PRELIMINARY DEVELOPMENT OF ENTERPRISING TONGKAT ALI HERBAL CAPSULE AUTHENTICATED TO BE ORIGINAL BY UNIVERSITI MALAYSIA PAHANG SCIENTISTS

PROJECT LEADER: DR JAYA VEJAYAN

COLLABORATORS:

PROF DATO' DR MASHITAH BINTI MOHD YUSOFF

&

DR AINI NORHIDAYAH BINTI MOHAMED

UNIVERSITI MALAYSIA PAHANG

2018

ACKNOWLEDGEMENT

The project leader and his collaborators wish to convey their utmost appreciations to the management of the university for the financial support to enable the successful completion of this project. Next, the project leader is indebted to the effort shown by the two collaborators throughout this project. The project itself could not have been completed without the contributions of many individuals included the undergraduates and postgraduate students, technical personnel, Dean and Deputy Dean Research of the Faculty of Industrial Sciences & Technology, testing laboratories in UMP and USM, National Pharmaceutical Regulatory Agency at the Ministry of Health, executives of Dong Foong Manufacturing Sdn Bhd, Madam Angie and Wan Ling of BioTree Biotechnology Sdn Bhd, the entrepreneur student candidate i.e.Fatin Hasnina binti Hafiz, indigenous person i.e. Cik Aida binti Yun etc.

ABSTRACT

Eurycoma longifolia is widely known for its aphrodisiac properties. Due to this, abundant of the *E. longifolia* based products are sold in the market without any specific regulation. To control the quality and authentication of *E. longifolia* product, the common marker used to authenticate *E. longifolia* is eurycomanone. This project is to develop *E. longifolia* product according to Malaysian Standard as well as to obtain approval of MAL number from National Pharmaceutical Regulations Agency (NPRA). HPLC analysis has been used as quantitative and qualitative analysis to determine the presence and quantity of eurycomanone content in water extract of *E. longifolia* derived Tongkat Ali Capsules by Indigenous People (TAC-IP) and Tongkat Ali Capsule in House Formulation (TAC-IHF). TAC-IP is herbal capsules manufactured by the indigenous people of Kampung Bukit Cermin, Perak without NPRA approval and sold to customers for the past 15 years. While TAC-IHF is a prototype manufactured in this project specifically to be standardized and tested prior to NPRA registration fulfillments. The result indicated the quantity of eurycomanone in *E. longifolia* root water extract is 1.24 (w/w) %. The formulation of TAC-IHF has been done by standardizing the percentage of eurycomanone content into 0.80 w/w %. This percentage was chosen as it is in accordance to the Malaysian Standard which stated to be in the range of 0.8 - 1.5 w/w% of eurycomanone. The formulation of TAC-IHF was to adopt the method used by the Indigenous people i.e. by sieving the powdered root. Several requirements of NPRA was fulfilled which is heavy metal test, microbial load test, uniformity of weight test and labeling process. Besides that, the additional test was conducted which is uniformity of eurycomanone content in each capsule by HPLC. It was conducted to gauge the percentage of eurycomanone in each capsule to be similar. Lastly, a survey to investigate the acceptance and effectiveness of TAC-IP was carried out. The proposed method and analyzing showed TAC-IHF to be standardized and in accordance to the Malaysian standard and partially fulfilled requirements of NPRA. Next, the powdered material weighing 1.5kg was submitted to Dong Foong Manufacturing Sdn Bhd (hired contract manufacturer) for the MAL number approval. After a series of documentation,

GMP capsulation, testing and many more procedures the manufactured capsules submitted to NPRA. Currently, the capsules obtained clearance at early phases and pending MAL number approval.

PREFACE

This project been designed to be three-way pyramid collaborations between indigenous people, scientists in UMP and an entrepreneur. The indigenous people expected to shared their knowledge in preparing their capsule preparations as well as sourcing original Tongkat Ali roots from the wild. The scientist ensured the roots were pulverized appropriately, authenticated, standardized, done preliminary tests likely to pass at NPRA such as microbial load, heavy metal, pH, moisture content etc. Also conducted a survey on the prototype with a small group of volunteer men to gauge the acceptance of the Tongkat Ali product. Once confident of the outcome of the tests a contract manufacturer was chosen from a number of manufacturers available in the market. Dong Foong Manufacturing Sdn Bhd was entrusted as the contract manufacturer to assist towards the successful approval of MAL number. The normal duration required to obtain MAL number from NPRA ranges from 6 to 10 months. The contract manufacturer duties included consultation, documentation, gamma radiation services, GMP capsulation, preliminary testing, submission and further dealings with NPRA and once MAL number been approved the capsules can only be manufactured within their GMP facility. The initial contribution of the student entrepreneur included in registering a company, obtain token required for NPRA registration from MSC Trustgate.com Sdn Bhd, setting up of a company website and perform preliminary sales of the prototype to the test the market for the product. It is expected once the MAL number been approved, the student is to make necessary efforts to market and achieve enough sales to continue the three-way pyramid collaborations with sufficient profit to the university and herself.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

NPRA	National Pharmaceutical Regulation Agency
MOH	Minister of Health
GMP	Good Manufacturing Practice
TAC-IHF	Tongkat Ali Capsules in House Formulation
MS	Malaysian Standard (MS2409:2011)
MAL	**Meditag Hologram labels**
TAC-IP	Tongkat Ali Capsule by Indigenous People
HPLC	High Performance Liquid Chromatography
DRGD	Drug Registration Guidance Document
CFU	Colony forming unit
TAMC	Total Aerobic Microbial Count
TYMC	Total Yeast and Mould Count

CHAPTER 1

INTRODUCTION

1.1 Background of Study

The studies of traditional plant, that has been used as herbal medicine which is famous in Malaysia, known as *Eurycoma longifolia* Jack *(E. longifolia*). This traditional plant most found in Southeast Asian countries such as Indonesia, Vietnam and Malaysia. Besides, it also can be found in a specific regions of Thailand, Cambodia, and Myanmar (Rowshanaie, Jaafar, Edaroyati, Wahab, & Rowshanaie, 2014). While in Indonesia known as "Pasak Bumi" and in Vietnam known as "Cay Ba Binh" (Chen et al., 2014).

Tongkat Ali (*Eurycoma longifolia)* wherein Ali means walking stick because of the presence of long twisted roots. The plant extract, especially at roots part, are unique used (traditionally) for men to help in enhancing testosterone levels. The plant extract (specific to roots part) have been used for its unique antimalarial, anti-diabetic, antiulcer, anticancer, anti-pyretic, cytotoxic and aphrodisiac properties. Roots extract (as a tea) have been used traditionally to fevers (mainly due to the presence of quassinoids) reduce blood pressure, and fatigue (Bhat & Karim, 2010).

Besides *Eurycoma longifolia* jack, there are three other plant species also known as "Tongkat Ali" which is *Polyathia bullata*, *Entomophthora apiculate* and *Goniothalamus sp*. *Eurycoma longifolia* is a shrub tree that will grows up to 10 meter in height, with green colour long leaves. The leaves are in pinnate shape and the flowers are dioecious, whereas the fruit is ovoid-shaped that turns to dark brown or dark red colour when its ripe (Mohd Effendy, Mohamed, Muhammad, Naina Mohamad, & Shuid, 2012).

A broad range of chemical compounds have been identified by isolated and characterized from *Eurycoma longifolia*, particularly from roots. The chemical compounds isolated from roots are include eurycomanol, eurycomanone, eurycomalactone, canthine-6-one alkaloid, 9-hydroxycathine-6-one, 14.15 β-

hydroxyklaineanone, quanissoids, phenolic components, tannins, and triterpenes. The *Eurycoma longifolia* also popular with its aphrodisiac property due its ability to stimulate the production or section of androgen hormones, especially testosterone. Furthermore, this plant has possess medicinal values such as anticoagulant for complications during child birth (Chen et al., 2014)(Mohd Effendy et al., 2012).

Recently, there has been great increase in demand for this plant and over 200 Tongkat Ali product are available in health market due to its aphrodisiac property. The products are available either as capsule (which some may mixed with other aphrodisiac herbal plants), as an addition mixed with coffee or in certain health product (supplement) as a replacement or ginseng or also in raw crude powder (roots) (Bhat & Karim, 2010).

Due to the high demanding production of Tongkat Ali, many product of Tongkat Ali have been commercialized. Unfortunately, the production of Tongkat Ali due to irresponsible traders may contain generic Viagra or other less popular plants such as *Tribulus terrestris* and has low quality in term of purity of aphrodisiac property. Some may even use the stem parts of Tongkat Ali instead of using the root parts. Based on the scientific research, the authentic *Eurycoma longifolia* can only be found in Asian country. The current project is to focus on the standardized product development by following the Malaysia Standard (MS) and National Pharmaceutical Regulation Agency (NPRA) (Pharmaceutical, Bureau, & Health, 1915).

1.2 Problem Statement

Tongkat Ali is the most famous herbal medicine in Asia. It is due to the high aphrodisiac properties in the root of Tongkat Ali. The development of Tongkat Ali product has been commercialized either in the capsule form, chips form or in the pre-mix coffee. The high demand of Tongkat Ali product due to health effect cause the high production of Tongkat Ali in the market. Unfortunately, the popularity of Tongkat Ali been exploited by certain manufactures and traders.

There has been issues about the use of fake Tongkat Ali in the production of capsule or medicine. While some other may also added the stem instead of the roots (which is has more aphrodisiac capabilities) or they may also replace the Tongkat Ali roots with other plant (*Tribulus terrestris*) which has similar aphrodisiac properties to the Tongkat Ali but less popular.

All these issues prompted the need of local university researchers to intervene in establishing authenticated Tongkat Ali products. The current project is the pre-commercialization efforts of a Tongkat Ali product i.e. mainly Tongkat Ali root obtained from indigenous people of Malaysia. Efforts done to derive a prototype containing herbal capsule of Tongkat Ali of our own. All efforts relevant to develope herbal capsuled Tongkat Ali conducted within this project.

In Malaysia, NPRA of Minister of Health (MOH) provides stringent guidelines for the formulation and approval of herbal capsules. This projects intends to develop Tongkat Ali capsules adhering appropriately towards this guideline. The fundamental regulatory requirements compulsory to be achieved currently by any herbal preparations included microbial load, heavy metal, GMP production and appropriate labelling procedures. These parameters are impossible to be achieved by the indigenous people due to their financial constraints and expertise. Hence, the indigenous people been very kind to assist us toward our endeavour in this project.

1.3 Objectives

- To develop the Tongkat Ali Capsules in House Formulation (TAC-IHF) by adapting the Indigenous People techniques and evaluate the potential marketability and effectiveness.
- To determine the safety of the products properties and achieve the standardization of Tongkat Ali product according to Malaysia Standard (MS) and National Pharmaceutical Regulatory Agency (NPRA).
- To evaluate the potential marketability and effectiveness of Tongkat Ali Capsules prototypes.

- To fulfil all requirements necessary in obtaining a MAL number

1.4 Scope of Study

Starts with the reliable sources of Tongkat Ali (*E. longifolia*) roots from indigenous people. The authentic Tongkat Ali can be determined by the simple preliminary bitter taste test on site. The bitter taste produced due to the quassinoid contain in the roots. Besides, the Tongkat Ali Capsule by Indigenous People (TAC-IP) was purchased from the indigenous people and has been tested according the Malaysia Standard (MS) and National Pharmaceutical Regulation Agency (NPRA). The extraction of *E. longifolia* was done by heating the powder under reflux process. The purpose of the extraction was to make sure that the eurycomanone content in the Tongkat Ali water extract follow the MS which is in the range of 0.8 – 1.5 w/w % in water extract form. HPLC technique has been used as a standard analysis to determine the set amount of Tongkat Ali capsule. Thus, it was also used to determine the amount of eurycomanone in the capsule that follow the MS and NPRA.

After the quality and quantity of the eurycomanone has been verified the conventional basic capsule marker was used to formulate the capsule by aseptic manner. The capsule was developed by adapting the indigenous people techniques. The capsule was formulated from the raw material as in powder form (pure Tongkat Ali root) without the other additional compounds or excipient. The parameters and testing included the microbial load, heavy metal, and uniformity of dosage unit, in term of weight variation and content uniformity (eurycomanone). Lastly, the labelling and packaging was carried out to fulfil the requirement for herbal medicine information as recommended by NPRA guidelines. Before this, crude extract was done by adding excipient but after discussion with the indigenous people pulverisation was decided instead for the preparation of powdered raw material. A professional designer hired to derive a label in accordance to NPRA regulations. The current project also will prove the effectiveness of Tongkat Ali Capsule by Indigenous People (TAC-IP) and evaluate the production of Tongkat Ali Capsule in House Formulation (TAC-IHF). The work ends by

providing the finished powdered Tongkat Ali authenticated to be original to a service provider for further evaluations towards obtaining NPRA approval.

CHAPTER 2

LITERATURE REVIEW

2.1 Taxonomy and Classification

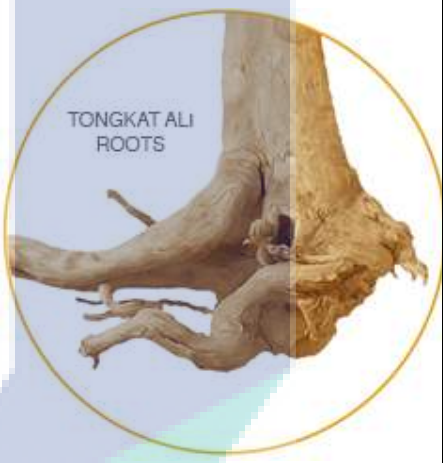
Table 2.1.1 The taxonomy and classification of *Eurycoma longifolia*.

Source: Adapted from (Bhat & Karim, 2010)

Kingdom	Plantae
Order	Sapindales
Family	Simaroubaceae
Genus	*Eurycoma*
Species	*longifolia*

According to the research, there has been reported that there were four different species of Tongkat Ali plant: *Eurycoma longifolia, Entomophthora apiculate, Polyathia bullata* and *Goniothalamus sp*. In traditional medicine purpose, Eurycoma longifolia is more preferred and used than other three species. The scientific name of Tongkat Ali is *Eurycoma longifolia*. Tongkat Ali also known as "Malaysia Ginseng" and popularly known as "Asian Viagra". There are several popular names of Tongkat Ali, which are: Long Jack, Local Ginseng, Tongkat Ali Hitam, Pasak Bumi, Pokok Syurga, Setunjang Bumi, Bedara Pahit, Payung Ali, Tongkat Baginda, Pokok Jelas, Natural Viagra, Cay Ba Binh, Jelaih and Ian-Don. Tongkat Ali is a flowering plant from the Simaroubaceae

family. It is native to the jungles and rainforests of Malaysia, Myanmar, Thailand, and Indonesia. Tongkat Ali is grown in Southeast Asia near the equator of the original tropical moist forest. *E. longifolia* (Tongkat Ali) is a slow-growing plant which attains a maximum height of 15-18m and stance fruits after nearly 2-3 years of cultivation. However, this plant might take up to 25 years to become completely matured. After the ripening, the fruit's colour changes from green to dark red or dark brown. The leaves are pinnate shape, spirally arranged and long between 10-15 inches with the 10-30 leaflets (Bhat & Karim, 2010).



(a) *Eurycoma longifolia* Plant

Source adapted from photo © 2011 MedicalHealthGuide.

(b) Root

Source adapted from Euryco® photo (www.javaplant.co.id)

Figure 2.1.1 The *Eurycoma longifolia* plant and root

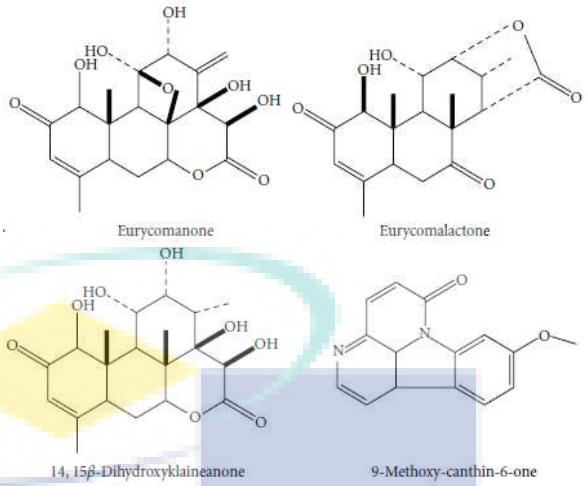
2.2 Chemical composition of *Eurycoma longifolia*

E. longifolia has wide range of chemical composition, especially at root part. There is some chemical composition that are commonly detected in *Eurycoma longifolia*, which; cantin-6-one alkaloids, quassinoid ditrpenoids, quassinoids, β-carboline alkaloids, Eurycomaoside, biphenylneolignans, tirucallane-type triterpenes, tannins, squalene derivatives, eurycomanone, eurycolactone, laurycolactone, eurycomalactone, and eurycomalactone (Mohd Effendy et al., 2012) (Chen et al., 2014).

Besides, some researches have reported the isolation from the roots of *E. longifolia* has almost 65 compounds and classified them by 1D and 2D NMR and by mass spectral data. From the isolates, the primary compounds recognized were four quassinoids diterpenoids acquired from natural sources, which is eurycomalide A, eurycomalide B, 13β, 21-dihydroxyeurycomanol and 5α, 14β, 15β-trihydroxyklaineanone. Other researchers, isolated a new quassinoid glycoside from eurycomanol-2-O-β-D-glycopyranoside and eurycomanol. The four quassinoids, pasakbumin-A, -B, -C and –D from Pasak Bumi (*E. longifolia*) were separated and revealed pasakbumin-A as eurycomannone and pasakbumin-B use to indicated potent antiulcer activity (Tada et al., 1991).

Morita et al. (1992), isolated novel isomeric from the wood of *E. longifolia*, known as 2,2′-dimethoxy-4-(3-hydroxy-1-propenyl)-4′-(1,2,3-trihydroxypropyl)diphenyl ethers, and two novel biphenyls,2-hydroxy-3,2′-dimethoxy-4′(2,3-epoxy-1-hydroxypropyl)-5-(3-hydroxy-1-propenyl)-biphenyl and 2-hydroxy-3,2′,6′-trimethoxy-4′-(2,3-epoxy-1-hydroxypropyl)-5-(3-hydroxy-1-ropenyl)-biphenyl (Morita, Kishi, Takeya, & Itokawa, 1992). Next, (Mitsunaga et al., 1994) discovery the isolation of 5 new canthin-6-one alkaloids (5,9-dimethoxycanthin-6-one, 9,10-dimethoxycanthin-6-one, 10-hydroxy-9-methoxycanthin-6-one, 9-methoxy-3-methylcanthin-5,6-dione and 11-hydroxy-10-methoxycanthin-6-one) from the wood and bark of Tongkat Ali (*Eurycoma longifolia)* (Bhat & Karim, 2010).

(a) Tongkat Ali root



(b) Some of chemical constituent

Figure 2.2.1 Several chemical constituents isolated from the root of *Eurycoma longifolia*

Source: Adapted from (Mohd Effendy et al., 2012)

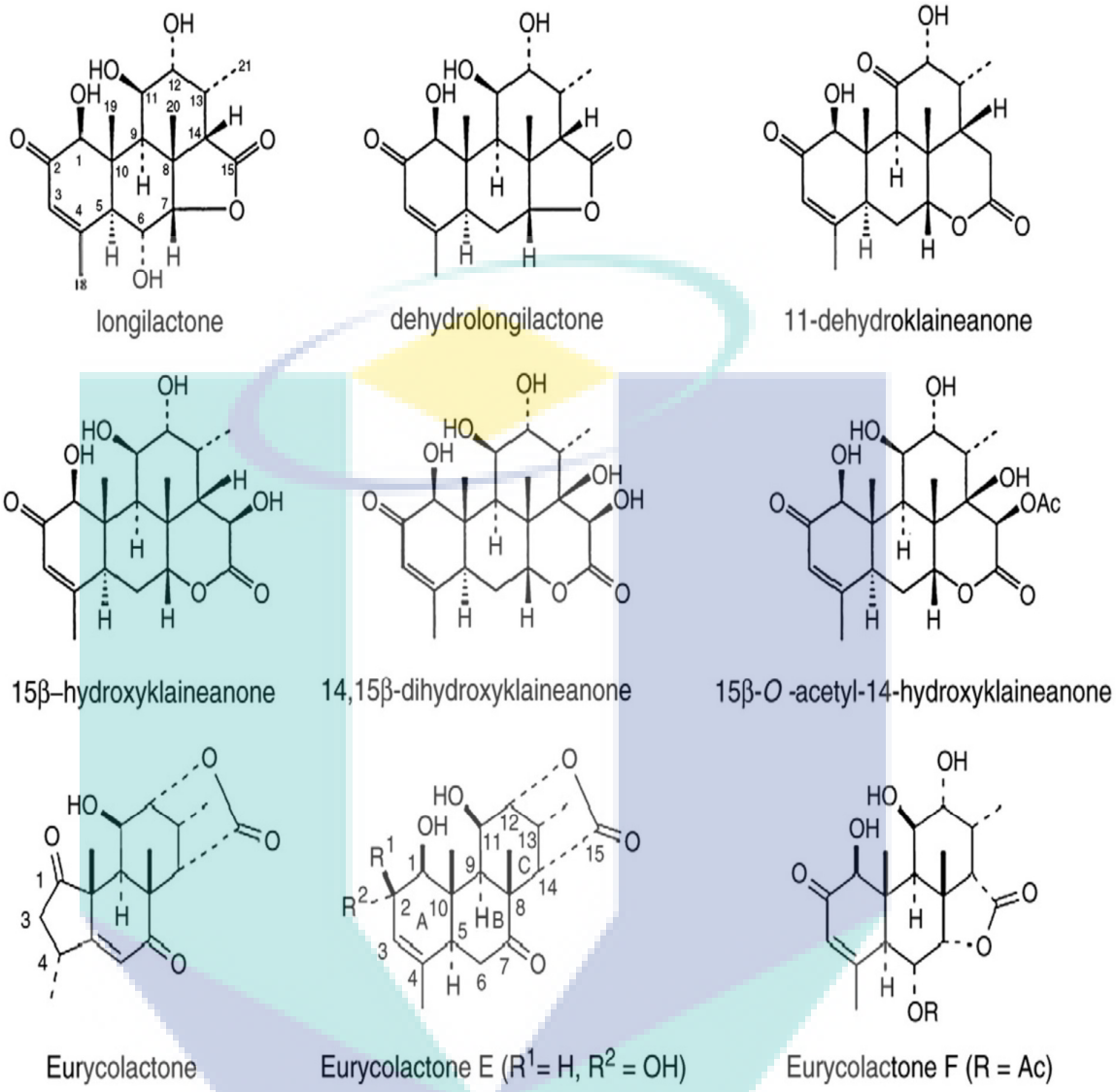


Figure 2.2.2 Some of quassinoids isolated from *E. longifolia*

Source: (Ang & Lee, 2002)

2.3 Eurycomanone (Major Quassinoids Compound)

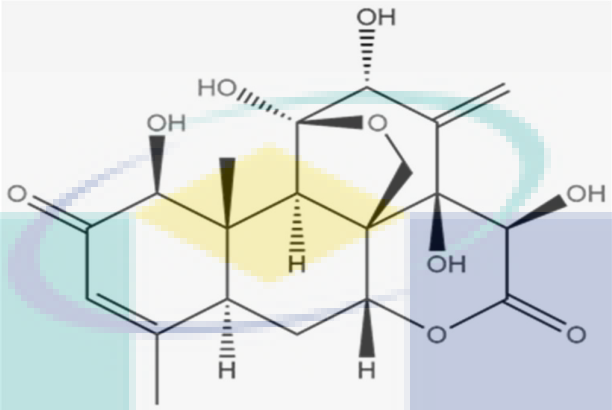


Figure 2.3.1 Chemical structure of Eurycomanone ($C_{20}H_{24}O_9$)

Source: Adapted from (Low, Choi, Abdul Wahab, Kumar Das, & Chan, 2013)

Table 2.3.1 Compound summary

Chemical name	Eurycomanone
CAS No.	84633-29-4
Item No.	FTC-RS-1702
Synonyms	Eurycomanone; Pasakbumin A; Eurycomanone; 84633- 29-4
Mole Formula	C20H24O9
Mole Weight	408.399
Purity	≥98%
Appearance	Off-white powder

Identify Grade	NMR/HPLC
Chemical Family	Diterpenoids

Source: Adapted from ForTopChem Technology

E. longifolia is locally known as Tongkat Ali in Asia, where many pharmaceutical preparations are freely available. *E. longifolia* is rich in quassinoids, triterpenes, squalene derivatives, biphenylneolignans, canthin-6-ones and β-carboline alkaloids. The quassinoids contributed to the bitter taste of the plant is. The majority of these components were found in the roots, witnessing the richness of secondary metabolites from this medicinal herbs plant. The major quassinoid, eurycomanone, and its derivative, eurycomanol were found in most of the collected root samples (Hajjouli et al., 2014). Eurycomanone, the highest concentrated quassinoid in the roots extract of *E. longifolia* improved fertility by increase the testosterone and spermatogenesis in men. The root extract has been reported to induce aphrodisiac properties, increase sperm quality and reversed estrogen-induced infertility (Low et al., 2013).

