kishi, K. Takeya and H. Itokawa, 1992. Biphenylneolignans from wood of *Eurycoma longifolia*. Phytochemistry, 31: 3993-3995.
36. Ang, H.H., Y. Hitotsuyanagi, H. Fukaya and K. Takeya, 2002. Quassinoids from *Eurycoma longifolia*. Phytochemistry, 59: 833-837.
37. Miyake, K., Y. Tezuka, S. Awale, F. Li and S. Kadota, 2009. Quassinoids from *Eurycoma longifolia*. J. Natural Prod., 72: 2135-2140.
38. Huyen, L.T., N.X. Nhiem, V.K. Thu, B.H. Tai and H.L.T. Anh *et al*., 2015. Quassinoids from *Eurycoma longifolia*. Vietnam J. Chem., 53: 82-85.
39. Bedir, E., H. Abou-Gazar, J.N. Ngwendson and I.A. Khan, 2003. Eurycomaoside: A new quassinoid-type glycoside from the roots of *Eurycoma longifolia*. Chem. Pharm. Bull., 51: 1301-1303.

40. Islam, A.K.M.S., Z. Ismail, B. Saad, A.R. Othman, M.N. Ahmad and A.Y.M. Shakaf, 2006. Correlation studies between electronic nose response and headspace volatiles of *Eurycoma longifolia* extracts. Sensor Actuators B: Chem., 120: 245-251.
41. Chan, K.L., M.J. O'Neill, J.D. Phillipson and D.C. Warhurst, 1986. Plants as sources of antimalarial drugs. Part 3[1] *Eurycoma longifolia*. Planta Med., 2: 105-107.
42. Kardono, L.B.S., C.K. Angerhofer, S. Tsauri, K. Padmawinata, J.M. Pezzuto and A.D. Kinghorn, 1991. Cytotoxic and antimalarial constituents of the roots of *Eurycoma longifolia*. J. Nat. Prod., 54: 1360-1367.
43. Wong, P.F., W.F. Cheong, M.H. Shu, C.H. Teh, K.L. Chan and S. AbuBakar, 2012. Eurycomanone suppresses expression of lung cancer cell tumor markers, prohibitin, annexin 1 and endoplasmic reticulum protein 28. Phytomedicine, 19: 138-144.
44. Yusuf, H., Mustofa, M.A. Wijayanti, R.A. Susidarti, P.B.S. Asih, Suryawati and Sofia, 2013. A new quassinoid of four isolated compounds from extract *Eurycoma longifolia*, jack roots and their *in-vitro* antimalarial activity. Int. J. Res. Pharmaceut. Biomed. Sci., 4: 728-734.
45. Park, S., N.X. Nhiem, P. Van Kiem, C. Van Minh and B.H. Tai *et al*., 2014. Five new quassinoids and cytotoxic constituents from the roots of *Eurycoma longifolia*. Bioorg. Med. Chem. Lett., 24: 3835-3840.
46. Kuo, P.C., L.S. Shi, A.G. Damu, C.R. Su and C.H. Huang *et al*., 2003. Cytotoxic and antimalarial β-carboline alkaloids from the roots of *Eurycoma longifolia*. J. Natl. Prod., 66: 1324-1327.
47. Al-Salahi, O.S.A., C. Kit-Lam, A.M.S.A. Majid, F.S.R. Al-Suede and S.A.M. Saghir *et al*., 2013. Anti-angiogenic quassinoid-rich fraction from *Eurycoma longifolia* modulates endothelial cell function. Microvasc. Res., 90: 30-39.
48. Meng, D., X. Li, L. Han, L. Zhang, W. An and X. Li, 2014. Four new quassinoids from the roots of *Eurycoma longifolia* Jack. Fitoterapia, 92: 105-110.
49. Ang, H.H., K.L. Chan and J.W. Mak, 1995. *In vitro* antimalarial activity of quassinoids from *Eurycoma longifolia* against Malaysian chloroquine-resistant *Plasmodium falciparum* isolates. Planta Medica, 61: 177-178.
50. Chan, K.L., S.P. Lee, T.W. Sam, S.C. Tan, H. Noguchi and U. Sankawa, 1991. 13β, 18-dihydroeurycomanol, a quassinoid from *Eurycoma longifolia*. Phytochemistry, 30: 3138-3141.
51. Morita, H., E. Kishi, K. Takeya, H. Itokawa and Y. Iitaka, 1993. Highly oxygenated quassinoids from *Eurycoma longifolia*. Phytochemistry, 33: 691-696.