Research constructed by (Hajjouli et al., 2014), found that eurycomanone inhibited NF-κB signaling through inhibition of IκBα phosphorylation and upstream mitogen activated protein kinase (MAPK) signaling, but not eurycomanol. The presence of the α,β-unsaturated ketone in eurycomanone could be prerequisite for the NF-κB inhibition (Beutler et al., 2009) Eurycomanone is the most active compounds in the roots of *E. longifolia* and shown to have *in vitro* anti-plasmodia activity. The investigation of stage specificity of eurycomanone at various stages of *P. falciparum* life cycles was performed on *P. falciparum* culture *in vitro* (Hajjouli et al., 2014).

Furthermore, the analysis of quantification of six major quassinoids of *E. longifolia* was carried out using the liquid chromatography with the tandem mass spectrometry method. Six quassinoids are eurycomanone, 13,21-dihydroeurycomanone, 13α (21)-epoxyeurycomanone, 14,15β-dihydroxyklaineanone, eurycomalactone, and longilactone. The developed method was used to measure the content of six quassinoids in dietary supplement or capsules containing Tongkat Ali (Han, Jang, Kim, Kim, & Yoo,

2015). As a result, the eurycomanone was showed to be the most abundant quassinoid among the tested samples (Darise, Kohda, Mizutani, & Tanaka, 1982).

2.4 Pharmaceutical Properties

E. longifolia was popularly used as an essential component, in herbal remedies for various illness including hypertension, fevers, sexual insufficiency, aches, vermifuge (an anthelmintic medicine), tuberculosis and as health dietary supplement. However, it has not been indicated or proved strongly for any specific illness. Thus, it is popular among Malaysia society for treating disease and health which sometimes referred as "Malaysia ginseng" (Talbott, Talbott, George, & Pugh, 2013).

In the previous text some of the existing reports pertaining close to the pharmacological potential of plant extract has been discussed. From the table, it shows an overview of some crucial works on pharmacological possessions, the isolated chemical component and their activity that is being held on the Tongkat Ali plant by its separate part (Bhat & Karim, 2010).

Table 2.4.1 An overview of several major works on pharmacological features of each parts of Tongkat Ali (*E. longifolia*) plant.

Plant part	**Activity**
Stem bark, roots	Anti-plasmodia activity (antimalarial)
Leaves (herb sample)	Anti-hyperglycemic activity
Roots	Cytotoxic, Anti-ulcer, Aphrodisiac activity
Leafs	Anti-tumor
Leafs, stem and roots	Anti-microbial

Source: Adapted from (Bhat & Karim, 2010)

2.4.1 Aphrodisiac Properties

For centuries, certain foods and substances have been rumored to have aphrodisiac properties. By definition, an aphrodisiac (named for the Greek goddess of love, Aphrodite) is a substance derived from animals, plants, or minerals and its function is to increase sexual desire especially for male. Enhanced sexual behavior may provide increased relationship satisfaction and self-esteem in humans. An aphrodisiac is explained as any drug or food that induces the induces venereal desire, sexual instinct, and increases pleasure and performance. A lot of natural substances have historically been known as aphrodisiacs in Africa and Europe, like *Pausinystalia yohimbe* tree and the mandrake plant, as well as ground rhinoceros horn in the Chinese culture and "Spanish fly" which is actually toxic. Even in today's culture, there are certain foods that are used as aphrodisiacs, including strawberries and raw oysters. Chocolate, coffee, and honey are also believed to have aphrodisiac potential. Although these natural items are claimed as aphrodisiacs, there is no or little scientific confirmation supporting those assertions.

The aphrodisiacs were classified into three types based on their mode of action, which are; Those that increase, potency, sexual pleasure or libido. Various substances of animal and plant origin have been used in folk medicines of different cultures to vitalize, improve sexual function, energize and physical performance in men, out of these very few have been identified pharmacologically. There are several herbal aphrodisiacs; *Chlorophytum borivilianum, Mondia whitei, Tribulus terrestris, Crocus sativus, Myristice fragrans, Phoenix dactylifera, Lepidium meyenii, Kaempferia parviflora, Satureja khuzestanica, Panax ginseng, Pausinystalia yohimbe, Fadogia agrestis, Montanoa tomentosa, Terminalia catappa, Casimiroa edulis, Turnera diffusa* and *E. longifolia* (Malviya, Jain, Gupta, & Vyas, 2011).

For this studies, we focus the aphrodisiac properties on *E. longifolia*. It has gained notoriety as a symbol of man's ego by the Malaysian men because it increases male sexual prowess and virility during sexual activities. The butanol, methanol, water, and chloroform extracts of the roots of *E. longifolia* Jack were studied by Ang *et al*. using diverse tests of effectiveness of treated male rats. From the studies, the results showed that *E. longifolia* developed a dose-dependent, recurrent and gradually increase in the

episodes of penile reflexes as evidenced by increases in quick flips, long flips and erections of the treated male rats during the 30 min examination period. These results provide further evidence that *E. longifolia* increases the aphrodisiac potency activity in treated animals. Therefore, this study lends further support to the use of the plant by indigenous populations as a traditional medicine for its aphrodisiac property (Ang & Sim, 1997).

The effects of butanol, methanol, water, and chloroform fractions of *E. longifolia* Jack on the levator ani muscle (most important muscles of the pelvis musculature) in both uncastrated and testosterone stimulated castrated entire male rats after dosing them for 12 consecutive weeks was evaluated. Results showed that all the fractions increased the levator ani muscle, when compared with the control (untreated) in the uncastrated intact male rats and testosterone-stimulated castrated intact male rats. thus, the pro-androgenic effect as proved by this study further encouragement the traditional use of this herbal plants as an aphrodisiac (Ang & Lee, 2006).

2.4.2 Anti-Malaria

Malaria is a mosquito-borne disease caused by Plasmodium parasite. The parasites are spread to people through the bites of infected female *Anopheles* mosquitoes. People with malaria often experience fever, chills, and flu-like illness. Left untreated, they may develop severe complications and die. Based on World Health Organization (WHO) and Centers of Disease Control and Prevention (CDC). In 2015, 91 countries and area had going malaria transmission. An estimated 214 million cases of malaria occurred worldwide and 438,000 people died, mostly children in the African Region. Almost 90 per cent of the deaths from malaria occur in sub-Saharan Africa, where the vulnerable groups are children under 5 years and the pregnant women**.** The control of malaria is pannier by the quick selection of parasites resistant to anti-malarial. Indeed, there is no sole antimalarial in clinical use to which the parasite has not yet grow resistance. Anti-malaria drug resistance has become one of the greatest challenges against malaria control. There is widespread multidrug resistance to common antimalarial drugs (Nafiu, Abdulsalam, & Akanji, 2013).

E. longifolia (Tongkat Ali) extract is traditionally used for fevers and has best anti-malarial effect against *P. falciparum*. Chan et al., tested the extracts of *E. longifolia* under *in vitro* conditions for anti-plasmodia activity against a multi-drug resistant Thailand's strain (K-1) of *P. falciparum*. From the plant there are several compound has showed the anti-malarial activities, which is isolated from 21, 331 12 of 31 10-hydroxycanthin-6-one, eurycomanone, eurycomalactone and eurycomanol (Chan, Choo, Rain, & Ismail, 2004). According to Kardono et al., there are two compounds; eurycomanone and 7-methoxy-β-carboline-1- propionic acid showed significant antimalarial activity against *P. falciparum* strains. Concluded by Low et al., that the regulation of the bioactive standardized extract Fr2 (200 mg/kg) viewed a good anti-malarial effect. 13α-(21)-epoxyeurycomanone and eurycomanone may be at most quassinoids contributing to the overall anti-malarial activity of *E. longifolia*. Ang et al., tested *E. longifolia* extract activity in vitro on Malaysian chloroquine-resistant Plasmodium falciparum culture (Bhat & Karim, 2010). They showed that the antimalarial activity of *Eurycoma longifolia* Jack was dose-dependent (Rehman, Choe, & Yoo, 2016).

2.4.3 Anti-Cancer (Cytotoxic to Cancer Cell)

In terms of the medical aspects, cancer known as a malignant neoplasm, is a wide group of diseases involving unregulated cells. Over 200 different known cancers that can affect humans was reported; and there are over sixty different organs in the body where a cancer can develop. Cancer has caused many deaths worldwide and cancer cases continue to increase (Rehman et al., 2016). A statistical report of the people diagnosed with cancer in the UK, for 2014, was reported that total 356,860 people while deaths cause by cancer were 163,444 people. The survival rate for cancer was half-half (50%).

However, cancer therapies are limited due to the adverse effect and development of drug resistance thus there is a need to find newer anti-cancer treatments. Plants are considered as promising anti-cancer candidate as they contain large amount of pharmacologically active and generally safe compounds. Ideally, the new cancer medicine derived from plants should be selectively cytotoxic towards the cancer cells, able to stop its proliferation and consequently induce cell death. Several studies reported the potential of *E. longifolia* as anti-cancer agents (Bhat & Karim, 2010).

E. longifolia has cytotoxicity and anti-proliferative effects on various human cancer cell lines, as well as various solid tumors, including breast, lung and cervical cancers. Cytotoxic effects of traditional medicines and novel drug entities are very essential to be investigated before testing to the further pharmacological activity. Eurycomanone found in *E. longifolia* is a cytotoxic bioactive ingredient, that has a cytotoxic response against many epithelial cell types. The anti-proliferative activity of eurycomanone was investigated on cancerous cell lines (HM3KO, HeLa, Caov-3, Hep G2, and MCF-7) and it was identified to be relatively nontoxic on noncancerous cell lines (MDBK, Vero). Eurycomanone showed to be cytotoxic towards HeLa cells by triggering apoptotic cell death.

Kuo et al., nearly 65 compounds were isolated and identified from the roots of *E. longifolia* and screened them for the possible anti-HIV and cytotoxicity activities by in vitro assays (Kuo, Damu, Lee, & Wu, 2004). According to Park et al., the compounds longilactone, eurycomalactone, and 14,15_-dihydroxyklaineanone showed remarkable cytotoxicity in both MCF-7 and A549, but the 13,21-dihydroeurycomanone was more particular against A549 and eurycomanone proved the cytotoxic effects only against MCF-7 (Rehman et al., 2016).

2.4.4 Anti-Diabetic Activity

Diabetes mellitus (chronic disease) is a group of metabolic disorders with different underlying etiologies, each characterized by hyperglycemia due to underutilization of glucose (Greenberg and Sacks, 2002). Diabetes influence the body's ability to use energy found in food. There type 1 diabetes, type 2 diabetes and gestational diabetes. Type 1 diabetes also known as insulin-dependent diabetes, which often begins in childhood. Type 2 diabetes mellitus or also known as adult-onset diabetes, which results from a composite of insulin resistance and insufficient secretion of insulin, is the most common form, accounting for 90–95% of diabetes in growing countries (England, 2001). Gestational diabetes was prompted by pregnancy (diagnosed in middle or late pregnancy). The pharmacological agents usually used for treatment of type 2 diabetes were include thiazolidinedione, acarbose, biguanide and sulfonylureas (Alberti &

Zimmet, 1998). Unfortunately, these type of agents have restricted usage due to several unpleasant side effects and fail to consequence alter the development of diabetic complications (Rang and Dale, 1991). Renewed attention to replacement medicines and natural therapies has reviving new wave of research interest in traditional implementation, and there is need to look for more efficacious agents with lesser side consequence (Husen, Hawariah, Pihie, & Nallappan, 2004)

Traditionally, daily consume of *E. longifolia* (Tongkat Ali) roots and leaves has been believed to control the levels of blood sugar. Despite, from the scientific sources available on this aspect related to human model is very limited, marking the need for elaborative research works to be handle in the future (Bhat & Karim, 2010). However, Husen et al have reported the viable hyper-glycemic activity of *E. longifolia* in the rat model system. The screening of aqueous extract (50,100, and 150 mg/kg BW) of freeze dried and spray dried samples of *E. longifolia* to determine the level of blood glucose lowering effect in normo-glycaemic and Streptozotocin-induced hyperglycaemic rats. However, the result that in the normo-glycaemic rats, no remarkable reduction to appear after administering extracts (Husen et al., 2004).

2.5 Toxicity Studies of *Eurycoma longifolia (E. longifolia)*

The disadvantage of using traditional herbal medicines is the insufficiency of supporting verification of scientific information on the levels of toxicity, quality and safety associated. Now, there are no available reports on assuring the safety and quality, also the side effect of long term consumption of Tongkat Ali plants. The Tongkat Ali extraction of the product to be commercialized may not meet the basic standard criteria related to the concentration of the bioactive constituent or no single pure constituent identified as of yet (Bhat & Karim, 2010).

Oral toxicity studies have purposeful the LD_{50} of Tongkat Ali extract as 2000 mg/kg body weight (acute) and the NOAEL (No Observed Adverse Effect Level) as greater than 1000 mg/kg body weight, resulting in a classification as Category 5 (highly safe) to the United Globally Harmonized System of Classification and Labelling Chemicals (GHS). Furthermore, the very high safety profile displayed in the rodent

toxicity studies, there are no proclaim adverse side effects in human studies of Tongkat Ali supplementation (Talbott, Talbott, & Pugh, 2013).

2.6 Herbal Drug Standardization and Quality Control

Throughout the history the use of herbs as medicine is the primary and oldest form of healthcare known to humanity and has been used in all cultures (Barnes et al., 2007). Herbal drugs use for the treatment and prevention of various health problem has been in practices from ancient times (Sahoo, Manchikanti, & Dey, 2010). Ancient people always knowledgeable by trial and error to differentiate useful plants with useful effects from those that were toxic or inactive. Also the processing or combinations methods had to be used to gain uniform and optimal results. Even in primitive cultures, tribal people methodically gathered the information on herbal plants and developed well-defined herbal pharmacopeias (Kunle, 2012). Medicinal plants or herbs are widely distributed throughout the world but most abundantly in tropical countries.

The knowledge gain from the plant-based drugs was developed stages by stages and was passed on, thus, create the basic foundation for many systems of traditional herbal medicine all over the world. In most countries, many herbal products are introduced into the market without proper or appropriate scientific evaluation, and without any toxicological studies and mandatory safety. The constant composition and well defined of the drug is one of the most crucial prerequisites for the production of a quality drug. The products nature from the plant origin, which are not usually fixed and are rely on and affected by many factors, ensuring consistent quality of products is important for the success and survival of the industry (Bauer, 1998).

Standardization of herbal medicines is one of the process of prescribing a set of inherent or standards characteristics, definitive qualitative, quantitative values and constant parameter that carry an assurance of quality, efficacy, reproducibility and safety. It is the process of agreeing and developing technical standards. Specific standards are worked out by observations and experimentation, which would bring to the process of prescribing a set of characteristics shown by the certain herbal medicine. Thus, the standardization is work as a tool in the quality control process. Stability, quality control

and standardization for herbal drugs are feasible, but complicate to accomplish. Furthermore, the rules of these drugs is not uniform and differences for each states. There are many variations in the methods and process used across medicine systems and countries in reaching the good quality control and stability.

The *E. longifolia* has been widely used as traditional herbal medicine. The products of Tongkat Ali can be found in a various form which is in capsule, energy drink, single or mixed with other herbs plant and available as additive in brewed coffee or tea (Mohd Effendy et al., 2012). The evaluation of heavy metal, microbial contamination, steroid and etc. must include in criteria of registration (Medicine Act, Advertisement and Sale, 1956 Revised 1983) in Malaysia (Ang & Lee, 2002). In addition, quality control and the standardization of herbal medicines also involve several other steps like source and quality of raw materials, good agricultural practices and good manufacturing practices. These practices acts as a main key in guaranteeing the stability and quality of herbal preparations (Loew & Kaszkin, 2002).

	
a) Nu-Prep® Lelaki with PhystaTongkat Ali (Source: Adapted from Biotropics Malaysia Berhad website)	b) Flavoured Drink With Tongkat Ali And Ginseng (Source: Adapted from Power Root website)

Figure 2.6.1 Some example of Tongkat Ali products

2.7 HPLC Analysis on *Eurycoma longifolia*

High Performance Liquid Chromatography (HPLC) is known as a form of column chromatography that pumps a sample mixture or analyte in a solvent (known as the mobile phase) at high pressure through a column with chromatographic packing material (stationary phase). The sample is carried by a moving carrier gas stream of helium or nitrogen. HPLC has the ability to separate, and identify compounds that are present in any sample that can be dissolved in a liquid in trace concentrations as low as parts per trillion. Because of this versatility, HPLC is used in a variety of industrial and scientific applications, such as pharmaceutical, environmental, forensics, and chemicals.

An analytical method that was developed to identified the quassinoid content from the *E. longifolia* Jack (Tongkat Ali) collected from various sources i.e. indigenous people was HPLC with UV detection. There were several chemical compound that has been isolated as reference standard which; eurycomanone, longilactone, 14,15β-dihydroxyklaineanone, 15β-acetyl-14-hydroxyklaineanone, 6α-hydroxyeurycoma-lactone, and eurycomalactone. Besides that, the synthesized 1β,12α,15β-triacetyleurycomanone (6, internal standard), were identified by NMR, UV, IR and MS spectroscopies. Their coefficient of variation values for 0.50-35 µg/mL (-1) concentrations of quassinoids and their retention times measured were small for both within-day and between-day. The recovery of the spiked quassinoids in *E. longifolia* samples and their detection limits at 8.5 times signal to noise ratio were 99.75-109.13% and 0.01 µg/mL (-1), respectively (Tong et al., 2015).

2.8 Guidelines of NPRA for The Labelling of Herbal Medicinal Capsule

According to NPRA, the minimum requirements that must be followed to register the health supplement or herbal capsule product are microbial load test, heavy metal and should be with the GMP certificate. In the labelling, the general guidelines should include a product name, dosage form, name of active substances, manufacturing date, expiry date, batch number, storage condition, logo, specific information, country registration number, route of administration, name and address of manufacturer and etc.

to make the standardize labelling for the herbal capsule. The purpose of labelling is to differ category of product based on its function. In addition, all labels and package inserts must be in Bahasa Malaysia or English and in the same size of fonts as a rule of labelling. (Pharmaceutical et al., 1915)

Table 2.8.1 Some of the major parameters required in labelling

No.	Parameters
1	Product name
2	Dosage form
3	Name of active substances
4	Strength of active ingredient in weight
5	Expiry date
6	Manufacturing date
7	Batch number
8	Dosage
9	Storage condition
10	Registration number (MAL)
11	Name & address of product registration holder
12	Name & address of manufacture
13	Warning label
14	Contraindication/ precaution

Source: Adapted from Drug Registration Guidance Document (DRGD), National Pharmaceutical Regulation Agency (NPRA), Ministry of Health Malaysia

2.9 Quality Control Test Accordance to NPRA Guidelines

According to the NPRA, for the development of herbal medicine must be followed the quality control test; included the heavy metal test, test for uniformity of weight and microbial load test. The testing was the important to ensure the safety of the product. The limit and guidelines for each testing can be refer to Drug Registration Guidance Document (DRGD) (Pharmaceutical et al., 1915).

2.9.1 Heavy Metal Test

Table 2.9.1.1 Limit for heavy metals

Lead	NMT 10.0 mg/kg or 10.0 mg/L (10.0 ppm)
Arsenic	NMT 5.0 mg/kg or 5.0 mg/L (5.0 ppm)
Mercury	NMT 0.5 mg/kg or 0.5 mg/L (0.5 ppm)
Cadmium	NMT 0.3 mg/kg or 0.3 mg/L (0.3 ppm)

*NMT: Not More Than Source: Drug Registration Guidance Document (DRGD)

2.9.2 Test for Uniformity of Weight

To ensure the consistency of dosage units, each unit in a batch should have a drug substance content within a narrow range around the label claim (Katori, Aoyagi, & Kojima, 2001). Dosage units are defined as dosage forms containing a single dose or a part of a dose of drug substance in each unit. The term "uniformity of dosage unit" is defined as the degree of uniformity in the amount of the drug substance among dosage units. Individual weight of the capsule to be within the limit of 90 % - 110 % of the average weight.

Table 2.9.2.1 The deviation of individual net weight should not exceed the limit given

Average net weight of capsule	Deviation (%)	Number of capsule
Less than 300 mg	± 10.0	Minimum 18
	± 20.0	Maximum 2
300 mg and more	± 7.5	Minimum 18
	± 15.0	Maximum 2

2.9.3 Microbial Load Test

Herbal medicine products containing, for example, extracts and/or herbal drugs, with or without excipients, where the method of processing or, where appropriate, in the case of herbal drugs, of pre-treatment reduces the levels of organism to below stated for this category

Table 2.9.3.1 Limit for microbial load

Microbiological control	Acceptance criteria
TAMC	NMT 5×10^4 CFU/g or CFU/mL
TYMC	NMT 5×10^2 CFU/g or CFU/mL
Bile-tolerant gram-negative bacteria	NMT 1×10^2 CFU/g or CFU/mL
Escherichia coli	Absence (1 g or 1 mL)
Salmonella	Absence (25 g or 25 mL)
Staphylococcus aureus	Absence (1 g or 1 mL)

*TAMC: Total Aerobic Microbial Count

*TYMC: Total Yeast and Mould Count

CHAPTER 3

METHODOLOGY

3.1 Introduction

According to the objectives and background of study, there will be two main parts which the analysis and evaluation on Tongkat Ali Capsule by Indigenous People (TAC-IP), and the development of Tongkat Ali Capsule in House Formulation (TAC-IHF) with the analysis and evaluation. TAC-IP was the capsule made by Indigenous People from Perak without any NPRA approval. While the TAC-IHF was the product that will be developed (for NPRA approval) by adapting the Indigenous peoples' techniques and in accordance to the Malaysia Standard (MS) and National Pharmaceutical Regulation Agency (NPRA). Both capsules were tested for their safety properties and achieved the standardization of Tongkat Ali products according to the MS and NPRA. As for the TAC-IP, analysis on eurycomanone compound, uniformity of eurycomanone content in capsule and weight of capsule, survey on effectiveness of capsule, microbial test and heavy metal test were conducted. However, for the TAC-IHF, similar procedures done except for the survey and also some additional method included grinding, sieving, capsule formulation and labelling of product prototype. Finally, the pulverised and tested powdered material will be sent to a service provider for NPRA approval.

3.2 Tongkat Ali Capsule by Indigenous People (TAC-IP)

The capsules were obtained from indigenous people in Kampung Bukit Cermin, Perak. based on indigenous people the capsule that they produced was 100% from Tongkat Ali root without any additional excipient or additives whether chemical or organic material. The capsules were manually produced by hand. In this project, the High Performance Liquid Chromatography (HPLC) analysis was used to determine the eurycomanone level in the capsule by comparing it with the calibration curve of eurycomanone standard. This to ensure to authenticated the Tongkat Ali capsules from

indigenous people. Certain safety investigation such as microbial load and heavy metal detection was successful done. Besides, to improve the effective and to evaluated the capsules produce by indigenous people, the survey was conducted for one-month duration with 20 voluntary respondents. The volunteers provided consent in a form generated.

3.3 Collection of Tongkat Ali Root

Tongkat Ali root *(E. longifolia* jack) was collected from the reliable source from indigenous residents that lives in Pahang, Malaysia. Simple test was performed to confirm Tongkat Ali obtained from indigenous people was genuine and not fake. By shaving the chip from the Tongkat Ali root and applied onto the tongue area which known to be sensitive for bitterness. This because the bitter taste of Tongkat Ali was contributed by the quassinoid contain in *E. longifolia* (Guo, 2005). The Tongkat Ali root obtained was used to produce the product of TAC-IHF by adapting techniques of indigenous people.

3.4 Pre-prepared Raw Material into Powder Form for Capsulation and Extraction

The preparation of raw material into the powdered form is for two purposed which is for capsulation and also for water extraction. The Tongkat Ali root was chopped into chip size by using the saw. Then spread them on the aluminium foil for drying in the oven at 35 to 45 °C. The drying procedure to remove any excess of water and to prevent any reproduction of microorganisms. After drying, the roots were ground into granules form with the help of electrical grinder. As for food powder, the powder size must be between 40-60 Mesh ("Volume: I: Issue-3: Nov-Dec -2010," 2010). Therefore, there will be further grinding which by using the herbal medicinal blender. Finally, the powder form of Tongkat Ali root was passed through the sieve size 50 Mesh which equal to 297 microns to get the standardize size of root powder particle size. This size of herbal powder been shown to be safe to the liver and kidney.

3.5 Extraction of Tongkat Ali Root

The 50 gm of *E. longifolia* chips was put into 500 mL deionized water. Then boiled it under reflux for five hours, followed by filtration with Whatman No1 filter paper and freeze drying accordingly. The extraction was carried out to test eurycomanone content in Tongkat Ali root water crude. According the Malaysia Standard (MS) the range for eurycomanone in water extract was between 0.8 – 1.5 % w/w. This extract is for authentication purposes to ensure the purity and originality of the Tongkat Ali root.

3.6 Formulation of Capsule (TAC-IHF)

The capsules were made from the Tongkat Ali root powder that has been standardize the particle size into 50 Mesh. The empty capsule was made up of gelatin. The capsule was manually produce by using the capsule maker size #1.

Material and apparatus

- Root powder
- Empty capsules
- Capsules maker size #1

After the capsule formulation, the capsules were tested the safety and effectiveness was evaluated.

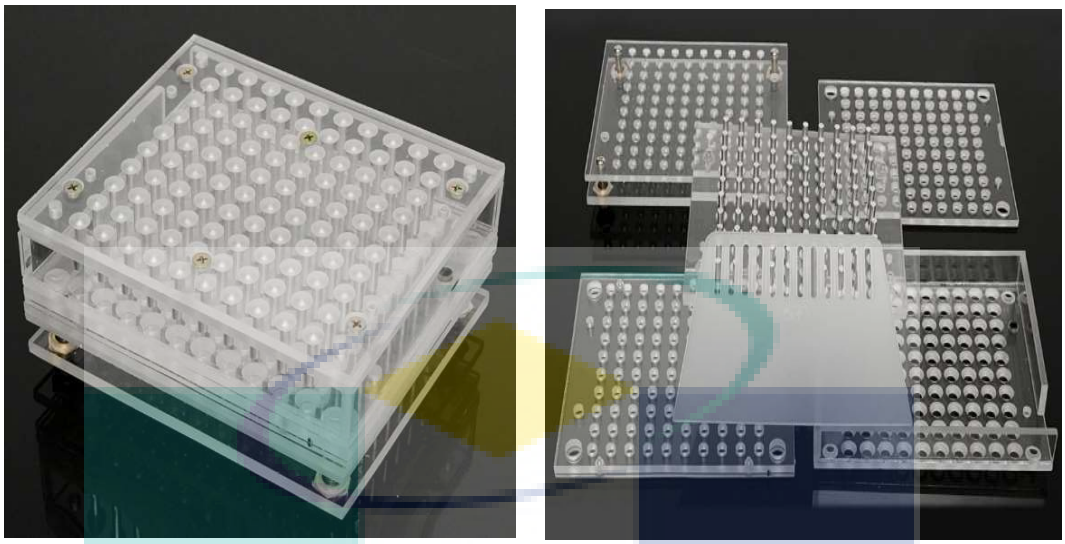


Figure 3.6.1 Capsule maker size #1

Figure 3.6.2 Empty gelatin capsule size #1 (Halal)

3.7 HPLC Analysis

HPLC was equipped with the basic set-up, HPLC Agilent 1260 Infinity with photodiode array. The column used was a Zorbex SB-C18 column (5µm, 250mm x 4.6 mm). The mobile phase contains of isocratic combination of water and acetonitrile (ratio

of 86: 14) and was filtered using 0.45 µm nylon membrane filter before used. The isocratic combination was the same ratio of the two solvents used from the starting to the end of sample running. The flow rate is 0.8 mL/min for 10 minutes. The 10µL of each samples were injected into the systems and the sample was run over 10 minutes because the eurycomanone component can be detected below the 10 minutes of time period. The detection wavelength is 245 nm using UV-V is wavelength. Data acquisition was performed by the ChemStation Data Software. The analysis was determined in HPLC instrument room at FIST laboratory.

3.8 Eurycomanone Standard

The eurycomanone standard reference was purchased from ChromaDex, USA while Acetonitrile (HPLC grade) was purchased from Sigma-Aldrich. Deionized water was prepared by using PURELAB Flex Ultra-Pure System to be use for HPLC analysis.

Material and Apparatus

- 1 mg of eurycomanone standard
- Deionized water
- 0.45 µL syringe filter
- 1 mL syringe
- Vials

The eurycomanone standard was dissolved in 1 mL deionized water to produce 1.0 mg/mL concentration. By using the 0.45µL syringe filter the solution was filtered and serially diluted was conducted to produce the differences concentration of the solution (0.5, 0.25, 0.125, 1.0625 and 0.03125 mg/mL). The eurycomanone content will be determined by using the standard calibration curve that generated from the eurycomanone standard compound.

3.9 Sample Preparation and Analysis

Five mg of each sample from both TAC-IP and TAC-IHF, were dissolved in 1 mL of deionized water at temperature of 37 °C for 10 minutes. The temperature of deionized water was set at 37 °C. The mixture was filtered through a 0.45 µm nylon membrane syringe filter. Then, 10 µL of each sample was injected into HPLC system and the analysis was run for 10 minutes. A set of triplicate was done for each sample.

3.10 Determination of Eurycomanone Concentration in Tongkat Ali Capsule

The content of eurycomanone was determined by the regression equation generated from the eurycomanone standard compound.

$$\text{Concentration (w/w\%)} = (Cf/Cs)*100 \qquad \text{Equation 3.1}$$

Cf = known concentration (from Eurycomanone standard calibration curve)

Cs = sample concentration

According to the Malaysian Standard (MS2409:2011), the level of eurycomanone concentration in *E. longifolia* water extract or product should be around 0.8 to 1.5 % w/w. By applying the guideline to the powder form of Tongkat Ali root as there is not specific standard for eurycomanone concentration in powder form.

3.11 Labelling Process in Accordance to NPRA

Guidelines for the labelling process has been provided by NPRA to be follow. Based on the NPRA guidelines, the brand name and product name has been set by our own. Also included the product description by describing visual and physical characteristics of product, i.e. shape, size, superficial markings, colour, odour, taste, type of coating, type of capsule etc., where applicable. Besides that, the labelling standard of the developed product was labelled as health supplement with no other

indications referring into specific medicinal activity such as aphrodisiac purposes. The dose instruction was fixed for customer to consumed. The precaution and side effect, the storage condition were also stated on the label to give detail information to the users (Pharmaceutical et al., 1915). In this study it is important to design a label in accordance to NPRA regulation and provided to the contract manufacturer before-hand to be included in the application of MAL number.

3.12 Uniformity Test

The capsules for both product, TAC-IP and TAC-IHF were testing for the uniformity test. The uniformity of dosage units for both products were demonstrated by two methods, Content Uniformity or Weight Variation (Airth, Bray, & Radecka, 1967) The content uniformity is the uniformity of active substance in the capsule which is known as eurycomanone. The concentration of eurycomanone for each capsule must be in average and equal quantities and qualities. The three capsules were chosen randomly for both products. Then the HPLC analysis will be conducted. While, for the weight variation requirement measures the variability in the amount of powder contained in each capsule. The average weight of capsule was determined by weighing randomly selected 20 capsules on electronic analytical balance Mettler Toledo™ MS-TS Analytical Balances. One capsule was weighed. The capsule was opened and the contents are removed as completely as possible. The emptied shells are weighed. The net weight of its contents are determined, that is by subtracting the weight of shells from the weight of the intact capsule (Katori et al., 2001).

The procedure is repeated with another 19 capsules. The average net weight is determined from the sum of individual net weights. The percentage of deviation from the average net weight for each capsules are determined. The deviation of individual net weight should not exceed the limits which is, not be less than 90 % and more than 110 % of the theoretically calculated weight of each unit (Pharmaceutical et al., 1915).

The percentage of deviation is calculated using the formula below.

$$\frac{net\ weight\ of\ capsules\ -\ average\ net\ weight\ of\ capsule}{average\ net\ weight\ of\ capsules} \times 100\%$$

3.13 Determining the Microbial Load and Heavy Metal Test

The capsules of TAC-IP and TAC-IHF, both was sent to the Central Laboratory UMP for the microbial load and heavy metal test. The heavy metal was analyzing by the ICP-OES and the Mercury element being tested by using Mercury Analyzer.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Bitterness Test of Tongkat Ali Root

Tongkat Ali root known for its unique and extreme bitterness. A feature only to test this plant due to high quassinoid content. The bitterness of the compound was different from normal bitter, it a bit unique bitter that can differentiate *E. longifolia* from other plants. The higher the bitterness the higher the value of the plant (Rehman et al., 2016). The taste was done on the root purchased from the indigenous person to confirm the authentic and verify it is in fact Tongkat Ali root. Such test at site of it is purchase required before proceed with the use of any analytical instrument such as HPLC in laboratory settings. The Tongkat Ali was tested by applying Tongkat Ali root chip at the bitter bud of tongue.

(a) Area of tongue

Source: Adapted from http://www.personal.psu.edu/afr3/blogs/SIOW/2011/09/sense-of-taste-wins-5th-place.html

(b) Tongkat Ali Chips

Figure 4.1.1 The picture of tongue area and Tongkat Ali root

4.2 Formulation of Standardize Tongkat Ali Capsule in House Formulation (TAC-IHF)

Our own standardised Tongkat Ali capsule was formulated based on the Indigenous people techniques. The capsule was formulated 100 % from the Tongkat Ali root powder without any other additional material or excipient. The capsule was pure 100 % contain Tongkat Ali root powder. The Tongkat Ali root was prepared by grinding and sieving according the standard for food powder. As for the capsule, the herbs powder size particle must be in the range of 40 – 60 Mesh ("Volume: I: Issue-3: Nov-Dec -2010," 2010). Therefore, for our formulation, we standardised the powder size to 50 Mesh. Since the eurycomanone compound in the root powder was determined by using HPLC, the content was fulfilled the Malaysia Standard (MS) which was calculated to be 0.80 %. By assume the MS range was 0.8 – 1.5 (w/w) % for water extract can also be guideline for the powder form as there is not specific regulation for powder form. (Malaysian Standard, MS 2409:2011)

The capsule was manually produce by using the capsule maker. The size for the capsule was #1 size, the standard size for capsule production in the Malaysia. Then, for the capsule shell, we used the capsule that made of gelatine. Generally, the gelatine is harmless and safe to consume. The gelatine capsules, also known as soft gels, are very cost effective and easy way to take on powdered herbal supplements. The present invention provided chewable, edible soft gelatine capsule which comprises a shell comprising water, gelatine, plasticizer, and an amount of hydrogenated starch hydrolysate effective to render said shell dispersible and soluble in the mouth of user. In addition, a soft gelatine capsule fill material in which an active ingredient, preferably a biologically-active agent, is dispersed or dissolved (Borkan et al, 1990). The shell is made of gelatin, usually derived from beef or pork, along with water and a plasticizing agent such as glycerin to provide durability with flexibility. The capsule we used was not from pork origin and its Halal to consumed. Gelatin capsules have advantages for both the drug and supplement manufacturer and the consumer. The advantages of using gelatine empty capsule was easy to swallow, help mask odours and bad tasting supplements and also easily dissolved after digested. The slippery structures of the capsule made it easy to shallow. The gelatine capsule can be dissolved within minutes of reaching the stomach.

(a) Empty gelatine capsule (b) TAC-IHF

Figure 4.2.1 The picture of empty gelatine capsule and finished product of Tongkat Ali Capsule in House Formulation (TAC-IHF)

4.3 Eurycomanone Standard

Eurycomanone compound is the organic marker as it is one of the major class of quassinoid found in the *E. longifolia* (Suan et al., 2011). The eurycomanone compound from the *E. longifolia* can be determined by the Reverse Phase- High Performance Liquid Chromatography (RP-HPLC). According to the Malaysia Standard, the eurycomanone in the *E. longifolia* can be determined by using the eurycomanone marker. The HPLC analysis was performed to determine the quality and quantity of eurycomanone compound in the *E. longifolia* by using the serial dilution that contained five different concentrations of eurycomanone standard to be able to determine the retention times and also peak areas of eurycomanone compound. From the HPLC analysis, the average retention time for eurycomanone standard was calculated 8.70 minutes. The result in the Table 4.1 showed, the higher the eurycomanone concentration, the higher the peak area generated a straight line passing the origin. The triplicate analysis was conducted for each concentration to form the accurate standard curve of eurycomanone standard. Based on the graph constructed, the regression coefficient (R2) is 0.9999 which indicated strong positive correlation while regression equation is y = 18229x - 49.059. Therefore, the determination of the quality and the quantity of the *E. longifolia* root extract, the Tongkat Ali Capsule by Indigenous People (TAC-IP) and standardized Tongkat Ali Capsule in

House Formulation (TAC-IHF) can be done by using the standard curve of eurycomanone.

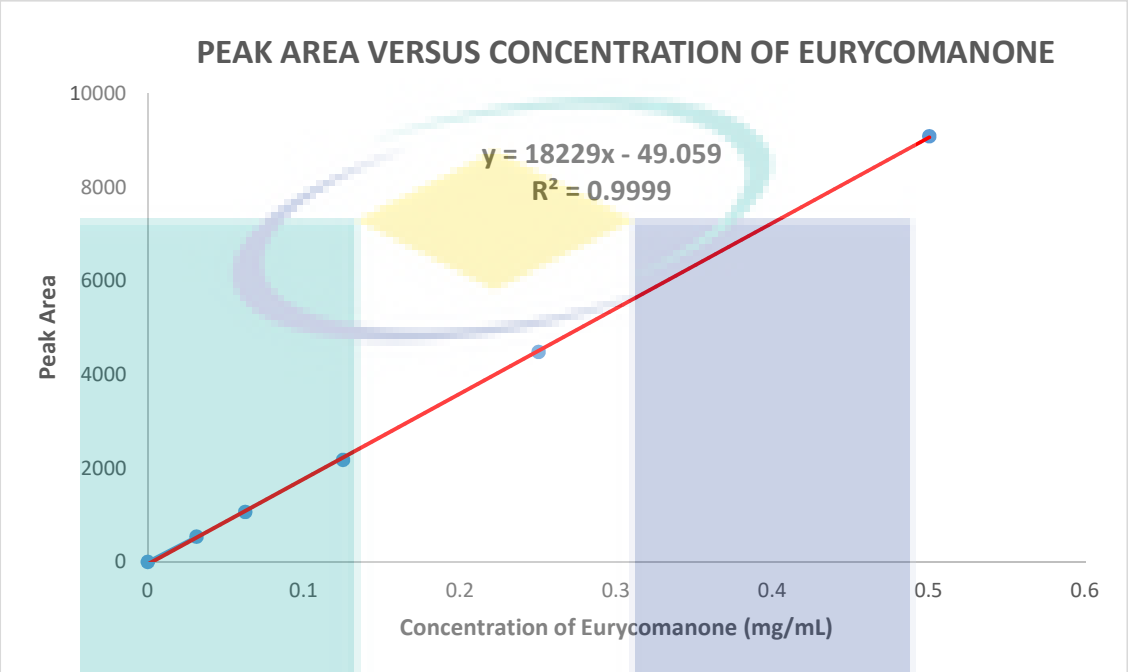


Figure 4.3.1 Calibration curve of Eurycomanone standard

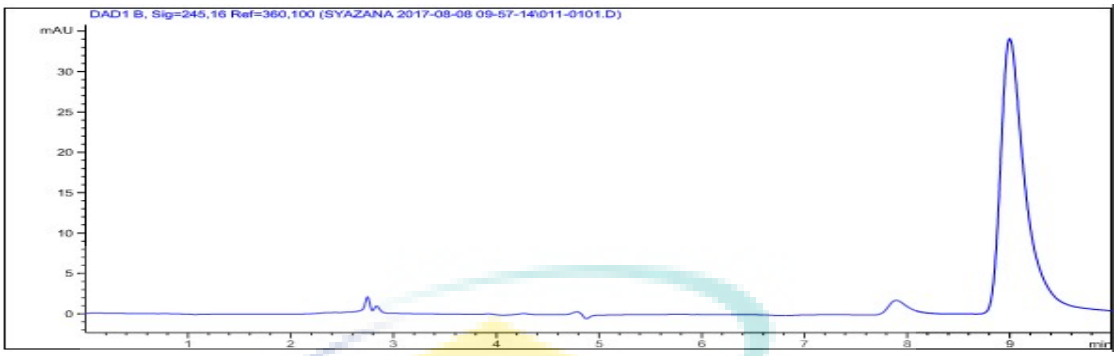
*Mobile phase ratio, Deionized water: Acetonitrile (86:14), Wavelength 245 nm, 10 µL injection for 10 minutes running time (triplicate), Room temperature

Table 4.3.1: Recovery analysis of Eurycomanone standard

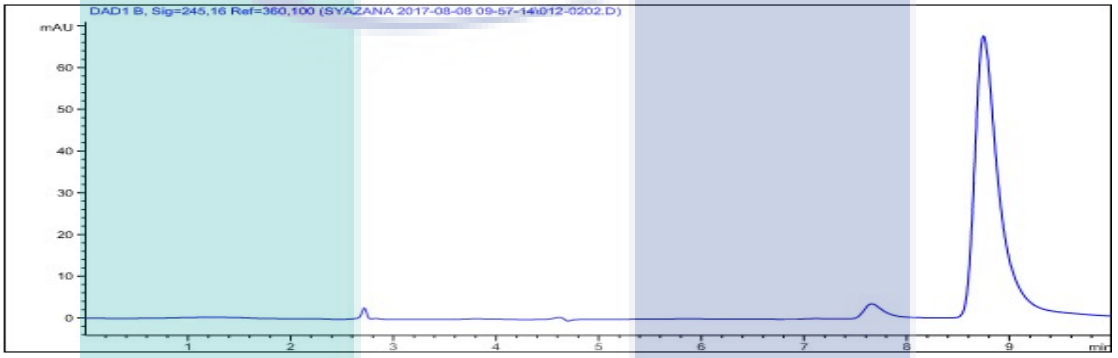
Concentration (mg/mL)	Retention Time (min)	Mean of Retention Time (min)	Peak Area	Mean of Peak Area
	8.99		545.29	
0.03125	8.92	8.92	538.34	540.34
	8.84		537.4	

	8.77		1069.43	
0.0625	8.75	8.74	1067.7	1068.92
	8.71		1069.62	
	8.68		2180.45	
0.125	8.67	8.67	2178.84	2180.51
	8.66		2182.23	
	8.64		4496.65	
0.25	8.61	8.61	4490.76	4483.78
	8.58		4463.93	
	8.56		9136.23	
0.5	8.54	8.54	9043.37	9091.13
	8.52		9093.79	

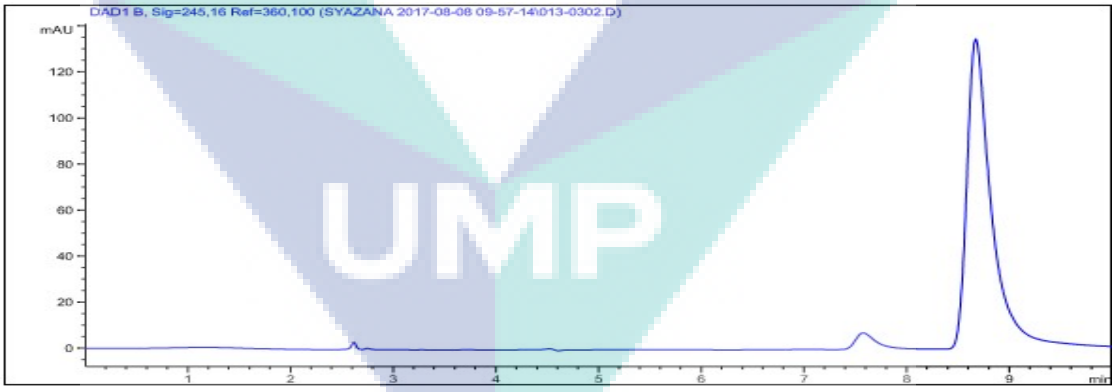
Average of retention time is 8.70 minutes



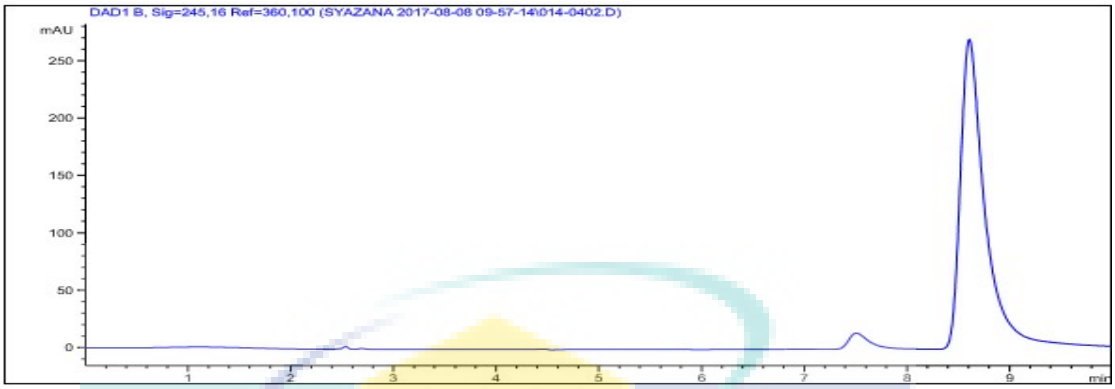
(a) 0.03125 mg/ml



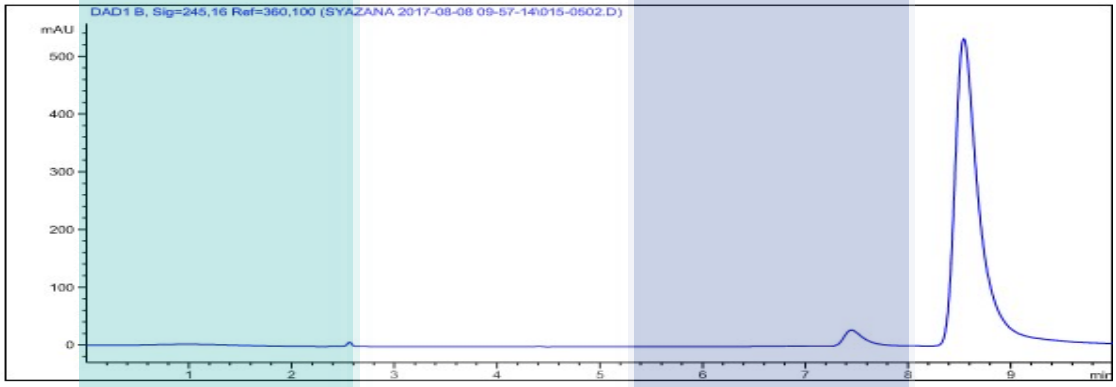
(b) 0.0625 mg/ml



(c) 0.125 mg/ml



(d) 0.25 mg/ml



(e) 0.5 mg/ml

Figure 4.3.2 The chromatograms of Eurycomanone standard by different concentration, (a)-(e) based on HPLC analysis

4.4 Determination of Eurycomanone Content

Eurycomanone content in the *E. longifolia* root water extract was analysed by using the HPLC. The water extract of eurycomanone was obtained from the extraction activity of Tongkat Ali root powder. The level of eurycomanone in the extract was calculated based on the calibration curve of eurycomanone standard in Figure 4.3.2. The peak area of eurycomanone in *E. longifolia* root water extract present in 7.47 minutes of retention time. However, the retention time was observed to be different from the standard which is 8.70 minutes. The percentages eurycomanone compound present in the *E. longifolia* root water extract was 1.24 (w/w) %. (Figure 4.4.2) The calculation of the percentage used the equation 3.1. The percentage obtained was following the Malaysia

Standard which the content of eurycomanone water extract must between the range of 0.8 – 1.5 (w/w) %. (Malaysia Standard, MS 2409:2011)

Besides, the eurycomanone content in the TAC-IP also was determined by HPLC. The level of eurycomanone was calculated (Equation 3.1), based on the calibration curve of eurycomanone standard in Figure 4.3.2. The peak area of eurycomanone in *E. longifolia* capsule present in 8.28 minutes of retention time. The retention time was found slightly different from the standard which is 8.70 minutes. The percentages eurycomanone compound present in the *E. longifolia* was 0.70 (w/w) %. Capsule. The percentage obtained was almost fulfil the following Malaysia Standard which the content of eurycomanone water extract must between the range of 0.8 – 1.5 (w/w) %. (Malaysia Standard, MS 2409:2011)

Furthermore, the eurycomanone content in the TAC-IHF also was determined by HPLC. The level of eurycomanone was calculated (Equation 3.1) based on the calibration curve of eurycomanone standard in Figure 4.3.2. The peak area of eurycomanone in TAC-IHF capsule present in 8.32 minutes of retention time. The retention time was found slightly different from the standard which is 8.70 minutes. The percentages eurycomanone compound present in the *E. longifolia* was 0.80 (w/w) %. The percentage obtained was within the range of the Malaysia Standard which the content of eurycomanone water extract must between the range of 0.8 – 1.5 (w/w) %. (Malaysia Standard, MS 2409:2011). This MS was constructed based on obtaining numerous Tongkat Ali samples and suggested to be guide merely and not a must to be adhered for the purpose of NPRA registration. The content of eurycomanone was determined by the regression equation generated from the eurycomanone standard compound:

$$\text{Concentration (w/w\%)} = (Cf/Cs)*100$$

Cf = known concentration (from Eurycomanone standard calibration curve)