52. Itokawa, H., E. Kishi, H. Morita, K. Takeya and Y. Iitaka, 1991. Eurylene, a new squalene-type triterpene from *Eurycoma longifolia*. Tetrahedron Lette., 32: 1803-1804.
53. Sheikh, N.S., 2014. Synthetic endeavours towards oxasqualenoid natural products containing 2, 5-disubstituted tetrahydrofurans-eurylene and teurilene. Natural Prod. Rep., 31: 1088-1100.
54. Tran, T.V., C. Malainer, S. Schwaiger, A.G. Atanasov, E.H. Heiss, V.M. Dirsch and H. Stuppner, 2014. NF-κB Inhibitors from *Eurycoma longifolia*. J. Nat. Prod., 77: 483-488.
55. Itokawa, H., X.R. Qin, H. Morita and K.J. Takeya, 1993. C18 and C19 quassinoids from *Eurycoma longifolia*. J. Nat. Prod., 56: 1766-1771.
56. Girish, S., S. Kumar and N. Aminudin, 2015. Tongkat Ali (*Eurycoma longifolia*): A possible therapeutic candidate against *Blastocystis* sp. Parasit. Vect., Vol. 8.
57. Itokawa, H., E. Kishi, H. Morita and K. Takeya, 1992. Cytotoxic quassinoids and tirucallane-type triterpenes from the woods of *Eurycoma longifolia*. Chem. Pharmaceut. Bull., 40: 1053-1055.
58. Miyake, K., F. Li, Y. Tezuka, S. Awale and S. Kadota, 2010. Cytotoxic activity of quassinoids from *Eurycoma longifolia*. Natural Prod. Commun., 5: 1009-1012.
59. Tada, H., F. Yasuda, K. Otani, M. Doteuchi, Y. Ishihara and M. Shiro, 1991. New antiulcer quassinoids from *Eurycoma longifolia*. Eur. J. Med. Chem., 26: 345-349.
60. Jiwajinda, S., V. Santisopasri, A. Murakami, N. Hirai and H. Ohigashi, 2001. Quassinoids from *Eurycoma longifolia* as plant growth inhibitors. Phytochemistry, 58: 959-962.
61. Chan, K.L., Y. Iitaka, H. Noguchi, H. Sugiyama, I. Saito and U. Sankawa, 1992. 6α-Hydroxyeurycomalactone, a quassinoid from *Eurycoma longifolia*. Phytochemistry, 31: 4295-4298.
62. Jiwajinda, S., V. Santisopasri, A. Murakami, M. Kawanaka and H. Kawanaka *et al*., 2002. *In vitro* anti-tumor promoting and anti-parasitic activities of the quassinoids from *Eurycoma longifolia*, a medicinal plant in Southeast Asia. J. Ethnopharmacol., 82: 55-58.
63. Kuo, P.C., C.R. Su, A.G. Damu and T.S. Wu, 2004. Eurycomalin A, a new dimeric dihydrobenzofuran from *Eurycoma longifolia*. Heterocycles, 63: 2123-2129.
64. Muhamad, S., A. Pihie, J. Latif, C.K. Rha and T.G. Sambandan, 2011. Induction of apoptosis in MCF-7 via the caspase pathway by longilactone from *Eurycoma longifolia* Jack. Res. Pharm. Biotechnol., Vol. 3. (In Press).
65. Morita, H., E. Kishi, K. Takeya, H. Itokawa and O. Tanaka, 1990. New quassinoids from the roots of *Eurycoma longifolia*. Chem. Lett., 19: 749-752.
66. Teh, C.H., H. Morita, O. Shirota and K.L. Chan, 2010. 2, 3-Dehydro-4α-hydroxylongilactone, a novel quassinoid and two known phenyl propanoids from *Eurycoma longifolia* Jack. Food Chem., 120: 794-798.
67. Han, L.F., J.L. Geng, D.L. Meng, N. Li and X. Li, 2011. Isolation and identification of chemical constituents from *Eurycoma longifolia* jack. J. Shenyang Pharmaceut. Univ., Vol. 7.
68. Chan, K.L., B.S. Low, C.H. Teh and P.K. Das, 2009. The effect of *Eurycoma longifolia* on sperm quality of male rats. Natural Prod. Commun., 4: 1331-1336.
69. Azmin, S.N.H.M., Z. Abdul Manan, S.R.W. Alwi, L.S. Chua, A.A. Mustaffa and N.A. Yunus, 2016. Herbal processing and extraction technologies. Separat. Purificat. Rev., 45: 305-320.

70. Tambi, M., M. Imran and R. Henkel, 2012. Standardised water soluble extract of *Eurycoma longifolia*, Tongkat Ali, as testosterone booster for managing men with late onset hypogonadism?. Andrologia, 44: 226-230.
71. Udani, J.K., A.A. George, M. Musthapa, M.N. Pakdaman and A. Abas, 2014. Effects of a proprietary freeze-dried water extract of *Eurycoma longifolia* (Physta) and *Polygonum minus* on sexual performance and well-being in men: A randomized, double-blind, placebo-controlled study. Evidence-Based Complement. Alternat. Med., Vol. 2014.
72. Ang, H.H., K.L. Lee and M. Kiyoshi, 2004. 2004. Sexual arousal in sexually sluggish old male rats after oral administration of *Eurycoma iongifolia* Jack. J. Basic Clin. Physiol. Pharmacol., 15: 303-309.
73. Solomon, M.C., N. Erasmus and R.R. Henkel, 2014. *In vivo* effects of *Eurycoma longifolia* Jack (Tongkat Ali) extract on reproductive functions in the rat. Andrologia, 46: 339-348.
74. George, A. and R. Henkel, 2014. Phytoandrogenic properties of *Eurycoma longifolia* as natural alternative to testosterone replacement therapy. Andrologia, 46: 708-721.
75. Kosalec, I., J. Cvek and S. Tomic, 2009. Contaminants of medicinal herbs and herbal products. Arch. Ind. Hyg. Toxcicol., 60: 485-501.
76. Khari, N., A.F. Aisha and Z. Ismail, 2014. Reverse phase high performance liquid chromatography for the quantification of eurycomanone in *Eurycoma longifolia* Jack (Simaroubaceae) extracts and their commercial products. Trop. J. Pharmaceut. Res., 13: 801-807.
77. Ang, H.H., 2004. An insight into Malaysian herbal medicines. Trends Pharmacol. Sci., 25: 297-298.
78. Mukherjee, P.K., S. Bahadur, S.K. Chaudhary, A. Kar and K. Mukherjee, 2015. Quality Related Safety Issue-Evidence-Based Validation of Herbal Medicine Farm to Pharma. In: Evidence Based Validation of Herbal Medicine, Mukherjee, P.K. (Ed.). Elsevier, Netherlands, USA, 405-425.
79. Bugno, A., A.A.B. Almodovar, T.C. Pereira, T.D.J.A. Pinto and M. Sabino, 2006. Occurrence of toxigenic fungi in herbal drugs. Braz. J. Microbiol., 37: 47-51.
80. Ernst, E., 2002. Toxic heavy metals and undeclared drugs in Asian herbal medicines. Trends Pharmacol. Sci., 23: 136-139.
81. Chau, W., R. Ross, J.Y. Li, T.Y. Yong, S. Klebe and J.A. Barbara, 2011. Nephropathy associated with use of a Chinese herbal product containing aristolochic acid. Med. J. Aust., 194: 367-368.
82. Muller, D., W. Weinmann and M. Hermanns-Clausen, 2009. Chinese slimming capsules containing sibutramine sold over the internet: A case series. Dtsch. Arztebl. Int., 106: 218-222.
83. Zhang, J., B. Wider, H. Shang, X. Li and E. Ernst, 2012. Quality of herbal medicines: Challenges and solutions. Complement. Ther. Med., 20: 100-106.
84. Ang, H.H., E.L. Lee and K. Matsumato, 2003. Analysis of lead content in herbal preparations in Malaysia. Hum. Exp.Toxicol., 22: 445-451.
85. Locatelli, C., D. Melucci and M. Locatelli, 2014. Toxic metals in herbal medicines: A review. Curr. Bioact. Compounds, 10: 181-188.
86. Ang, H.H., E.L. Lee and H.S. Cheang, 2004. Determination of mercury by cold vapor atomic absorption spectrophotometer in tongkat ali preparations obtained in Malaysia. Int. J. Toxicol., 23: 65-71.
87. Calixto, J.B., 2000. Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents). Braz. J. Med. Biol. Res., 33: 179-189.
88. Newmaster, S.G., M. Grguric, D. Shanmughanandhan, R. Sathishkumar and S. Ragupathy, 2013. DNA barcoding detects contamination and substitution in North American herbal products. BMC Med., Vol. 11. 10.1186/1741-7015-11-222.