Cs = sample concentration

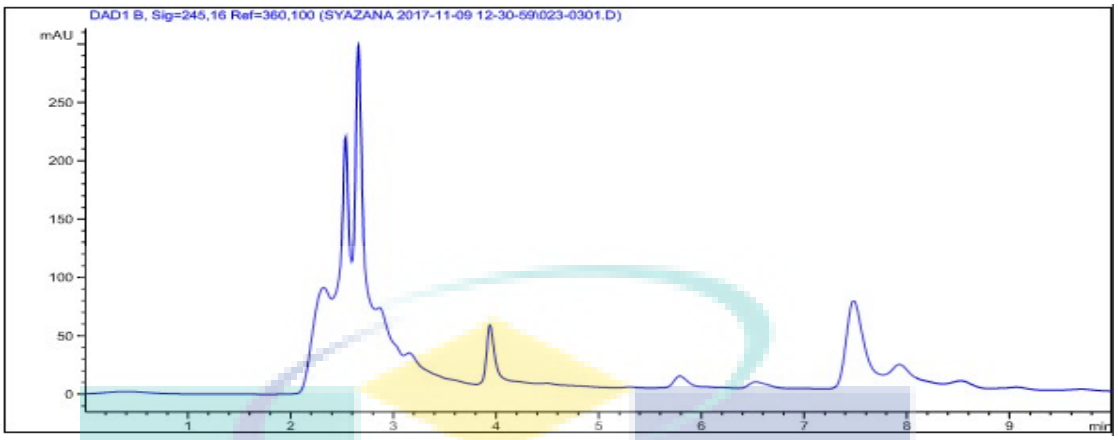


Figure 4.4.1 The chromatogram of *E. longifolia* water extract

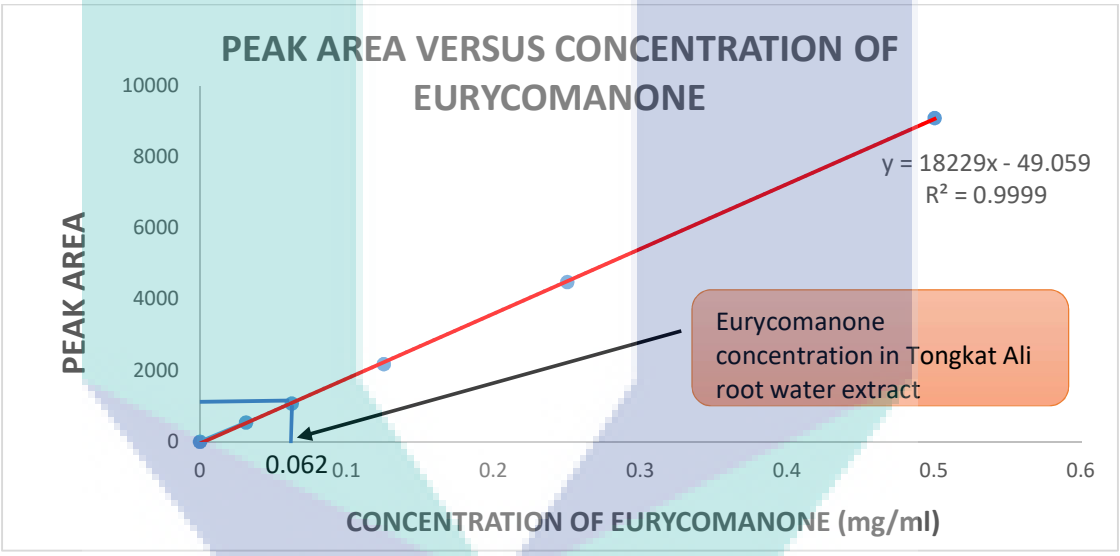


Figure 4.4.2 Calibration curve of Eurycomanone standard with Tongkat Ali root water extract

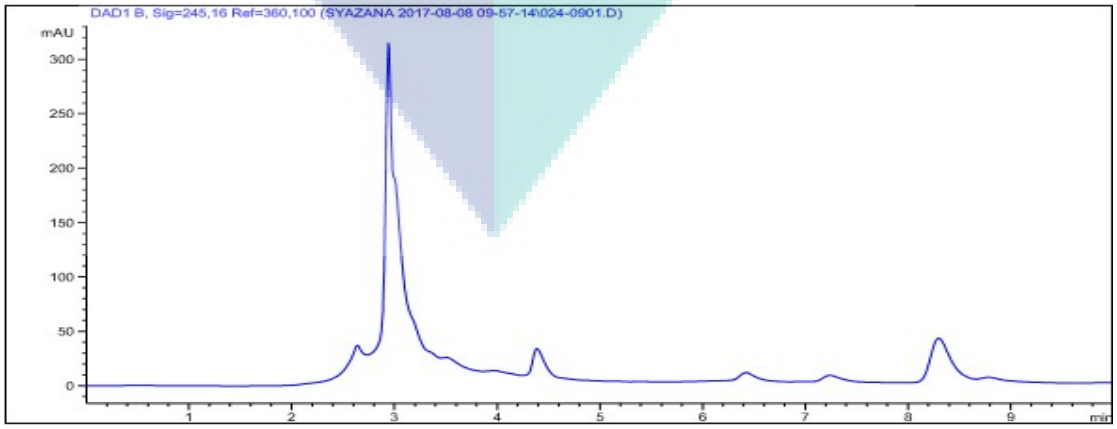


Figure 4.4.3 The chromatogram of TAC-IP

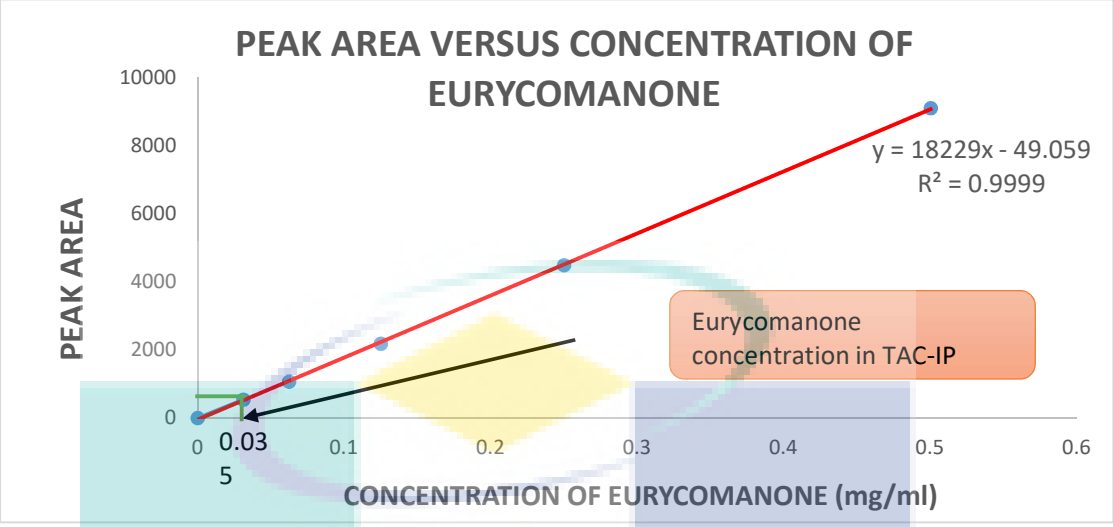


Figure 4.4.4 Calibration curve of Eurycomanone standard with Tongkat Ali Capsule by Indigenous People (TAC-IP)

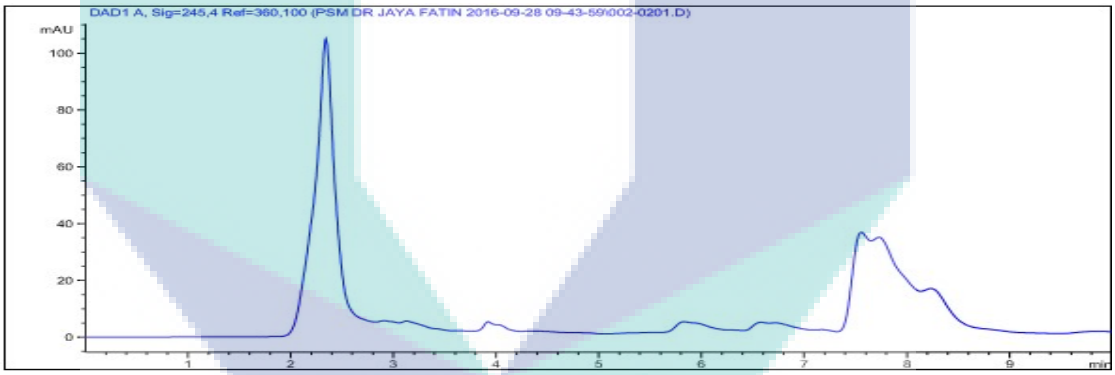


Figure 4.4.5 The chromatogram of TAC-IHF

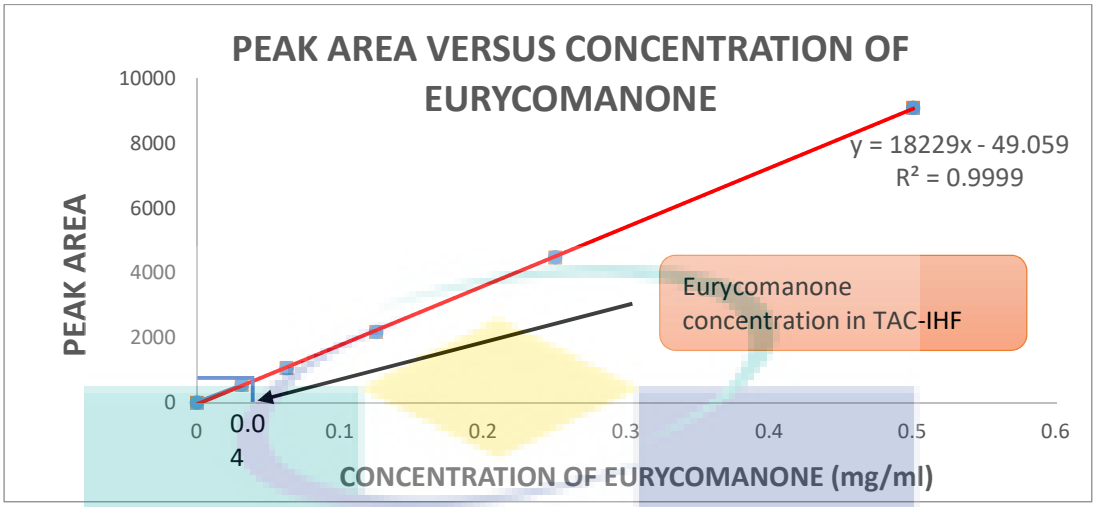


Figure 4.4.6 Calibration curve of Eurycomanone standard with Tongkat Ali Capsule in House Formulation (TAC-IHF)

Based on Table 4.2, the amount of 1.24 % (w/w) found in crude water extract of Tongkat Ali upon extraction by reflux. Such amount indicated the maximum available Eurycomanone within the root powder. Able to provide benefit to a person consuming the capsule. However, it is not often that the full extent of this amount will be supplied within a human body due to the constrains of the biological environment and of the liver enzymes on the Tongkat Ali capsules content.

Table 4.4.1 Recovery analysis of Eurycomanone content in Tongkat Ali root water extract

Retention Time (min)	Mean Retention Time (min)	Peak Area	Mean Peak Area	% of eurycomanone in Tongkat Ali root water extract based on standard curve of eurycomanone
7.48		965.24		
7.47	7.47	1092.06	1086.41	1.24
7.46		1201.93		

Table 4.4.2 Recovery analysis of eurycomanone content in TAC-IP

Retention Time (min)	Mean Retention Time (min)	Peak Area	Mean Peak Area	% of eurycomanone in TAC-IP based on standard curve of eurycomanone
8.30		587.35		
8.27	8.28	596.45	590.73	0.70
8.26		588.40		

Table 4.4.3 Recovery analysis of Eurycomanone content in TAC-IHF

Retention Time (min)	Mean Retention Time (min)	Peak Area	Mean Peak Area	% of Eurycomanone in TAC-IHF based on the standard curve of eurycomanone
8.34		751.37		
8.32	8.32	688.37	688.26	0.80
8.31		625.03		

*Concentration (w/w%) = (Cf/Cs)*100

Cf = known concentration (from Eurycomanone standard calibration curve)

Cs = sample concentration

4.5 Uniformity of Eurycomanone Content in Capsules (TAC-IP and TAC-IHF)

The uniformity of eurycomanone compound for each capsule must be standardised to ensure the efficiency and also the quality and quantity of the capsules. The simple test was carried out by taken three capsules randomly. Then the eurycomanone was determined by using the HPLC and calculated the percentage by referring the calibration curve of Eurycomanone standard.

HPLC analysis was conducted for three of capsules of TAC-IP. The result shows in Table 4.6 the first, second, and third capsules are 0.70. 0.68. and 0.72, respectively which is calculated by using the calibration curve of eurycomanone standard. The average was taken, it shows that the percentages of eurycomanone is 0.70 w/w (%) and approaching one percent of Eurycomanone. This analysis was conducted to investigate the uniformity of amount of Eurycomanone between capsules prepared in the same batch.

In meantime, three capsules from TAC-IHF also being analysed for the eurycomanone content by HPLC. The result shows in Table 4.7, the first, second, and third capsules are 0.82. 0.84. and 0.80, respectively which is calculated by using the calibration curve of eurycomanone standard. The average was taken, it shows that the percentages of eurycomanone is 0.82 w/w (%) and approaching one percent of Eurycomanone. This analysis was conducted to investigate the uniformity of amount of Eurycomanone between capsules prepared in the same batch. It is expected through this result, consumers and buyers can have confidence to use UMP standardize Tongkat Ali as their daily supplement

Table 4.5.1 Recovery analysis of TAC-IP

Capsule	Retention Time (min)	Mean of Retention Time (min)	Peak Area	Mean of Peak Area	Percentage of Eurycomanone	Mean Percentage of Eurycomanone
1	7.72	7.73	583.91	588.20	0.70	0.7
	7.73		588.40			
	7.74		592.28			
2	7.61	7.58	576.17	569.36	0.72	
	7.57		542.00			
	7.55		590.09			
3	7.42	7.38	591.85	603.72	0.68	
	7.38		632.46			
	7.35		586.92			

*Concentration (w/w%) = (Cf/Cs)*100

Cf = known concentration (from Eurycomanone standard calibration curve)

Cs = sample concentration

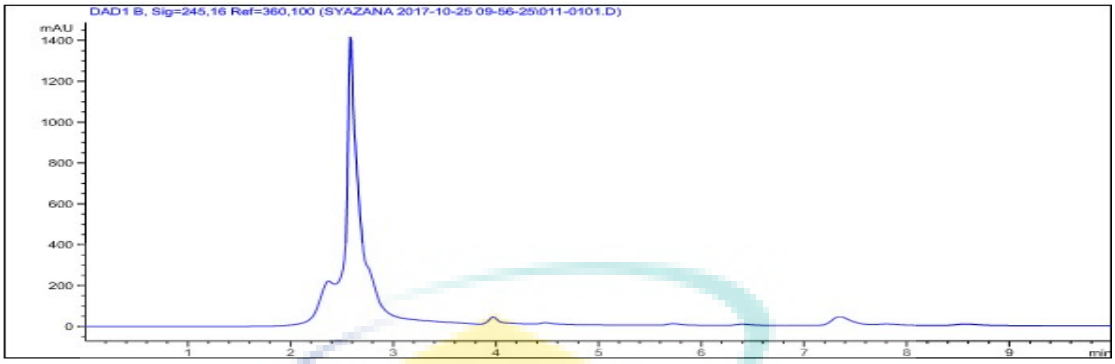


Figure 4.5.1 The chromatogram of TAC-IP (Uniformity test)

*Others chromatograms can refer APPENDIX G

Table 4.5.2 Recovery analysis of TAC-IHF

Capsule	Retention Time (min)	Mean of Retention Time (min)	Peak Area	Mean of Peak Area	Percentage of Eurycomanone	Mean Percentage of Eurycomanone
1	7.81	7.84	708.39	695.96	0.82	0.82
	7.86		697.17			
	7.85		682.80			
2	7.72	7.72	648.73	718.72	0.84	
	7.71		787.48			
	7.72		719.94			
3	7.62	7.60	738.67	698.96	0.80	
	7.62		675.90			
	7.57		682.62			

*Concentration (w/w%) = (Cf/Cs)*100

Cf = known concentration (from Eurycomanone standard calibration curve)

Cs = sample concentration

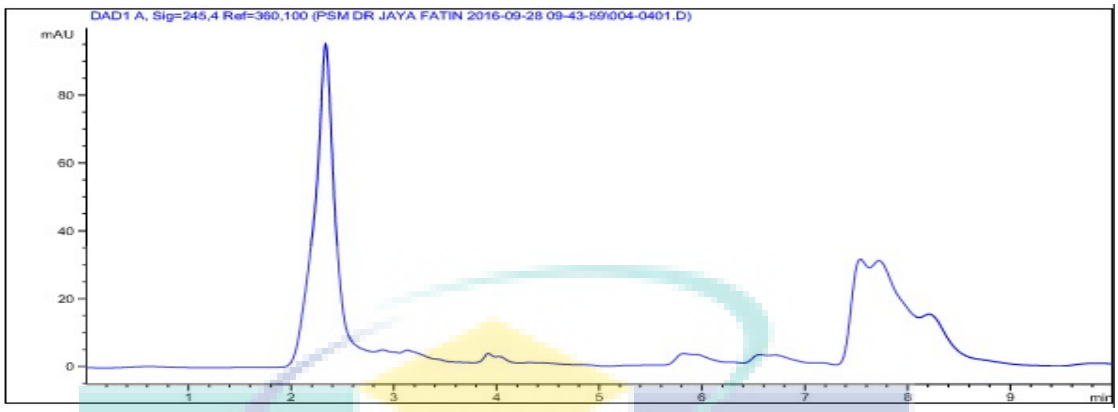


Figure 4.5.2 The chromatogram of TAC-IHF (Uniformity test)

*Others chromatograms can refer APPENDIX H

4.6 Uniformity weight of the capsule

To ensure the consistency of dosage units, each unit in a batch should have a drug substance content within a narrow range around the label claim. Dosage units are defined as dosage forms containing a single dose or a part of a dose of drug substance in each unit. The uniformity of dosages unit specification is not intended to apply to suspensions, emulsions, or gels in unit-dose containers intended for topical administration. The term "uniformity of dosage unit" is defined as the degree of uniformity in the amount of the drug substance among dosage units. The individual weight of capsule to be within the limit of 90 – 110 % of the average weight. The percentage of deviation is calculated using the formula as below.

$$\frac{net\ weight\ of\ capsules\ -\ average\ net\ weight\ of\ capsule}{average\ net\ weight\ of\ capsules} \times 100\%$$

Based on the result (Figure 4.16), the uniformity of weight of TAC-IP was determined by using capsules in order to make sure that the dosage units are consistent. The capsules have an average weight of 206.77 mg which is under the category of "less than 300 mg". all the 20 capsules that have been carried out did not exceed the limit of

± 10.0% and therefore, it considered to pass the test. The overall result of all 20 capsule can be seen at APPENDIX I.

While from the result on Figure 4.17, the uniformity of weight TAC-IHF was determined by using capsules in order to make sure that the dosage units are consistent. The capsules have an average weight of 162.80 mg which is under the category of "less than 300 mg". all the 20 capsules that have been carried out did not exceed the limit of ± 10.0% and therefore, it considered to pass the test. The overall result of all 20 capsule can be seen at APPENDIX J.

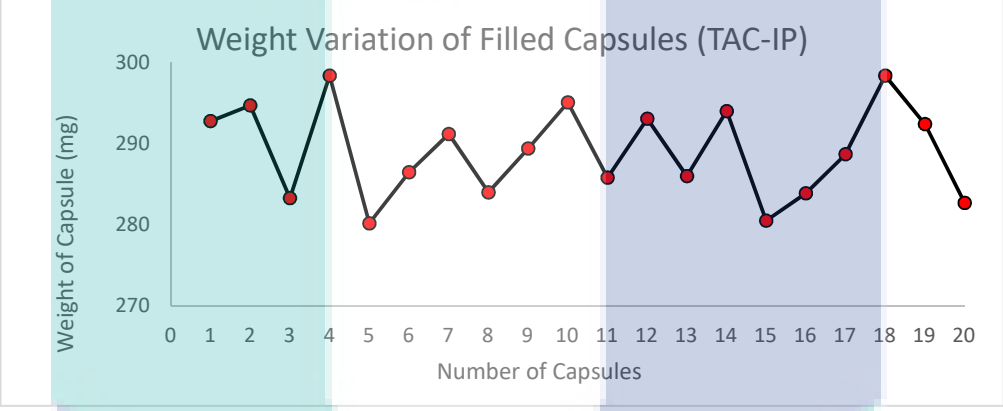


Figure 4.6.1 Measurement of weight variation of TAC-IP

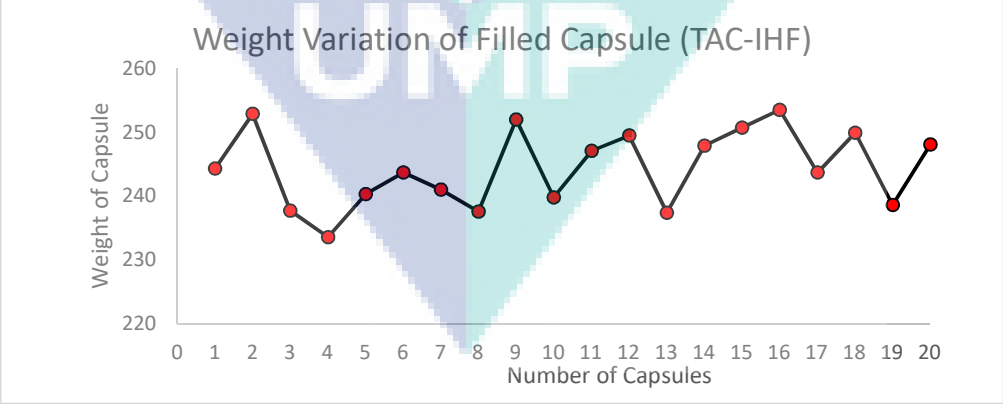


Figure 4.6.2 Measurement of weight variation of TAC-IHF

4.7 Heavy Metal Test

Analysis of heavy metal tests have been done in the Central Laboratory of the UMP. Based on the result obtained, for both TAC-IP and TAC-IHF, the three elements of heavy metal (Arsenic, Cadmium and Lead) were not detected which is less than 0.1 ppm. Mercury also was not detected which less than 10 ppb for both TAC-IP and TAC-IHF. Therefore, both TAC-IP and TAC-IHF do not exceed the minimum requirements set by MS and NPRA. The result for both capsules can be compared with the limit stated by Drug Registration Guidance Document (DRGD) in Table 4.7. The result for TAC-IP and TAC-IHF were in Table 4.8 and Table 4.9 respectively.

Table 4.7.1 Limit for heavy metals

Lead	NMT 10.0 mg/kg or 10.0 mg/L (10.0 ppm)
Arsenic	NMT 5.0 mg/kg or 5.0 mg/L (5.0 ppm)
Mercury	NMT 0.5 mg/kg or 0.5 mg/L (0.5 ppm)
Cadmium	NMT 0.3 mg/kg or 0.3 mg/L (0.3 ppm)

*NMT: Not More Than Source: Drug Registration Guidance Document (DRGD)

Table 4.7.2 Heavy metal testing for TAC-IP

No	Parameter	Results	Unit
1.	Cadmium (Cd)	Not detected (less than 0.1)	ppm
2.	Lead (Pb)	Not detected (less than 0.1)	ppm

3.	Arsenic (As)	Not detected (less than 0.1)	ppm
4.	Mercury (Hg)	Not detected (less than 10)	ppb

Source: APPENDIX K (Sample description: Tongkat Ali 1)

Table 4.7.3 Heavy metal testing for TAC-IHF

No	Parameter	Results	Unit
1.	Cadmium (Cd)	Not detected (less than 0.1)	ppm
2.	Lead (Pb)	Not detected (less than 0.1)	ppm
3.	Arsenic (As)	Not detected (less than 0.1)	ppm
4.	Mercury (Hg)	Not detected (less than 10)	ppb

Source: APPENDIX K (Sample description: Tongkat Ali 2)

4.8 Microbial Load Test

The microbial analysis included in the NPRA requirement and important as the quality control of the product. According to the DRGD, the microbial test for both capsule; TAC-IP and TAC-IHF were followed the B category requirement based on the technique and method used in producing the capsules. The B category requirement is the herbal medicinal products containing, for example, extracts and/or herbal drugs,

with or without excipients, where the method of processing or, where appropriate, in the case of herbal drugs, of pre-treatment reduces the level of organism as stated in the category. The Table 4. 10 showed the limit for microbial load that need to be followed (Pharmaceutical et al., 1915).

According to the guidelines the product should not contain microbial contamination such as *E. coli, Staphylococcus aureus, Salmonella sp.*, bile tolerant gram negative bacteria and fungi. Therefore, from the result obtained for both capsules, TAC-IP and TAC-IHF, the bacteria were present. As conclusion, the microbial load testing was failed and not fulfil the requirement. However, in the future development, our sample will be done in a GMP facility at Dong Foong Manufacturing Sdn Bhd. The contract manufacturer will be helping in obtaining the KKM MAL number by making the capsule under GMP environment to avoid any possible contamination. A contract manufacturer required as UMP laboratory does not have a GMP certified laboratory yet.