89. Chan, K., 2003. Some aspects of toxic contaminants in herbal medicines. Chemosphere, 52: 1361-1371.
90. Norhidayah, A., J. Vejayan and M.M. Yusoff, 2015. Detection and quantification of eurycomanone levels in Tongkat Ali herbal products. J. Applied Sci., 15: 999-1005.
91. Abdul Rahman, A.S., M.M.S. Yap, A.Y.M. Shakaff, M.N. Ahmad, Z. Dahari, Z. Ismail and M.S. Hitam, 2004. A microcontroller-based taste sensing system for the verification of *Eurycoma longifolia*. Sensors Actuator. B: Chem., 101: 191-198.
92. Han, Y.M., M. Jang, I.S. Kim, S.H. Kim and H.H. Yoo, 2015. Simultaneous quantitation of six major quassinoids in Tongkat Ali dietary supplements by liquid chromatography with tandem mass spectrometry. J. Separat. Sci., 38: 2260-2266.
93. Shuid, A.N., L.K. Siang, T.G. Chin, N. Muhammad, N. Mohamed and I.N. Soelaiman, 2011. Acute and subacute toxicity studies of *Eurycoma longifolia* in male rats. Int. J. Pharmacol., 7: 641-646.
94. Zhu, X., S. Xiao, B.O. Chen, F. Zhang and S. Yao *et al*., 2005. Simultaneous determination of sildenafil, vardenafil and tadalafil as forbidden components in natural dietary supplements for male sexual potency by high-performance liquid chromatography-electrospray ionization mass spectrometry. J. Chromatogr. A, 1066: 89-95.
95. Zou, P., S.S.Y. Oh, P. Hou, M.Y. Low and H.L. Koh, 2006. Simultaneous determination of synthetic phosphodiesterase-5 inhibitors found in a dietary supplement and pre-mixed bulk powders for dietary supplements using high-performance liquid chromatography with diode array detection and liquid chromatography-electrospray ionization tandem mass spectrometry. J. Chromatogr. A, 1104: 113-122.
96. Singh, S., B. Prasad, A.A. Savaliya, R.P. Shah, V.M. Gohil and A. Kaur, 2009. Strategies for characterizing sildenafil, vardenafil, tadalafil and their analogues in herbal dietary supplements, and detecting counterfeit products containing these drugs. TrAC Trends Anal. Chem., 28: 13-28.

97. Gilard, V., S. Balayssac, A. Tinaugus, N. Martins, R. Martino and M. Malet-Martino, 2015. Detection, identification and quantification by 1 H NMR of adulterants in 150 herbal dietary supplements marketed for improving sexual performance. J. Pharmaceut. Biomed. Anal., 102: 476-493.
98. Stief, C.G., S. Uckert, A.J. Becker, M.C. Truss and U. Jonas, 1998. The effect of the specific phosphodiesterase (PDE) inhibitors on human and rabbit cavernous tissue *in vitro* and *in vivo*. J. Urol., 159: 1390-1393.
99. Said, M.M., S. Gibbons, A.C. Moffat and M. Zloh, 2014. Rapid detection of sildenafil analogue in *Eurycoma longifolia* products using a new two-tier procedure of the near infrared (NIR) spectra database. Food Chem., 158: 296-301.
100. Champagne, A.B. and K.V. Emmel, 2011. Rapid screening test for adulteration in raw materials of dietary supplements. Vibrat. Spectrosc., 55: 216-223.
101. Ekor, M., 2014. The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol., Vol. 4.
102. Abubakar, B.M., F.M. Salleh, M.S.S. Omar and A. Wagiran, 2017. DNA barcoding and chromatography fingerprints for the authentication of botanicals in herbal medicinal products. Evidence-Based Complement. Alternat. Med., Vol. 2017.
103. Sahoo, N., P. Manchikanti and S. Dey, 2010. Herbal drugs: Standards and regulation. Fitoterapia, 81: 462-471.
104. Wah, C.L., S.C. Hock and T.K. Yun, 2012. Current scientific status and regulatory control of traditional/herbal medicinal products: Globalization challenges. Pharmaceut. Eng., Vol. 32.
105. Fan, T.P., G. Deal, H.L. Koo, D. Rees and H. Sun *et al*., 2012. Future development of global regulations of Chinese herbal products. J. Ethnopharmacol., 140: 568-586.