Table 4.8.1 Limit for microbial load

Microbiological control	Acceptance criteria
TAMC	NMT 5×10^4 CFU/g or CFU/mL
TYMC	NMT 5×10^2 CFU/g or CFU/mL
Bile-tolerant gram-negative bacteria	NMT 1×10^2 CFU/g or CFU/mL
Escherichia coli	Absence (1 g or 1 mL)
Salmonella	Absence (25 g or 25 mL)
Staphylococcus aureus	Absence (1 g or 1 mL)

*TAMC: Total Aerobic Microbial Count

*TYMC: Total Yeast and Mould Count

Source: Drug Registration Guidance Document (DRGD)

Table 4.8.2 The microbial load result of TAC-IP

Microbiological control	Acceptance criteria
TAMC	7.5×10^3 CFU/g
TYMC	5.0×10^2 CFU/g
Bile-tolerant gram-negative bacteria	Present in 1 g
Escherichia coli	Present in 1 g
Salmonella	Present in 1 g
Staphylococcus aureus	Present in 1 g

Table 4.8.3 The microbial load result of TAC-IHF

Microbiological control	Acceptance criteria
TAMC	5.0×10^3 CFU/g
TYMC	5.5×10^2 CFU/g
Bile-tolerant gram-negative bacteria	Present in 1 g
Escherichia coli	Present in 1 g
Salmonella	Present in 1 g
Staphylococcus aureus	Present in 1 g

4.9 Labelling in accordance to the NPRA Guidelines

The guidelines from the Drug Registration Guidance Document (DRGD) was followed in the labelling process (APPENDIX B). According to the NPRA, the product brand to be suggested by us however the list of main material must be included within the brand name. Product description, pack size, active substances, dosage form, precautions, storage condition, ingredients, manufacturing date, expired date and address also included in the label. The product stated as traditional medicine. Dosage that required are 2 capsules once daily after meal. Due to the unavailability of a certified GMP plant for herbal manufacturing in UMP, Dong Foong Manufacturing Sdn Bhd will be identified as the manufacturer in the label. Some empty space been included within the label to include MAL number once it has been approved. Figure 4.9.1 showed the final design of the label submitted to NPRA.

Figure 4.9.1 The label of TAC-IHF

4.10 Survey on the effectiveness of TAC-IP

The survey conducted for one-month period. There were 20 respondents involved in this survey and consented to the form given in APPENDIX L and APPENDIX M. This survey was done voluntarily and with consent to evaluate the effectiveness of the capsule.

The respondent behavioural changes before the daily intake of the Tongkat Ali capsule and after 30 days by taking the capsule twice daily. The capsule made by the Indigenous people was 100 % pure from Tongkat Ali root powder without any additional excipient and others herbs plant. Before taken the capsule, all the respondent required to answer the pre- trial question which is how there feel before consume this capsules. Based on the result obtained, mostly the respondent chose the scores of neutral for each question. Then, after one month consuming the capsule, once again the respondents required to answer the section of post-trial and overall questions. As for the post- trial, the result was quite different from pre- trial. Most the respondent chose the score of satisfied and the score of very satisfied with the product. Moreover, there is no complains of any side effect while taking this capsule, and by consuming the product, most respondent agree that their energy level increased or much better than before. The difference between the pre- trial and post- trial result can been seen at Figure 4.11(a) and Figure 4.17(b) respectively.

Respondent were recruited from volunteers around Pahang and Johor state. We screened approximately 20 respondents for the survey on effectiveness of TAC-IP. The 20 respondent were all male within the average age of 18 to 50 years old. Moreover, the respondents were all Malay. A quarter from the respondents has been tried the Tongkat Ali product before this. By comparing the Tongkat Ali product they have tried before, they stated that this product (TAC-IP) is better and effective than other product they have tried before. Others respondent who not even tried once of the Tongkat Ali product stated that 'they never have need it'. The demographic information of the respondent has been sorted out in the Table 4.11. The all 20 respondents were received 60 capsules of TAC-IP for 4 week durations. The 4 week durations were selected as more representative of energy change.

Based on the result obtained, from the Figure 4.18, the survey was promising overall. The response was encouraging whereby almost all respondents were acknowledging the effectiveness of the product and requested to continue consuming it. None of the respondents provided any negative feedback or complaint, as such that the Tongkat Ali capsule produce by the Indigenous people was proven to be authenticated as *E. longifolia* and preferred by respondents. The capsule made by Indigenous people was powdered finely by using a spice grinder. This technique used to ensure that the capsule

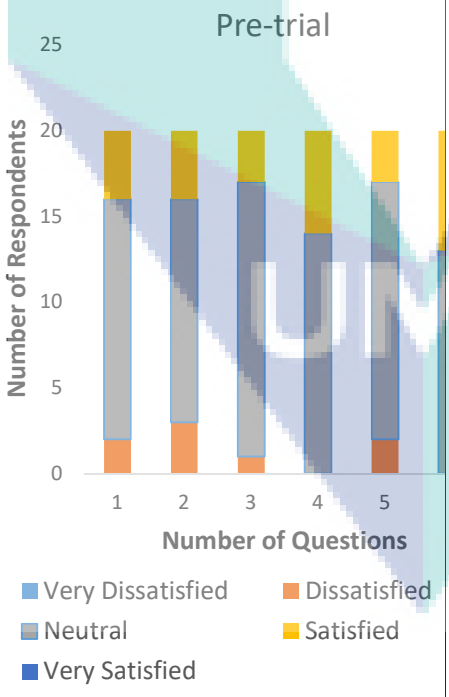
produce contain 100 % of Tongkat Ali root. Before this, ancient people consumed the Tongkat Ali as in the tea form which is the root was boiled (Bhat & Karim, 2010). Now, by adapting the modern technology, they invent the Tongkat Ali root in the form of capsule which is easier to consume and effective. The advantages of this grinding method that, the capsule will not need any excipient as additional material to formulated the capsule. Then, it will restore the originality and purity of the product.

We realised based on the survey and also communications with the indigenous people the technique of making the root into fine powder were acceptable by the respondents. According to the indigenous people they have been selling the capsule in this manner for almost 15 years with none of the customers ever complained of any liver or kidney failures due to consumption of the Tongkat Ali capsule over long period. Some manufacturer nevertheless preferred to extract before making into capsule. This technique known to be time consuming and even likely to be costly especially with Tongkat Ali root requiring reflux by boiling. By adopting the Indigenous people method, we can reduce these issues and avoid the use of foreign material such as excipients. NPRA allows both types of raw material for capsules i.e. either powdered or extracted.

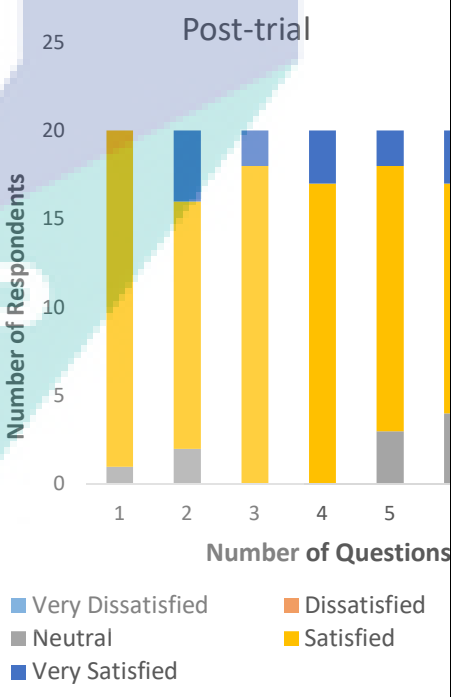
Table 4.10.1 Demographic part of 20 respondents

No.	Parameters	Results
1.	Gender	Male
2.	Religion	Islam
3.	Age	18 – 25 (5 people) 26 – 35 (5 people) 36 – 45 (5 people) 46 – 55 (5 people)
4.	Have try any Tongkat Ali product before?	Yes (5 people) No (15 people)

5.	Product that have been tried.	Nu-Prep Vigor Jamu for Men Tongkat Ali Plus Petani Yusmira Tongkat Ali VIMAX
6.	Reason for not tried any Tongkat Ali products.	Never have need for it (13 people) Tried other men's energy pills (2 people)



(a)



(b)

Figure 4.10.1 **(a)** The Pre-Trial result of the survey by 20 voluntary respondents and **(b)** The Post-Trial result of the survey by 20 voluntary respondents

Table 4.10.2 The lists of questions for Pre-trial and Post-trial section

No.	Questions
1	How satisfied are you with your energy?
2	How satisfied are you with your ability to perform your daily living activities?
3	How satisfied are you with your capacity for work?
4	How satisfied are you with yourself?
5	How satisfied are you with your personal relationships?
6	How satisfied are you with your sex life?

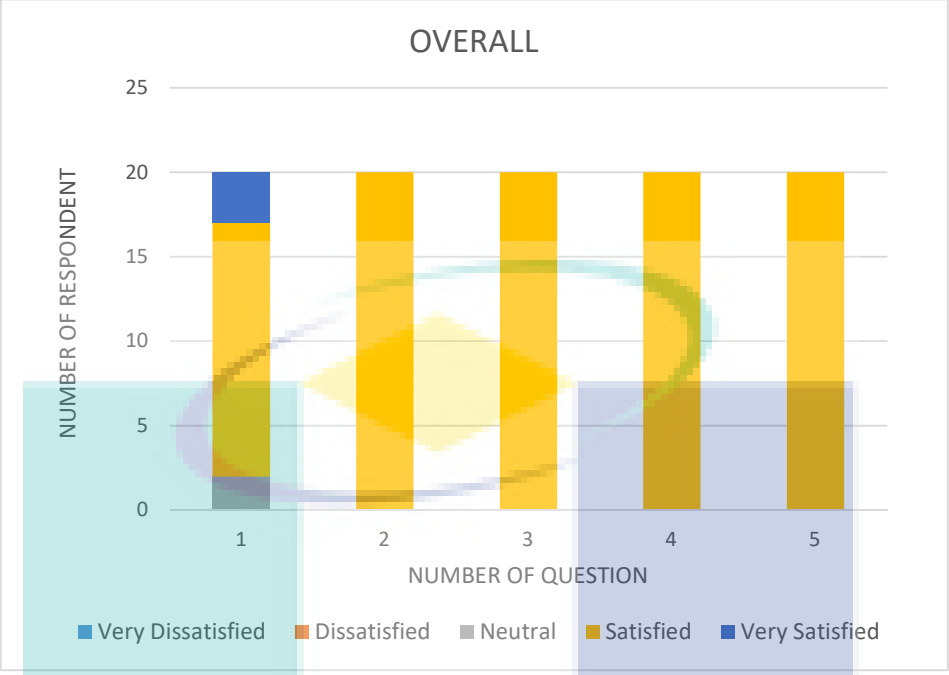


Figure 4.10.3 The overall result of the survey by 20 voluntary respondents

Table 4.10.3 The lists of overall question section

No.	Questions
1	How satisfied or dissatisfied are you with the ability of the product to enhance the sexual activity?
2	How satisfied or dissatisfied are you with the amount of time it takes the product to start working?
3	How convenient or inconvenient is it to take the product as instructed?
4	Overall, how confident are you that taking this product is a good thing for you?

5	How confident that you will recommend this product to your friends?

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusion

In conclusion, 1.24 w/w % of eurycomanone was detected in water extract of *E. longifolia* root powder by using HPLC analysis. The value obtained was to verified the purity and originality of Tongkat Ali root. The Tongkat Ali Capsule in House Formulation (TAC-IHF) was formulated from the Tongkat Ali root powder by adopting the Indigenous people way of preparing the raw material for the capsules. Each capsule contains 0.8 w/w % of eurycomanone which incidentally fulfilled the suggested Malaysia Standard range, 0.8 – 1.5 w/w %. The survey on effectiveness of Tongkat Ali Capsule by Indigenous People (TAC-IP) was carried out with positive feedback overall. Several requirements of NPRA was fulfilled which were heavy metal test, uniformity of weight, and label as required by NPRA. Although the microbial evaluations showed some microbial contamination but expected to be resolved by Dong Foong Manufacturing Sdn Bhd. A total 1.5kg of the powdered root raw material was send to the contract manufacturer for purpose of NPRA registration.

5.2 Recommendations

The pre-commercialization project achieved the intended objectives and likely to obtain the MAL number within a period of 4-6 months. However in order to ensure the next phase for the success of this product required the commitment of the student entrepreneur whereby certain skills of marketing should be learned. One advantage of this product compared to other herbals is the huge market it is having. Example a search of the keyword of "Tongkat Ali" at Amazon.com provided total hits of over 900 products

while only 8 hits if searched as “Misai kucing” or its genus species i.e. “*Orthosiphon aristatus*” with slightly over 100 products. This is evident that aphrodisiac products have a huge following by the male gender looking for physical enhancement, libido improvement or improvement in their sexual performances.

REFERENCES

Airth, J. M., Bray, D. F., & Radecka, C. (1967). Variability of uniformity of weight test as an indicator of the amount of active ingredient in tablets. *Journal of Pharmaceutical Sciences*, *56*(2), 233–235. https://doi.org/10.1002/jps.2600560217

Alberti, K. G. M. M., & Zimmet, P. Z. (1998). Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus. Provisional report of a WHO Consultation. *Diabetic Medicine*, *15*(7), 539–553. https://doi.org/10.1002/(SICI)1096-9136(199807)15:7<539::AID-DIA668>3.0.CO;2-S

Ang, H. H., & Lee, K. L. (2002). Effect of Eurycoma longifolia Jack on orientation activities in middle-aged male rats. *Fundamental & Clinical Pharmacology*, *16*(6), 479–483. https://doi.org/10.1046/j.1472-8206.2002.00106.x

Ang, H. H., & Lee, K. L. (2006). Contamination of mercury in tongkat Ali hitam herbal preparations, *44*, 1245–1250. https://doi.org/10.1016/j.fct.2006.01.014

ANG, H. H., & SIM, M. K. (1997). Eurycoma longifolia Jack Enhances Libido in Sexually Experienced Male Rats. *Experimental Animals*, *46*(4), 287–290. https://doi.org/10.1538/expanim.46.287

Beutler, J. A., Kang, M.-I., Robert, F., Clement, J. A., Pelletier, J., Colburn, N. H., … Henrich, C. J. (2009). Quassinoid Inhibition of AP-1 Function Does Not Correlate with Cytotoxicity or Protein Synthesis Inhibition. *Journal of Natural Products*, *72*(3), 503–506. https://doi.org/10.1021/np800732n

Bhat, R., & Karim, A. A. (2010). Tongkat Ali (Eurycoma longifolia Jack): A review on its ethnobotany and pharmacological importance. *Fitoterapia*, *81*(7), 669–679. https://doi.org/10.1016/j.fitote.2010.04.006

Chan, K., Choo, C., Rain, N., & Ismail, Z. (2004). Antiplasmodial studies of Eurycoma longifolia Jack using the lactate dehydrogenase assay of Plasmodium falciparum, *92*(785), 223–227. https://doi.org/10.1016/j.jep.2004.02.025

Chen, C. K., Wan Mohamad, W. M. Z., Ooi, F. K., Ismail, S. B., Abdullah, M. R., & George, A. (2014). Supplementation of Eurycoma longifolia Jack extract for 6 weeks does not affect urinary testosterone: Epitestosterone ratio, liver and renal

functions in male recreational athletes. *International Journal of Preventive Medicine*, *5*(6), 728–733.

Darise, M., Kohda, H., Mizutani, K., & Tanaka, O. (1982). Eurycomanone and eurycomanol, quassinoids from the roots of Eurycoma longifolia. *Phytochemistry*, *21*(8), 2091–2093. https://doi.org/https://doi.org/10.1016/0031-9422(82)83050-1

England, T. N. (2001). Numb Er 18 Prevention Of Type 2 Diabetes Mellitus By Changes In Lifestyle Among Subjects With Impaired Glucose Tolerance, *344*(18), 1343–1350.

GUO, Z. (2005). Biologically active quassinoids and their chemistry : potential leads for drug design. *Curr Med Chem*, *12*, 173–190. Retrieved from http://ci.nii.ac.jp/naid/10031088071/en/

Hajjouli, S., Chateauvieux, S., Teiten, M. H., Orlikova, B., Schumacher, M., Dicato, M., … Diederich, M. (2014). Eurycomanone and eurycomanol from Eurycoma longifolia jack as regulators of signaling pathways involved in proliferation, cell death and inflammation. *Molecules*, *19*(9), 14649–14666. https://doi.org/10.3390/molecules190914649

Han, Y. M., Jang, M., Kim, I. S., Kim, S. H., & Yoo, H. H. (2015). Simultaneous quantitation of six major quassinoids in Tongkat Ali dietary supplements by liquid chromatography with tandem mass spectrometry. *Journal of Separation Science*, *38*(13), 2260–2266. https://doi.org/10.1002/jssc.201500207

Husen, R., Hawariah, A., Pihie, L., & Nallappan, M. (2004). Screening for antihyperglycaemic activity in several local herbs of Malaysia, *95*, 205–208. https://doi.org/10.1016/j.jep.2004.07.004

Katori, N., Aoyagi, N., & Kojima, S. (2001). The study of the applicability of content uniformity and weight variation test--the state of commercial tablets and capsules in Japan. *Chemical & Pharmaceutical Bulletin*, *49*(11), 1412–9. https://doi.org/10.1248/cpb.49.1412

Kunle. (2012). Standardization of herbal medicines - A review. *International Journal of Biodiversity and Conservation*, *4*(3), 101–112. https://doi.org/10.5897/IJBC11.163

Kuo, P. C., Damu, A. G., Lee, K. H., & Wu, T. S. (2004). Cytotoxic and antimalarial

constituents from the roots of Eurycoma longifolia. *Bioorganic and Medicinal Chemistry*, *12*(3), 537–544. https://doi.org/10.1016/j.bmc.2003.11.017

Loew, D., & Kaszkin, M. (2002). Approaching the problem of bioequivalence of herbal medicinal products. *Phytotherapy Research*, *16*(8), 705–711. https://doi.org/10.1002/ptr.1248

Low, B. S., Choi, S. B., Abdul Wahab, H., Kumar Das, P., & Chan, K. L. (2013). Eurycomanone, the major quassinoid in Eurycoma longifolia root extract increases spermatogenesis by inhibiting the activity of phosphodiesterase and aromatase in steroidogenesis. *Journal of Ethnopharmacology*, *149*(1), 201–207. https://doi.org/10.1016/j.jep.2013.06.023

Malviya, N., Jain, S., Gupta, V. B., & Vyas, S. (2011). RECENT STUDIES ON APHRODISIAC HERBS FOR THE MANAGEMENT OF MALE SEXUAL DYSFUNCTION ñ A REVIEW, *68*(1), 3–8.

Mitsunaga, K., Koike, K., Tanaka, T., Ohkawa, Y., Kobayashi, Y., Sawaguchi, T., & Ohmoto, T. (1994). Canthin-6-one alkaloids from Eurycoma longifolia. *Phytochemistry*, *35*(3), 799–802. https://doi.org/https://doi.org/10.1016/S0031-9422(00)90609-5

Mohd Effendy, N., Mohamed, N., Muhammad, N., Naina Mohamad, I., & Shuid, A. N. (2012). Eurycoma longifolia: Medicinal plant in the prevention and treatment of male osteoporosis due to androgen deficiency. *Evidence-Based Complementary and Alternative Medicine*, *2012*. https://doi.org/10.1155/2012/125761

Morita, H., Kishi, E., Takeya, K., & Itokawa, H. (1992). Biphenylneolignans from wood of Eurycoma longifolia. *Phytochemistry*, *31*(11), 3993–3995. https://doi.org/https://doi.org/10.1016/S0031-9422(00)97570-8

Nafiu, M. O., Abdulsalam, T. A., & Akanji, M. A. (2013). Phytochemical analysis and antimalarial activity aqueous extract of lecaniodiscus cupanioides root. *Journal of Tropical Medicine*, *2013*. https://doi.org/10.1155/2013/605393

Pharmaceutical, N., Bureau, C., & Health, M. O. F. (1915). Drug Registration. *The Boston Medical and Surgical Journal*, *172*(January 2013), 948–948. https://doi.org/10.1056/NEJM191506241722519

Rehman, S. U., Choe, K., & Yoo, H. H. (2016). Review on a traditional herbal medicine, eurycoma longifolia Jack (Tongkat Ali): Its traditional uses, chemistry, evidence-based pharmacology and toxicology. *Molecules*, *21*(3). https://doi.org/10.3390/molecules21030331

Rowshanaie, H., Jaafar, H. Z., Edaroyati, P., Wahab, M., & Rowshanaie, O. (2014). Impact of Different Water Levels on Growth , Plant Water Relations and Leaf Characteristics in Seedling of Tongkat Ali (EURYCOMA LONGIFOLIA JACK), *1*(4), 197–201. https://doi.org/10.15764/WPT.2014.03002

Sahoo, N., Manchikanti, P., & Dey, S. (2010). Herbal drugs: Standards and regulation. *Fitoterapia*, *81*(6), 462–471. https://doi.org/10.1016/j.fitote.2010.02.001

Suan, L., Amaiza, N., Amin, M., Chun, J., Neo, H., Hun, T., … Abdul, R. (2011). LC – MS / MS-based metabolites of Eurycoma longifolia (Tongkat Ali) in Malaysia (Perak and Pahang). *Journal of Chromatography B*, *879*(32), 3909–3919. https://doi.org/10.1016/j.jchromb.2011.11.002

Tada, H., Yasuda, F., Otani, K., Doteuchi, M., Ishihara, Y., & Shiro, M. (1991). New antiulcer quassinoids from Eurycoma longifolia. *European Journal of Medicinal Chemistry*, *26*(3), 345–349. https://doi.org/https://doi.org/10.1016/0223-5234(91)90069-Y

Talbott, S. M., Talbott, J. A., George, A., & Pugh, M. (2013). Effect of Tongkat Ali on stress hormones and psychological mood state in moderately stressed subjects. *Journal of the International Society of Sports Nutrition*, *10*(1), 28. https://doi.org/10.1186/1550-2783-10-28

Talbott, S. M., Talbott, J. A., & Pugh, M. (2013). Effect of Magnolia officinalis and Phellodendron amurense (Relora®) on cortisol and psychological mood state in moderately stressed subjects. *Journal of the International Society of Sports Nutrition*, *10*(1), 37. https://doi.org/10.1186/1550-2783-10-37

Tong, K. L., Chan, K. L., AbuBakar, S., Low, B. S., Ma, H. Q., & Wong, P. F. (2015). The in Vitro and in Vivo Anti-Cancer Activities of a Standardized Quassinoids Composition from Eurycoma longifolia on LNCaP Human Prostate Cancer Cells. *PLoS ONE*, *10*(3), 1–21. https://doi.org/10.1371/journal.pone.0121752

Volume: I: Issue-3: Nov-Dec -2010. (2010), (3), 960–967.

APPENDIX A

NATIONAL PHARMACEUTICAL REGULATORY DIVISION
MINISTRY OF HEALTH, MALAYSIA

DRUG REGISTRATION GUIDANCE DOCUMENT (DRGD)

Second Edition – September 2016, revised March 2017

Address:

Lot 36, Jalan Universiti, 46200 Petaling Jaya, Selangor Darul Ehsan, Malaysia

+ 603-7883 5400

+ 603-7956 2924, 7955 7075

http://npra.moh.gov.my/

Please visit the NPRA website for the latest updates

APPENDIX B

2.7 LABELLING REQUIREMENT

a) The following information as shown in **Table 9** shall be included in the product label. Please refer example of label for natural products approved by the Authority, as shown below.

No.	Items	Immediate Label	Outer Label	Package Insert	Blister Pack
1.	Product name	√	√	√	√
2.	Dosage Form	√	√	√	√
3.	Name of active ingredients, including part of plant used	√	√	√	
4.	Strength of active ingredient in weight	√	√	√	
5.	Indication	√	√		
6.	Batch number	√	√		√
7.	Manufacturing date	√	√		
8.	Expiry date	√	√		√
9.	Dosage/ Use instruction	√	√	√	
10.	Storage condition(s) - state temperature used in the stability study - state "Protect from light and moisture" (If product is not packed in moisture resistant container)	√	√	√	
11.	Registration number (MAL)	√	√		√

No.	Items	Immediate Label	Outer Label	Package Insert	Blister Pack
12.	Name and address of product registration holder (Example: Product Registration Holder: XXXXX)	√	√		
13.	Name and address of manufacturer (Example: Manufacturer: XXXXX)	√ At least name of town/ city and country of manufacturer	√ At least name of town/ city and country of manufacturer	√	
14.	Warning label (if applicable) e.g. Ginseng, Bee Pollen etc. as required under 2.7.2 Specific Labelling Statements/ Warning & Precautions *Note: Please refer Appendix 9: Labelling Requirements*	√	√	√	
15.	Pack size (unit/ volume)	√	√	√	
16.	Name and strength of preservative	√	√	√	
17.	Name and content of alcohol,where present	√	√		
18.	To declare source of ingredients derived from animal origin (active and excipient) including starting materials and gelatine (capsule shell).	√	√		
19.	Additional statement (if applicable)	√	√	√	

No.	Items	Immediate Label	Outer Label	Package Insert	Blister Pack
20.	Contraindication/ Precaution (if any)	√	√	√	
21.	Security Label (Hologram)		√ #		

b) All labels and package inserts must be in *Bahasa Malaysia* or English. In additional to this, translation to another language will be allowed.

c) # In case of a product without an outer carton, the security label shall be applied onto the immediate label. The security label shall however not be applied onto the outer shrink wrap of the product.

d) Font size of the product name on the label, including alphabets and numbers, should be equal in size.

e) For a product containing 2 or more active ingredients, font size of each active ingredient that is highlighted on the inner/ outer carton must be of equal size and equal prominence (Note: this is not referring to the product name, but the statement made on the label).Justification for highlighting certain ingredients only on the product name / label must be provided and subject to approval by the Evaluation Committee.

f) Please ensure all requirements as specified below are stated on the labels and package inserts:

- State the weight per dosage form
- State the quantity/ content of active ingredients per dosage form
- For products in liquid form (syrup), content of active ingredients shall be stated as follows:

  "Each ____ml (per dosage) product contains extract of the following ingredients"

  Herb X = ___mg

  Herb Y = ___mg
- Check and correct all spelling/ grammar and translations.

g) For products meant for traditional practitioner/ physician use, please state its primary use by the related traditional physician/ practitioner on the label.

For example: 'For Chinese Physician Use Only' OR
'For Ayurvedic Practitioner Use Only'.

Example of label approved by the Authority:

This is a traditional medicine

Please consult your pharmacist/ doctor before taking this product

Jauhkan daripada capaian kanak-kanak
Keep out of reach of children

Indication: Traditionally used for women's health

Warning: Pregnancy and breastfeeding: Insufficient reliable data

Keep below 30 ° celcius
Protect from light and moisture

Manufacturing date:
Expiry date:

KAPSUL PQR
500MG

MALXXXXXXXXT

50 CAPSULE

Hologram

Each Capsule (Vegetable capsule) contains :

Folium XX	200mg
Fructus QY	300mg

Dosage : 2 capsule taken twice a day after food

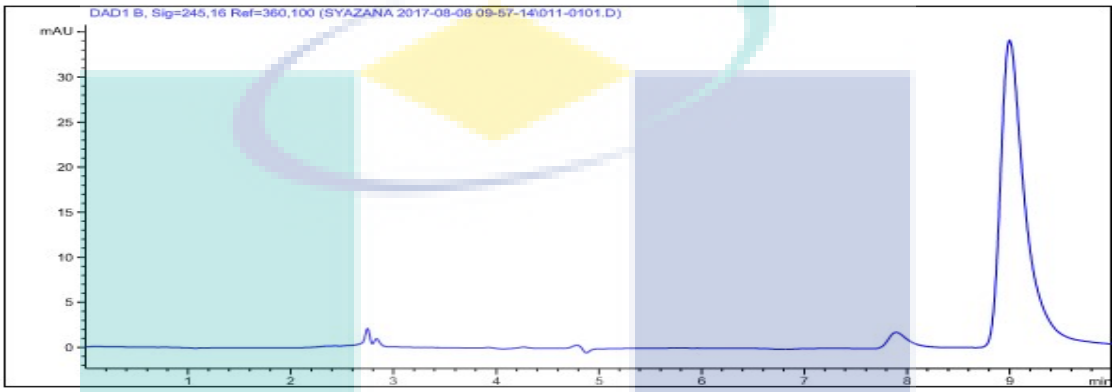
Marketing authorization holder:
Syarikat XYZ Sdn Bhd
18, Jalan Utama
47000 Sungai Buloh
Selangor

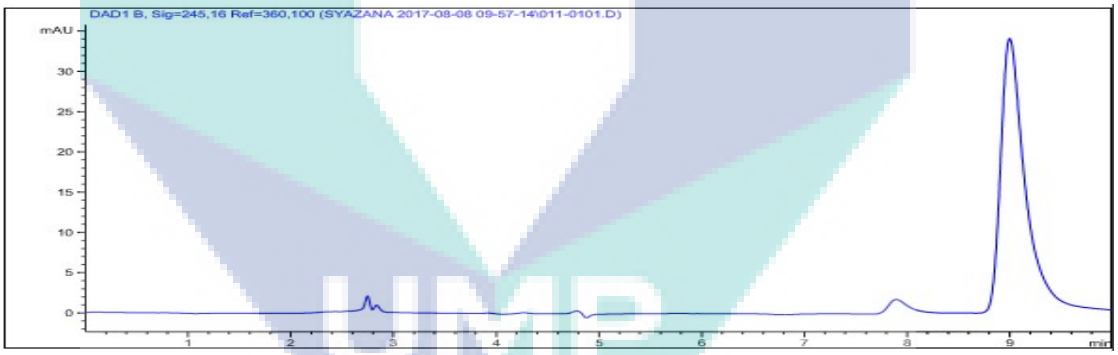
Manufactured by:
Syarikat ABC Sdn Bhd
3, Jalan Universiti
46730 Petaling Jaya

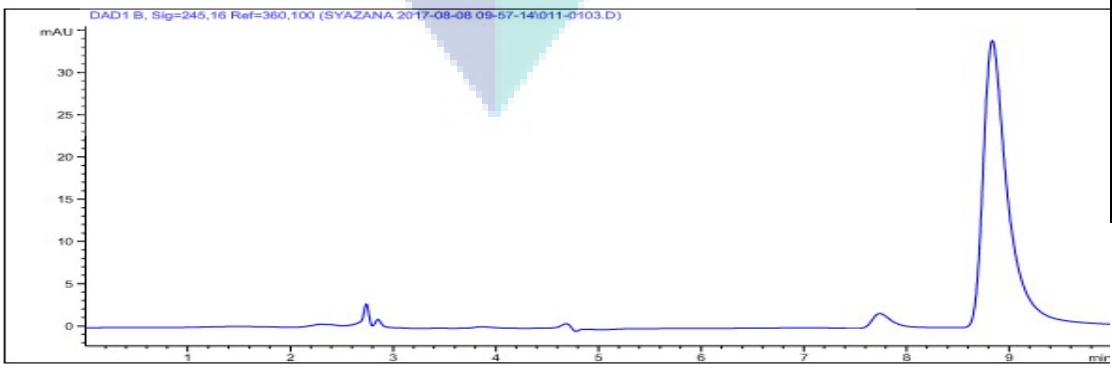
APPENDIX C

THE CHROMATOGRAMS OF STANDARD EURYCOMANONE

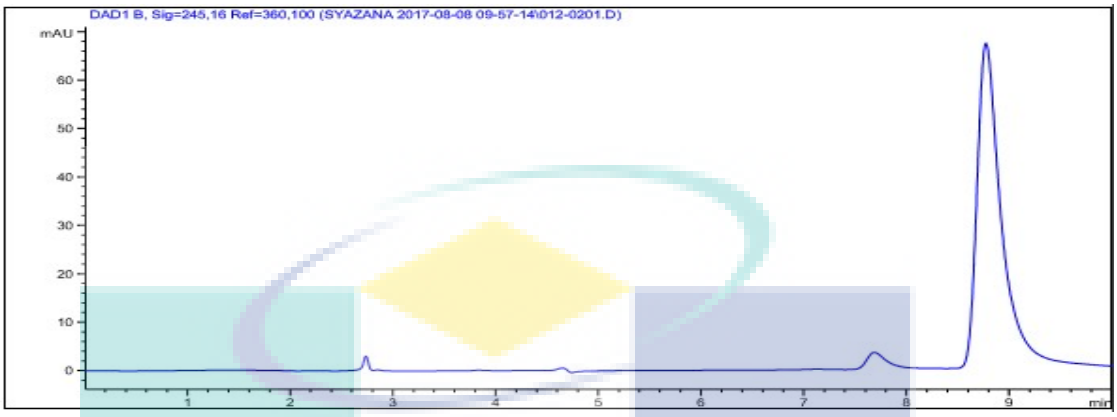
0.03125 gm/ml

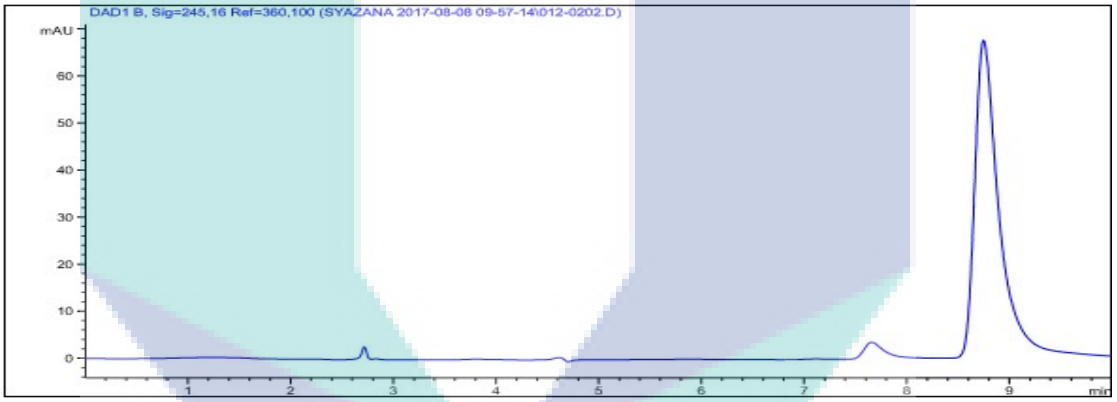


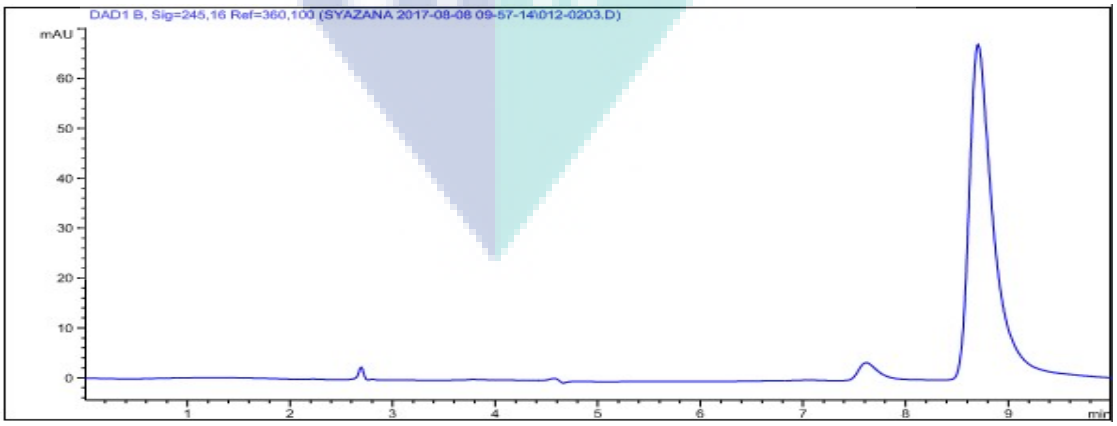




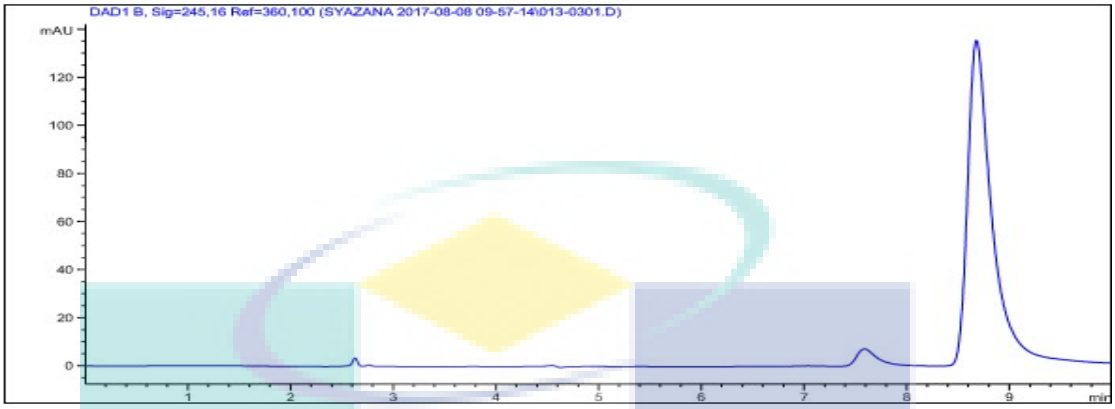
0.0625 mg/ml

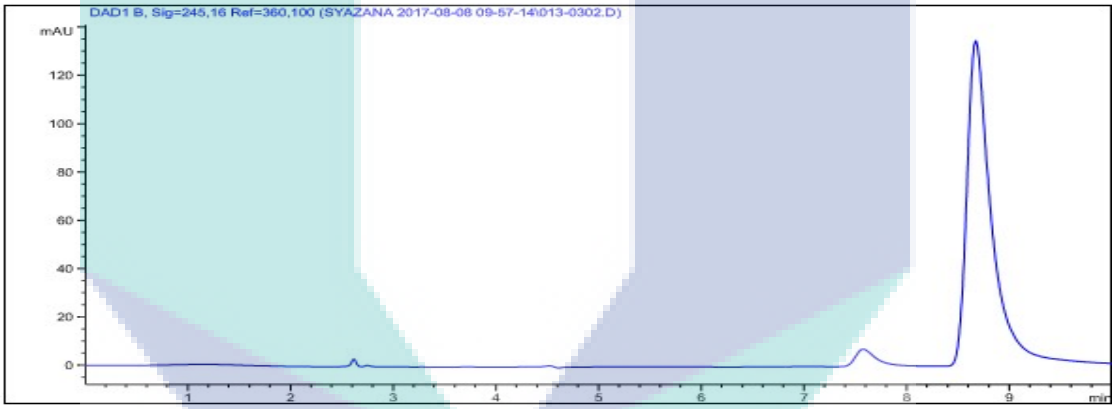

DAD1 B, Sig=245,16 Ref=360,100 (SYAZANA 2017-08-08 09-57-14\012-0201.D)
mAU
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
min

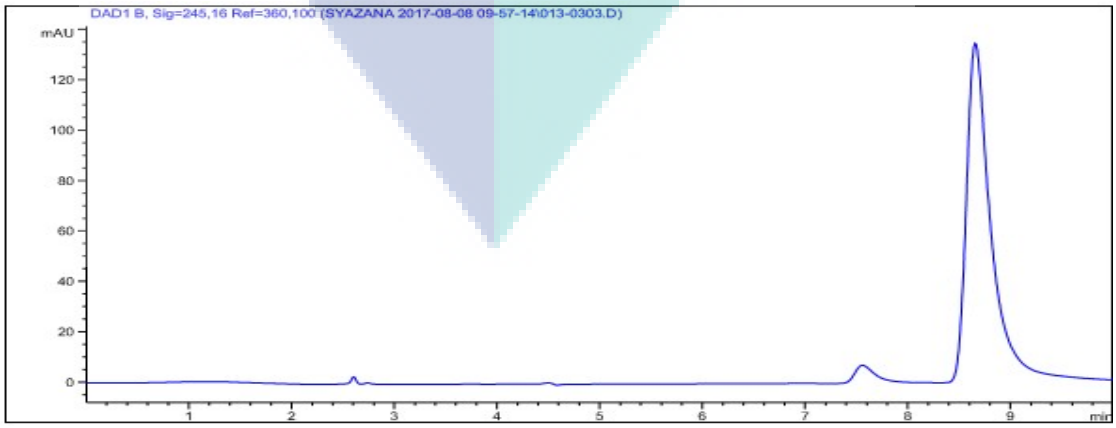

DAD1 B, Sig=245,16 Ref=360,100 (SYAZANA 2017-08-08 09-57-14\012-0202.D)
mAU
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
min


DAD1 B, Sig=245,16 Ref=360,100 (SYAZANA 2017-08-08 09-57-14\012-0203.D)
mAU
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
min

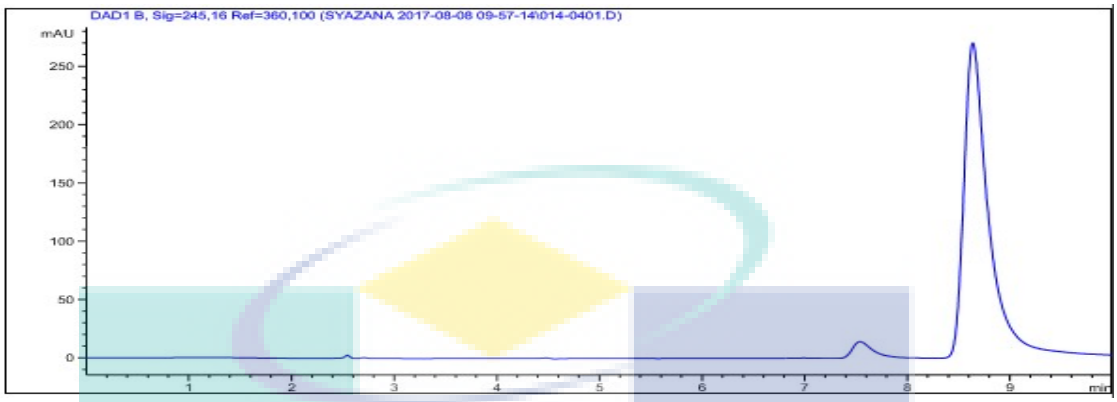
0.125 mg/ml

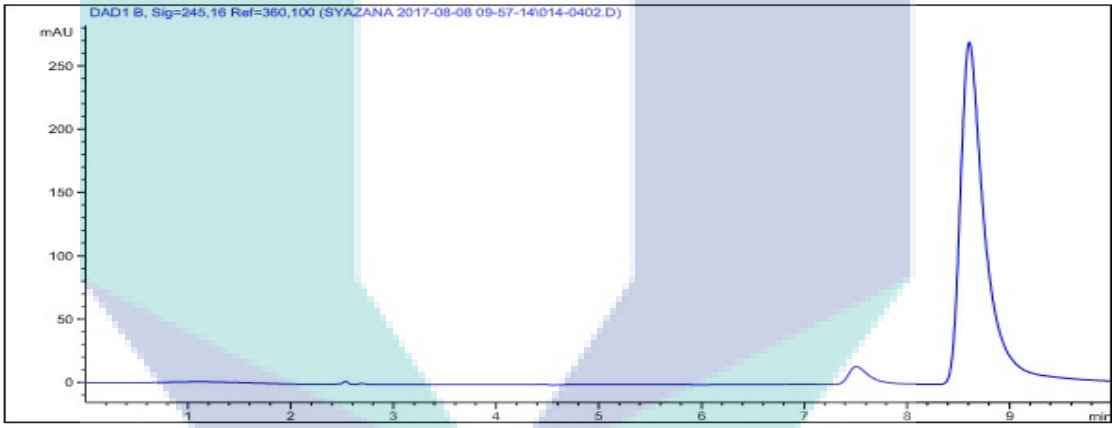

DAD1 B, Sig=245,16 Ref=360,100 (SYAZANA 2017-08-08 09-57-14\013-0301.D)
mAU
120
100
80
60
40
20
0
1
2
3
4
5
6
7
8
9
min

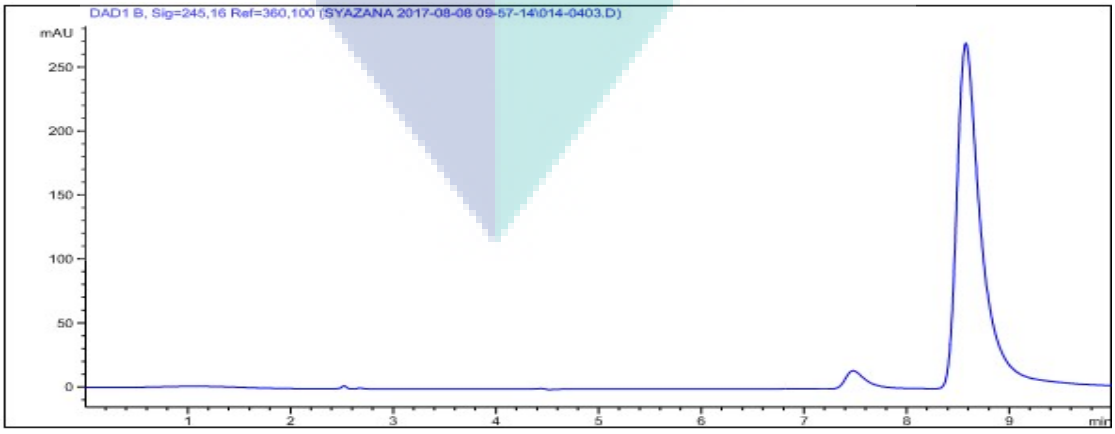

DAD1 B, Sig=245,16 Ref=360,100 (SYAZANA 2017-08-08 09-57-14\013-0302.D)
mAU
120
100
80
60
40
20
0
1
2
3
4
5
6
7
8
9
min


DAD1 B, Sig=245,16 Ref=360,100 (SYAZANA 2017-08-08 09-57-14\013-0303.D)
mAU
120
100
80
60
40
20
0
1
2
3
4
5
6
7
8
9
min

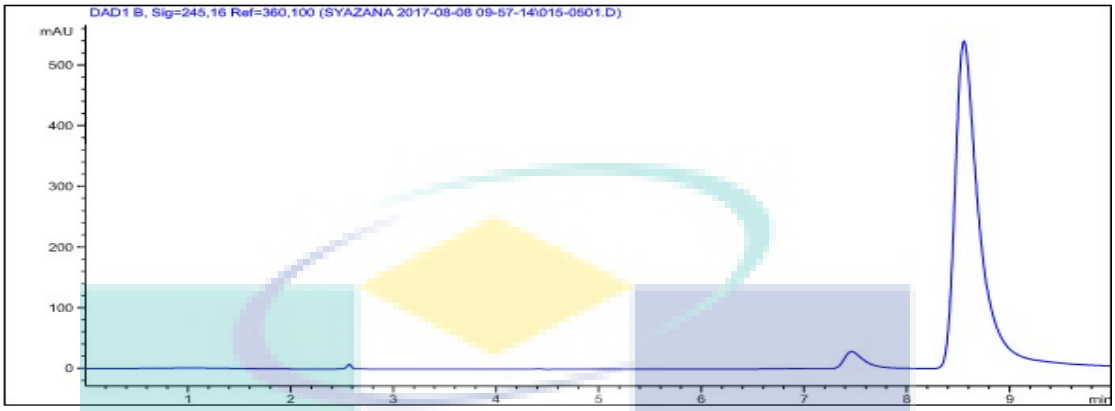
0.25 mg/ml

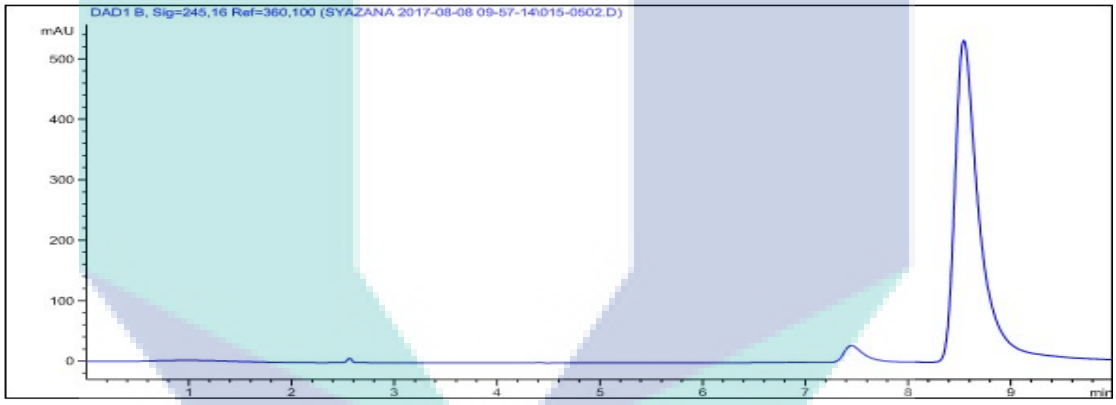

DAD1 B, Sig=245,16 Ref=360,100 (SYAZANA 2017-08-08 09-57-14\014-0401.D)
mAU
250
200
150
100
50
0
1
2
3
4
5
6
7
8
9
min

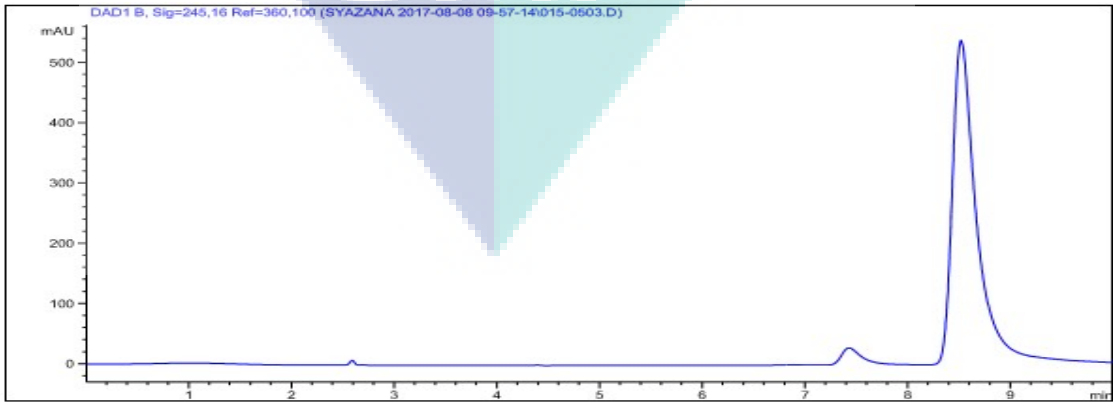

DAD1 B, Sig=245,16 Ref=360,100 (SYAZANA 2017-08-08 09-57-14\014-0402.D)
mAU
250
200
150
100
50
0
1
2
3
4
5
6
7
8
9
min


DAD1 B, Sig=245,16 Ref=360,100 (SYAZANA 2017-08-08 09-57-14\014-0403.D)
mAU
250
200
150
100
50
0
1
2
3
4
5
6
7
8
9
min

0.5 mg/ml


DAD1 B, Sig=245,16 Ref=360,100 (SYAZANA 2017-08-08 09-57-14\015-0501.D)
mAU
500
400
300
200
100
0
1
2
3
4
5
6
7
8
9
min

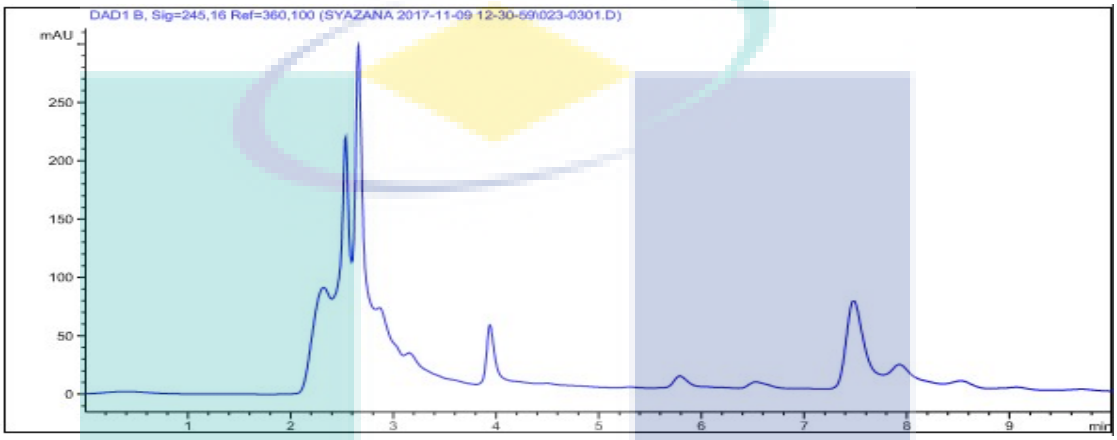

DAD1 B, Sig=245,16 Ref=360,100 (SYAZANA 2017-08-08 09-57-14\015-0502.D)
mAU
500
400
300
200
100
0
1
2
3
4
5
6
7
8
9
min

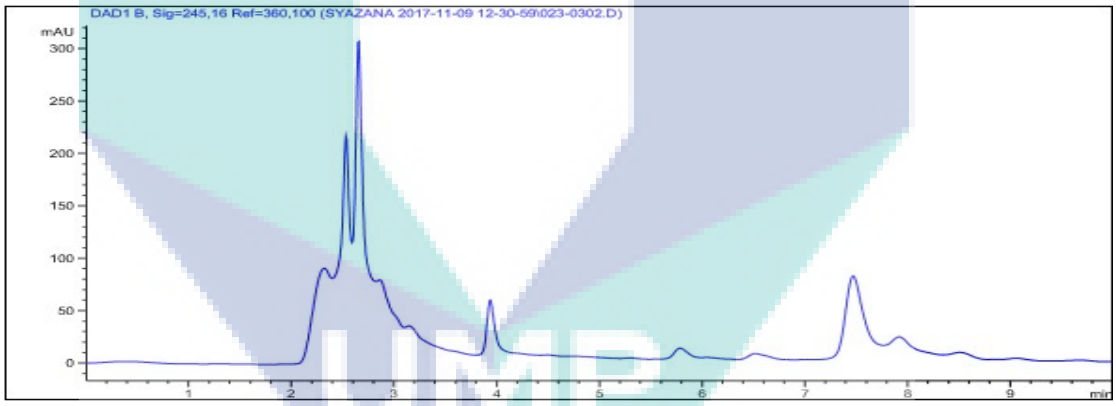

DAD1 B, Sig=245,16 Ref=360,100 (SYAZANA 2017-08-08 09-57-14\015-0503.D)
mAU
500
400
300
200
100
0
1
2
3
4
5
6
7
8
9
min

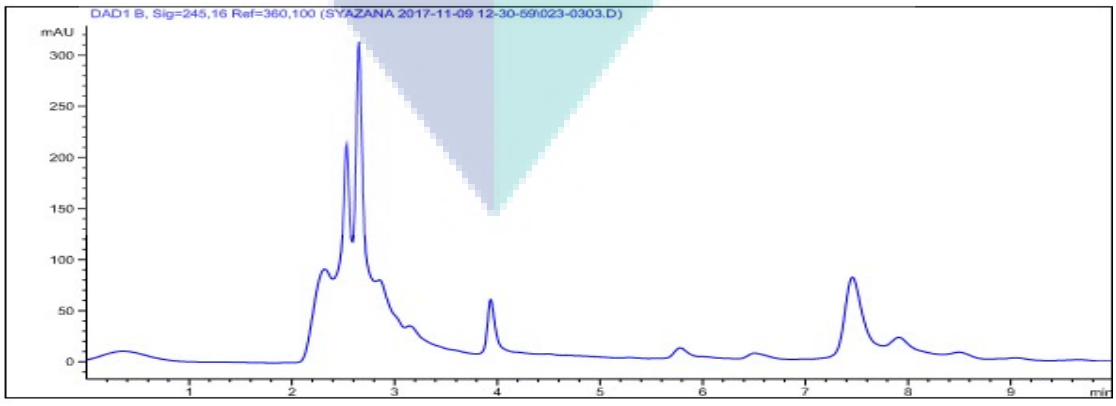
APPENDIX D

THE CHROMATOGRAMS OF TONGKAT ALI WATER EXTRACT

5 mg/ml



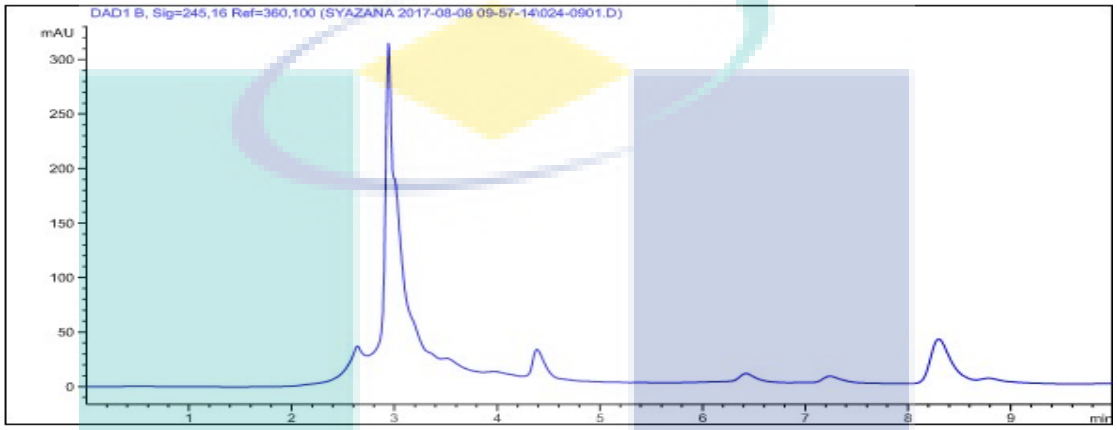


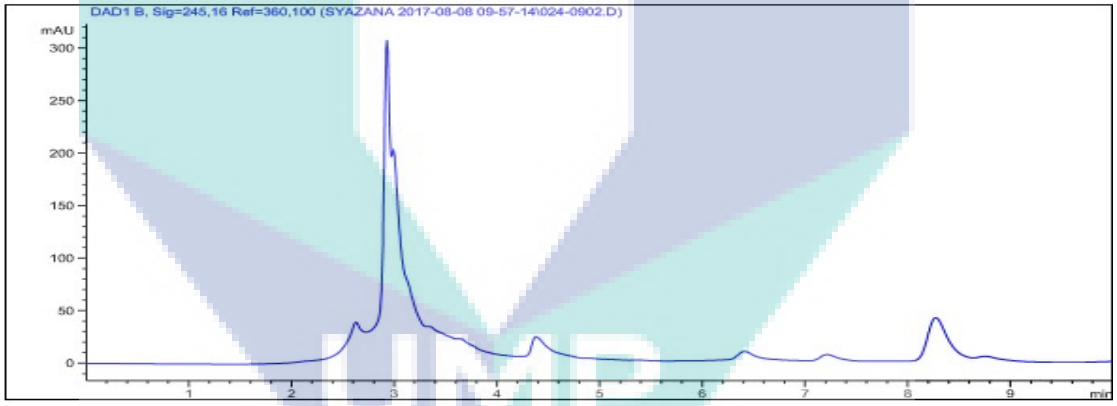


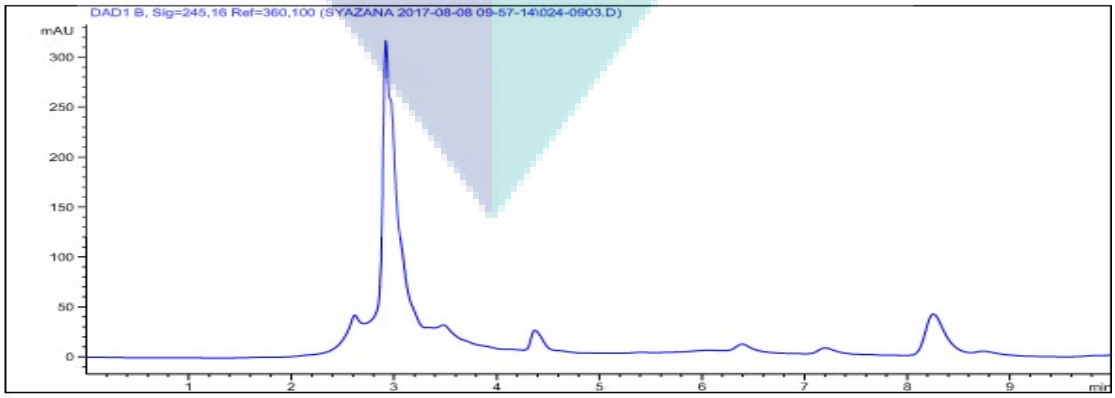
APPENDIX E

THE CHROMATOGRAMS OF TAC-IP

5 mg/ml



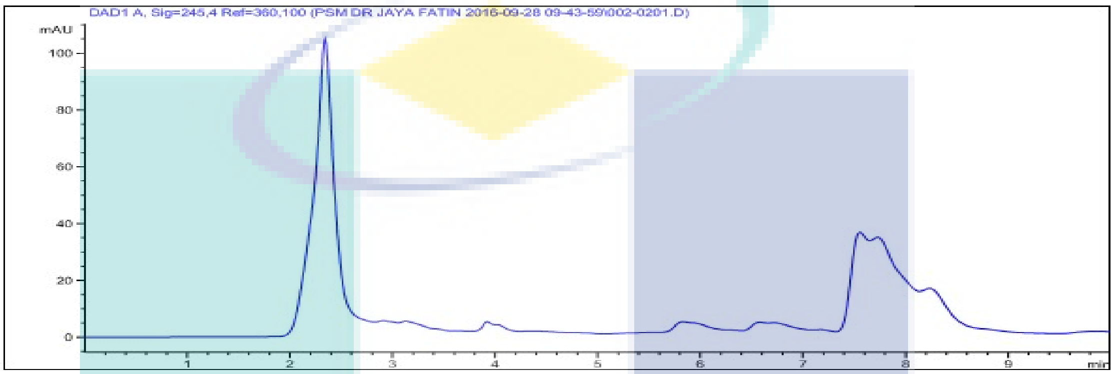


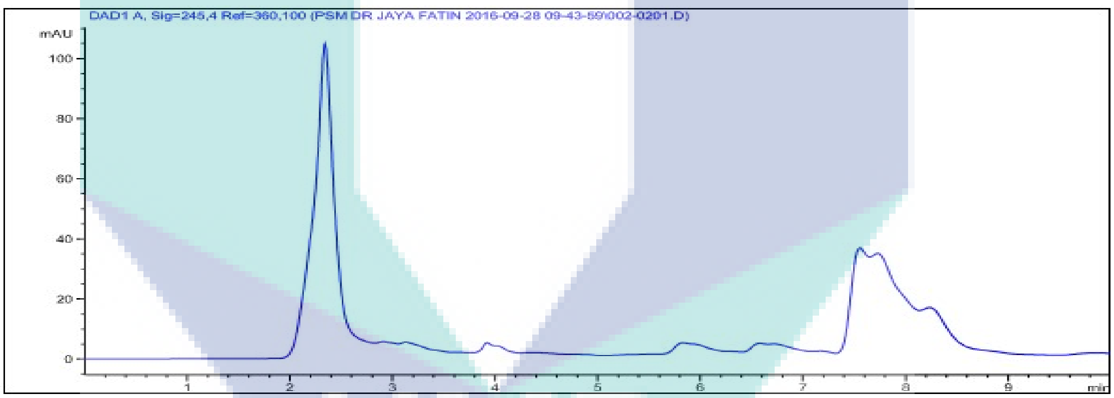


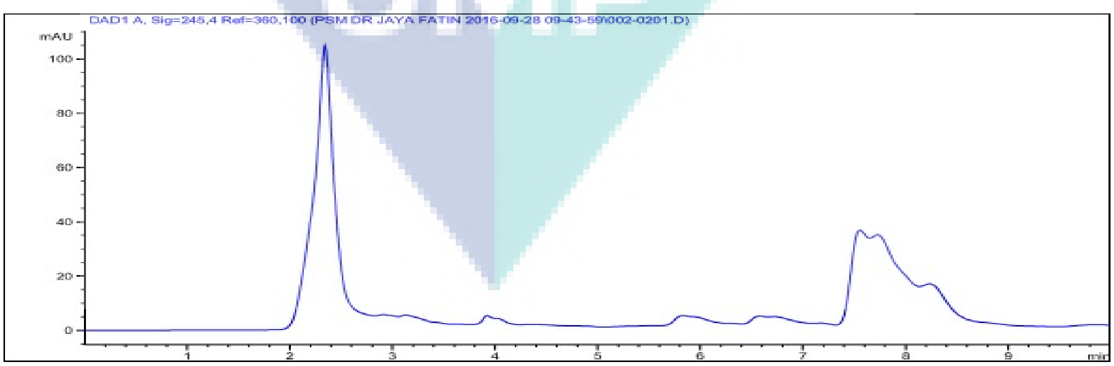
APPENDIX F

THE CHROMATOGRAMS OF TAC-IHF

5 mg/ml



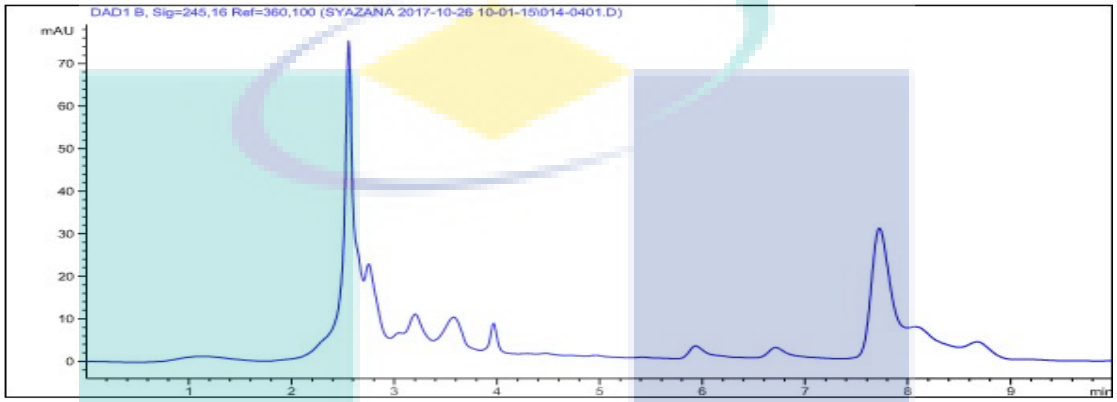


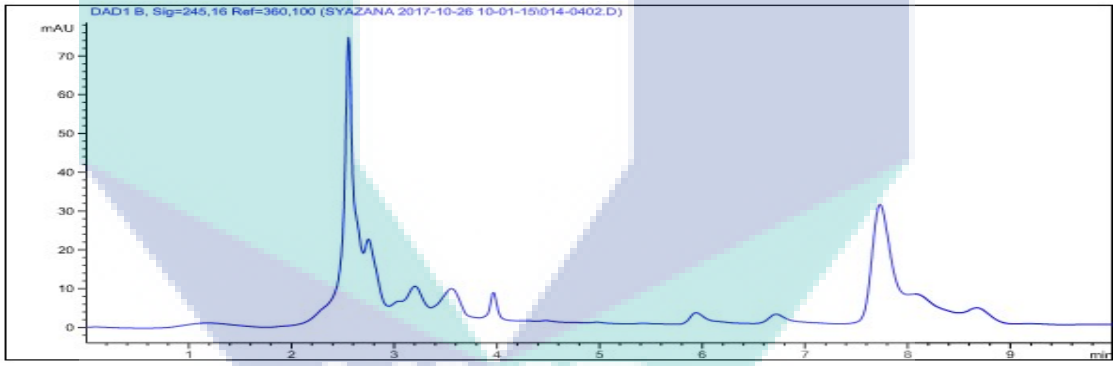


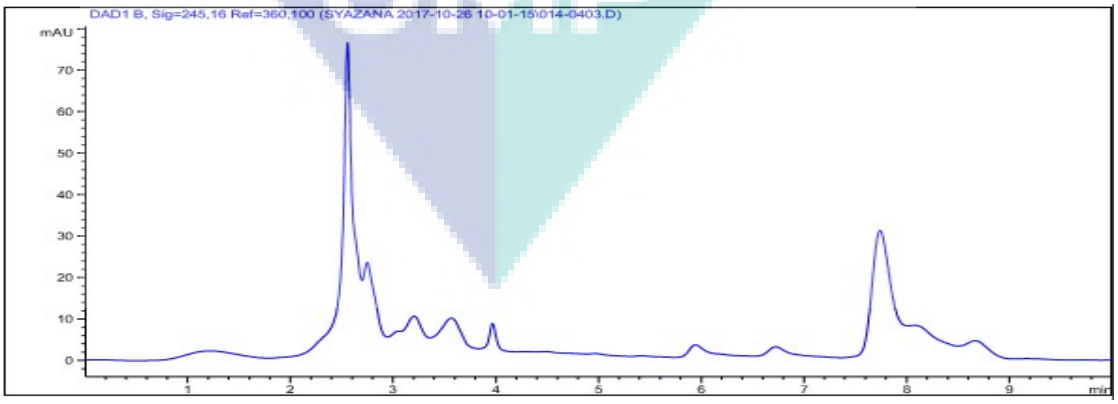
APPENDIX G

THE CHROMATOGRAMS OF UNIFORMITY CONTENT OF EURYCOMANONE IN TAC-IP

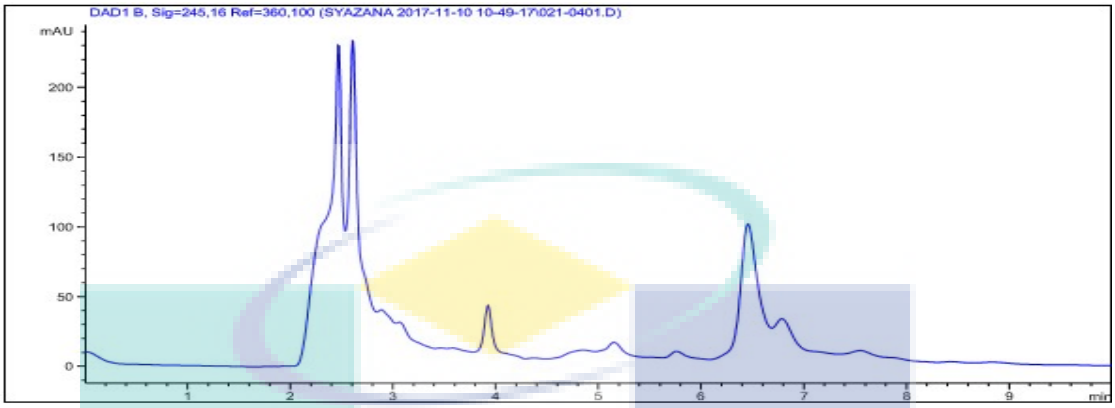
Capsule 1

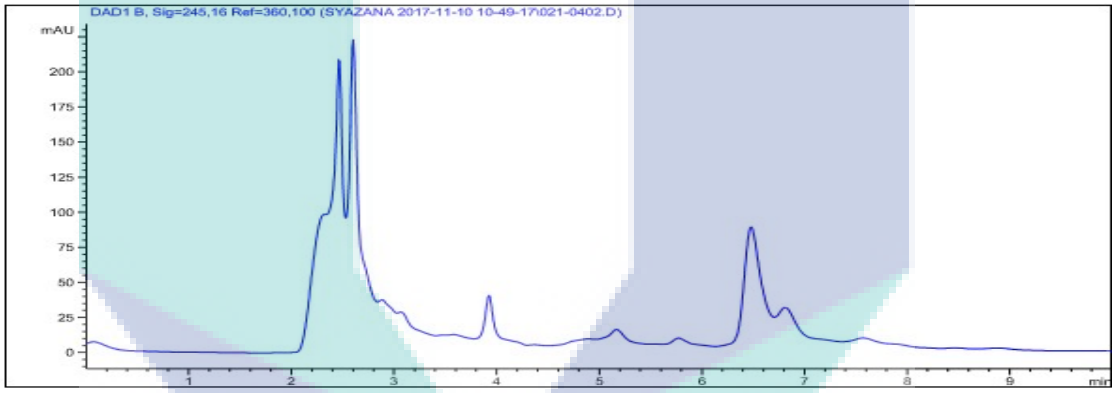


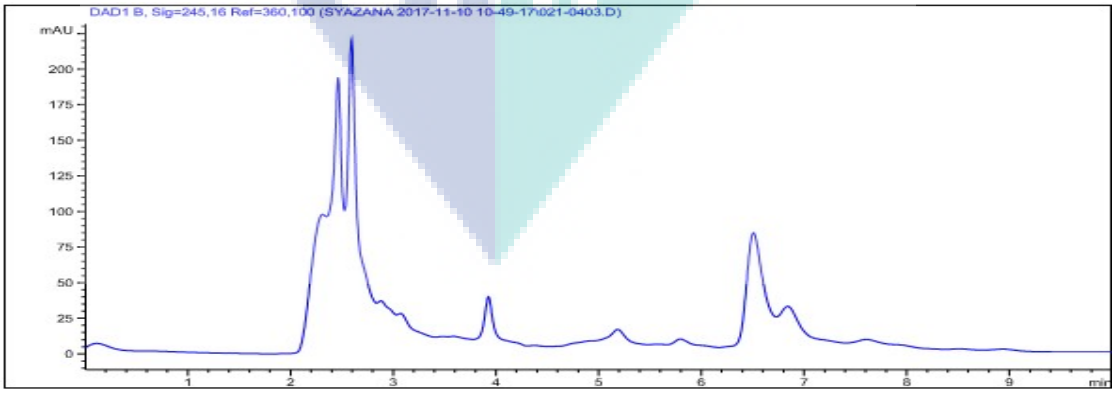




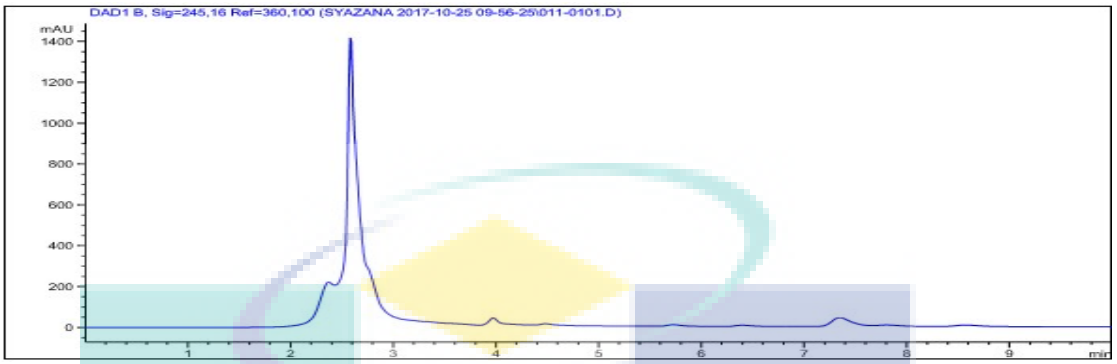
Capsule 2

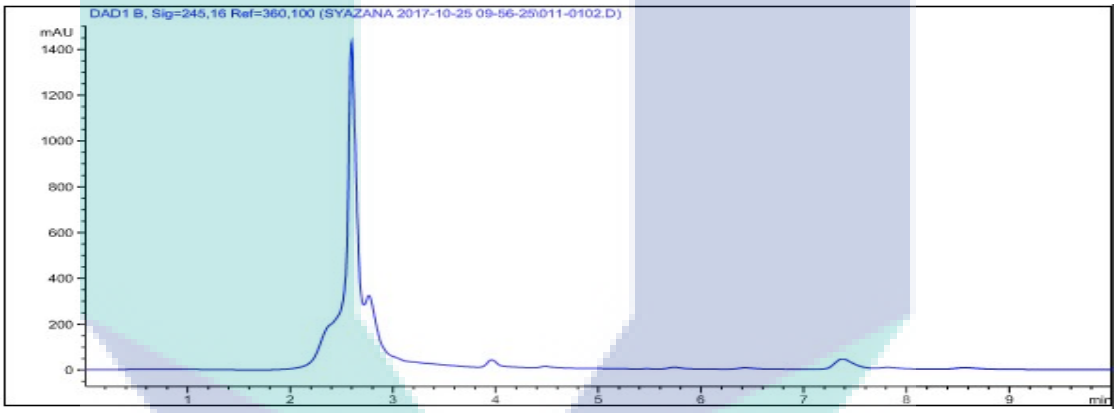

DAD1 B, Sig=245,16 Ref=360,100 (SYAZANA 2017-11-10 10-49-17\021-0401.D)
mAU
200
150
100
50
0
1
2
3
4
5
6
7
8
9
min

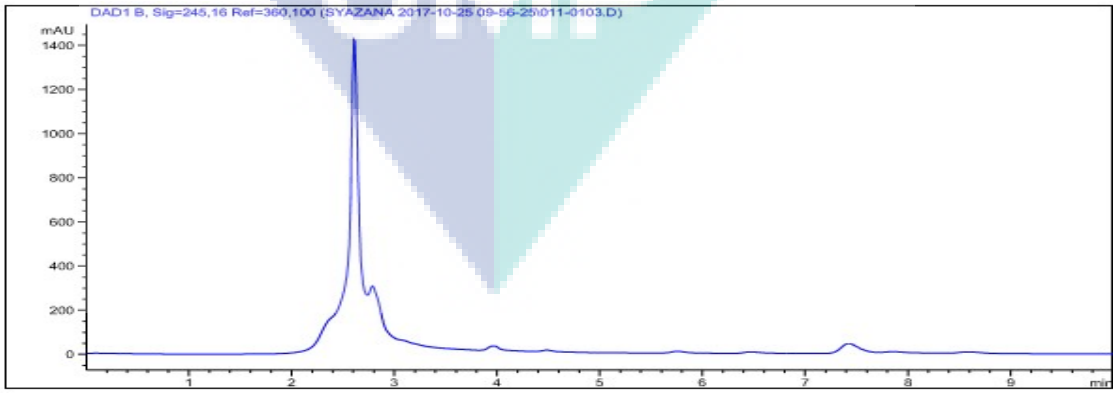

DAD1 B, Sig=245,16 Ref=360,100 (SYAZANA 2017-11-10 10-49-17\021-0402.D)
mAU
200
175
150
125
100
75
50
25
0
1
2
3
4
5
6
7
8
9
min


DAD1 B, Sig=245,16 Ref=360,100 (SYAZANA 2017-11-10 10-49-17\021-0403.D)
mAU
200
175
150
125
100
75
50
25
0
1
2
3
4
5
6
7
8
9
min

Capsule 3


DAD1 B, Sig=245,16 Ref=360,100 (SYAZANA 2017-10-25 09-56-25\011-0101.D)
mAU
1400
1200
1000
800
600
400
200
0
1
2
3
4
5
6
7
8
9
min

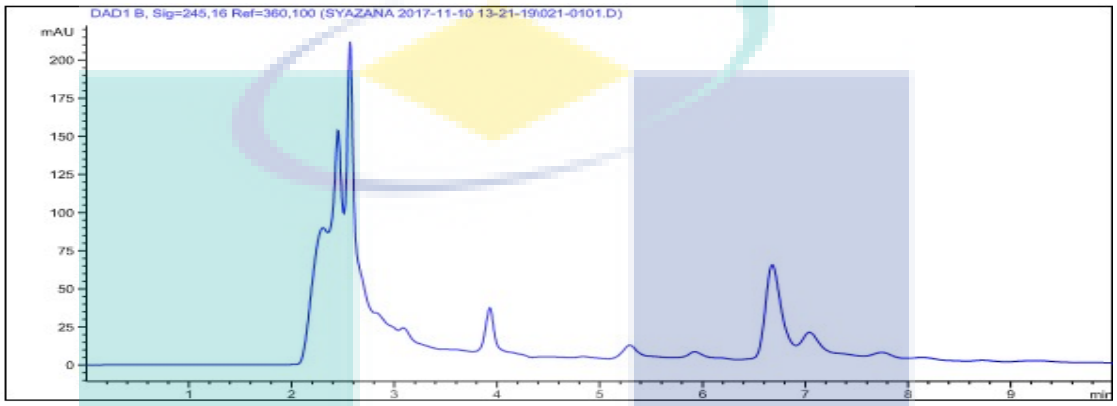

DAD1 B, Sig=245,16 Ref=360,100 (SYAZANA 2017-10-25 09-56-25\011-0102.D)
mAU
1400
1200
1000
800
600
400
200
0
1
2
3
4
5
6
7
8
9
min

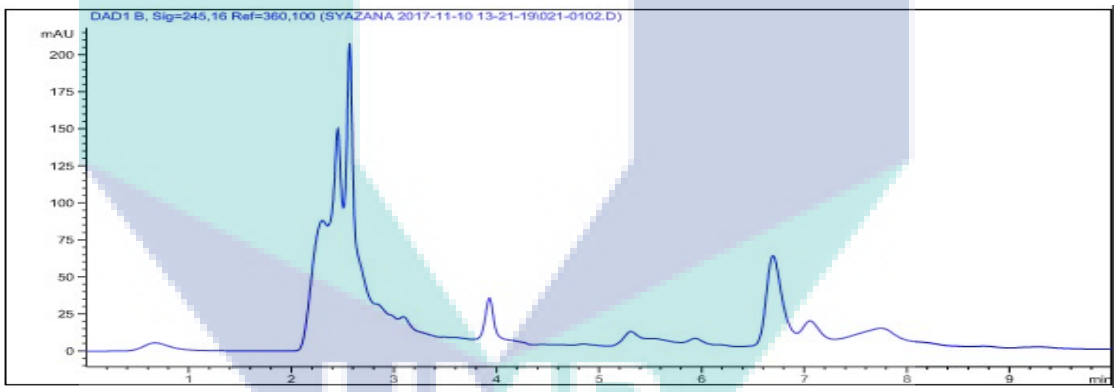

DAD1 B, Sig=245,16 Ref=360,100 (SYAZANA 2017-10-25 09-56-25\011-0103.D)
mAU
1400
1200
1000
800
600
400
200
0
1
2
3
4
5
6
7
8
9
min

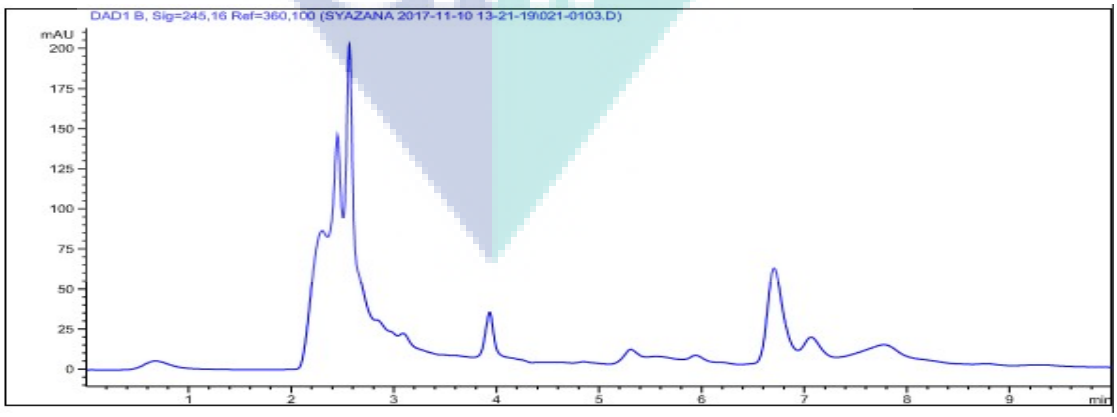
APPENDIX H

THE CHROMATOGRAMS OF UNIFORMITY CONTENT OF EURYCOMANONE IN TAC-IHF

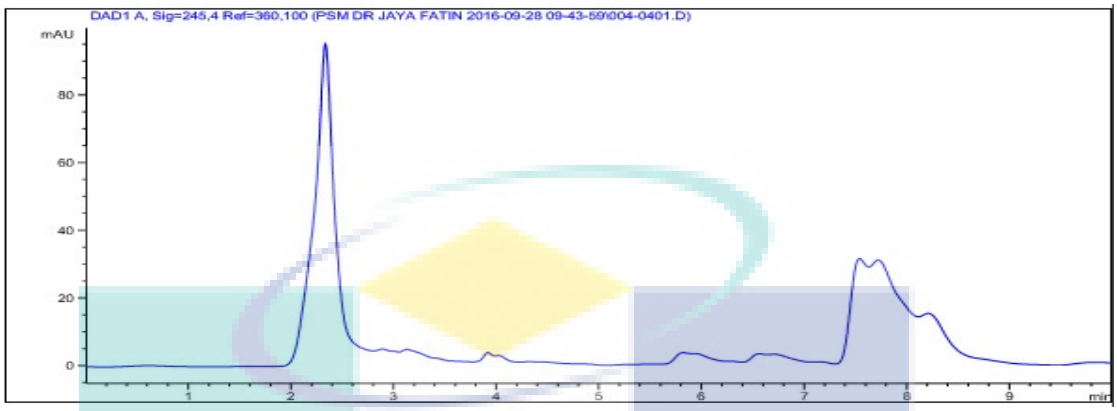
Capsule 1

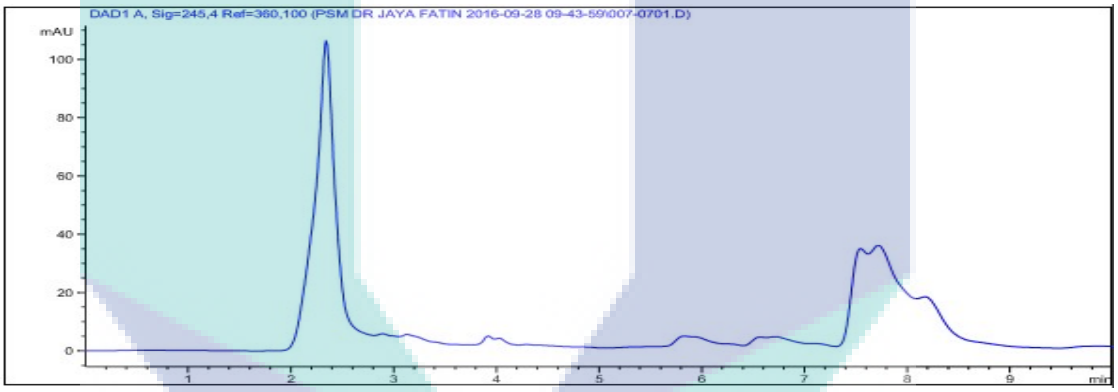


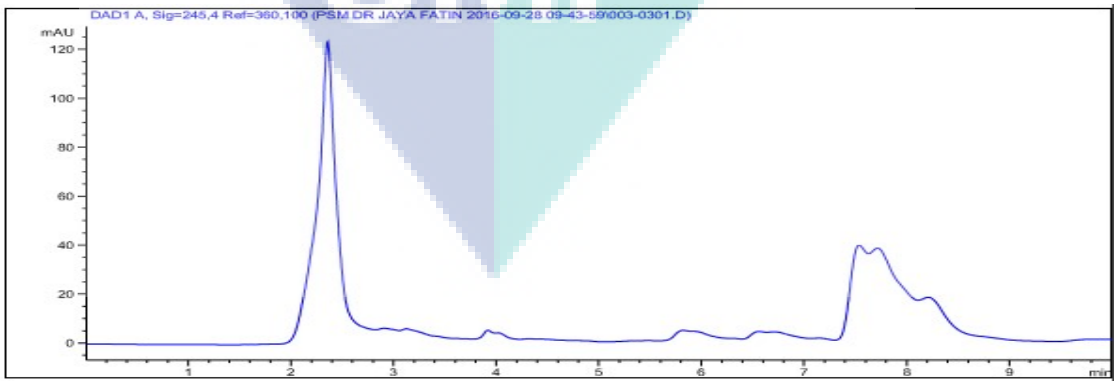




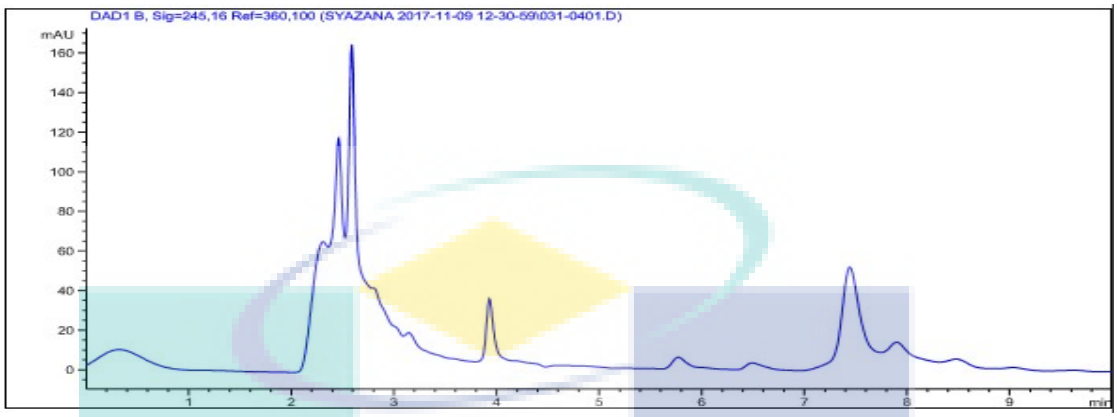
Capsule 2

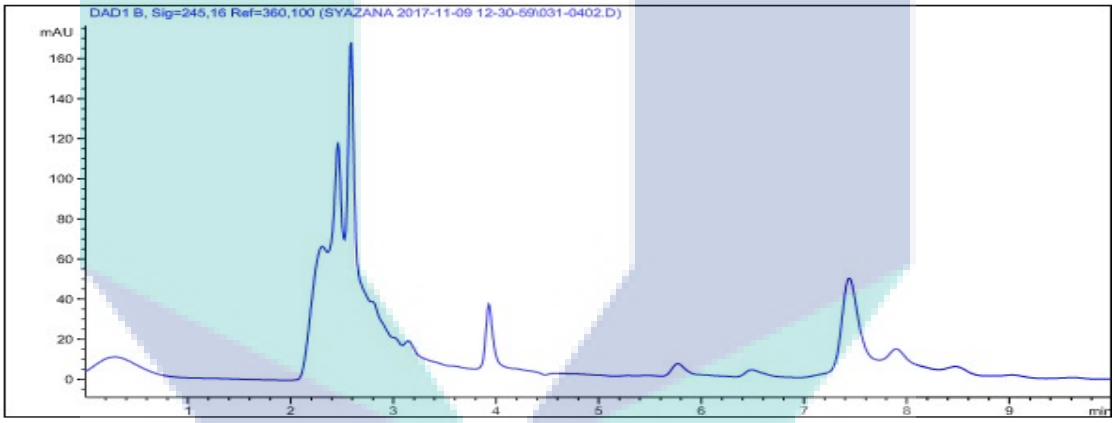

DAD1 A, Sig=245,4 Ref=360,100 (PSM DR JAYA FATIN 2016-09-28 09-43-59\004-0401.D)
mAU
min

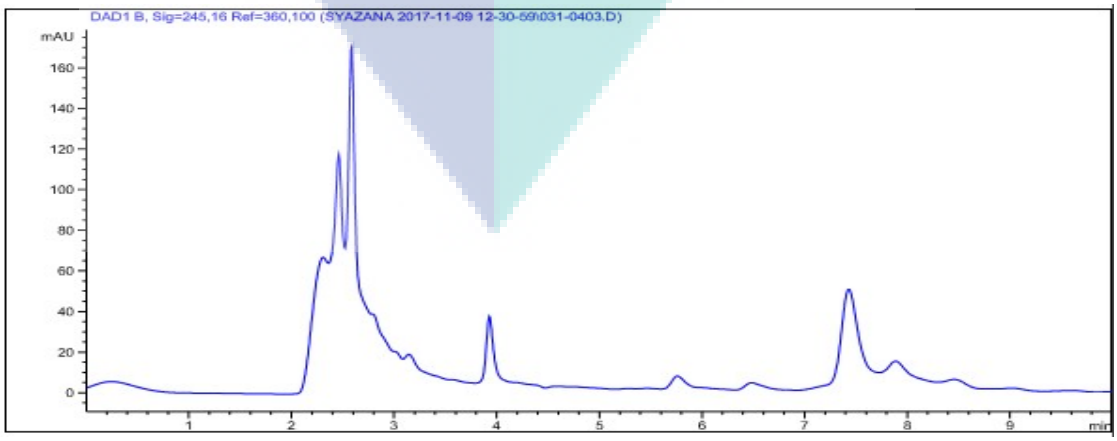

DAD1 A, Sig=245,4 Ref=360,100 (PSM DR JAYA FATIN 2016-09-28 09-43-59\007-0701.D)
mAU
min


DAD1 A, Sig=245,4 Ref=360,100 (PSM DR JAYA FATIN 2016-09-28 09-43-59\003-0301.D)
mAU
min

Capsule 3


DAD1 B, Sig=245,16 Ref=360,100 (SYAZANA 2017-11-09 12-30-59\031-0401.D)
mAU
min


DAD1 B, Sig=245,16 Ref=360,100 (SYAZANA 2017-11-09 12-30-59\031-0402.D)
mAU
min


DAD1 B, Sig=245,16 Ref=360,100 (SYAZANA 2017-11-09 12-30-59\031-0403.D)
mAU
min

APPENDIX I

THE UNIFORMITY WEIGHT OF TAC-IP

The uniformity weight of 20 capsules

Capsules	Weight (mg)	Weight of Shell (mg)	Net Weight mg)	Percentage of Deviation (%)
1	292.8	86.6	206.2	0.28
2	294.7	79.8	214.9	3.93
3	283.3	83.1	200.2	3.18
4	298.4	80.0	218.4	5.62
5	280.2	81.3	198.9	3.81
6	286.5	78.5	208.0	0.59
7	291.2	85.9	205.3	0.71
8	284.0	82.3	201.7	2.45
9	289.4	81.7	207.7	0.45
10	295.1	84.5	210.6	1.85
11	285.8	81.6	204.2	1.24
12	293.1	85.5	207.6	0.40
13	286.0	80.1	205.9	0.42
14	294.0	85.3	208.7	0.93
15	280.5	86.5	194.0	6.18
16	283.9	82.4	201.5	2.55
17	288.7	78.4	210.3	1.71
18	298.4	80.4	218.0	5.43
19	292.4	84.8	207.6	0.40
20	282.7	77.1	205.6	0.57

APPENDIX J

THE UNIFORMITY WEIGHT OF TAC-IHF

The uniformity weight of 20 capsules

capsule	weight (mg)	weight of shell (mg)	net weight mg)	percentage of deviation (%)
1	244.3	80.8	163.5	0.43
2	252.9	81.3	171.6	5.41
3	237.7	81.9	155.8	4.30
4	237.4	81.2	156.2	4.05
5	240.3	81.5	158.8	2.46
6	237.7	81.3	156.4	3.93
7	233.6	79.1	154.5	5.10
8	237.6	80.9	156.7	3.75
9	252.0	82.7	169.3	3.99
10	239.8	82.5	157.3	3.38
11	247.1	81.9	165.2	1.47
12	249.5	82.3	167.2	2.70
13	243.7	79.9	163.8	0.61
14	247.9	82.8	165.1	1.41
15	250.7	81.4	169.3	3.99
16	253.5	82.4	171.1	5.10
17	241.0	79.6	161.4	0.86
18	249.9	81.0	168.9	3.75
19	238.6	82.7	155.9	4.24
20	248.1	80.9	167.2	2.70

APPENDIX K

HEAVY METAL ANALYSIS

CENLAB | F / 007

Universiti Malaysia PAHANG

CENTRAL LABORATORY

Universiti Malaysia Pahang, Lebuhraya Tun Razak,
26300 Gambang, Kuantan, Pahang Darul Makmur.
Tel : 09-5493351 Fax : 09-5493353
E-mail : ucl@ump.edu.my

CERTIFICATE OF ANALYSIS (COA)

To / Attn	RST / Nursyazana Binti Rosli		
Address	UMP, Gambang		
Tel No	011-16547132	Fax No	
Sample Lab No	2017/505	No of sample	2

Date of sample received : 23-11-2017
Date reported : 04-12-2017
RESULT:

Sample marking : 2017/505 (1)
Sample description : Tongkat Ali 1

No	Parameter	Results	Unit	Test Method
1.	Cadmium (Cd)	Not Detected (Less than 0.1)	ppm	In-house Method based on APHA 3010
2.	Lead (Pb)	Not Detected (Less than 0.1)	ppm	In-house Method based on APHA 3010
3.	Arsenic (As)	Not Detected (Less than 0.1)	ppm	In-house Method based on APHA 3010
4.	Mercury (Hg)	Not Detected (Less than 10)	ppb	In-house Method using Milestone DMA-80

Sample marking : 2017/505 (2)
Sample description : Tongkat Ali 2

No	Parameter	Results	Unit	Test Method
1.	Cadmium (Cd)	Not Detected (Less than 0.1)	ppm	In-house Method based on APHA 3010
2.	Lead (Pb)	Not Detected (Less than 0.1)	ppm	In-house Method based on APHA 3010
3.	Arsenic (As)	Not Detected (Less than 0.1)	ppm	In-house Method based on APHA 3010
4.	Mercury (Hg)	Not Detected (Less than 10)	ppb	In-house Method using Milestone DMA-80

The above analysis is based on the sample submitted by the customer.

.....
ABU UMAR BIN RAMLI
SCIENCE OFFICER

APPENDIX L

MICROBIAL LOAD TEST

CENLAB / F / 007

Universiti Malaysia PAHANG

CENTRAL LABORATORY

Universiti Malaysia Pahang, Lebuhraya Tun Razak,
26300 Gambang, Kuantan, Pahang Darul Makmur.
Tel : 09-5493351 Fax : 09-5493353
E-mail : ucl@ump.edu.my

CERTIFICATE OF ANALYSIS (COA)

To / Attn	Nursyazana Binti Rasdi		
Address	Faculty Science and Technology Industry, Universiti Malaysia Pahang, Lebuhraya Tun Razak, 26300 Gambang, Kuantan, Pahang		
Tel No	019-9955010	Fax No	
Sample Lab No	2017/468	No of sample	2

Sample marking : 2017/468 (1)
Sample description : Tongkat Ali Capsule In House
Date of sample received : 01-11-2017
Date reported : 07-11-2017

RESULTS:

No	Parameter	Results	Unit	Test Method
1.	Total Aerobic Microbial Count	5.0×10^3	cfu/g	CENLAB/WI/BIOTECH-TM/001 (Appendix XVI B, BP 2009 Harmonised Method)
2.	Total Yeast & Mould Colunt	5.5×10^2	cfu/g	CENLAB/WI/BIOTECH-TM/002 (Appendix XVI B, BP 2009 Harmonised Method)
3.	Detection of *Escherichia coli*	Present	in 1g	CENLAB/WI/BIOTECH-TM/004 (Appendix XVI B, BP 2009 Harmonised Method)
4.	Detection of *Staphylococcus aureus*	Present	in 1g	CENLAB/WI/BIOTECH-TM/005 (Appendix XVI B, BP 2009 Harmonised Method)
5.	Detection of *Salmonella sp*	Present	in 1g	CENLAB/WI/BIOTECH-TM/006 (Appendix XVI B, BP 2009 Harmonised Method)
6.	Detection of *Pseudomonas aeruginosa*	Present	in 1g	CENLAB/WI/BIOTECH-TM/007 (Appendix XVI B, BP 2009 Harmonised Method)
7.	Detection of Bile Tolerant Gram Negative Bacteria	Present	in 1 g	CENLAB/WI/BIOTECH-TM/003 (Appendix XVI B, BP 2009 Harmonised Method)

Sample marking : 2017/468 (2)
Sample description : Tongkat Ali Capsule Indigeneous Formulation
Date of sample received : 01-11-2017
Date reported : 07-11-2017

RESULTS:

No	Parameter	Results	Unit	Test Method
1.	Total Aerobic Microbial Count	7.5×10^1	cfu/g	CENLAB/WI/BIOTECH-TM/001 (Appendix XVI B, BP 2009 Harmonised Method)
2.	Total Yeast & Mould Count	5.0×10^1	cfu/g	CENLAB/WI/BIOTECH-TM/002 (Appendix XVI B, BP 2009 Harmonised Method)
3.	Detection of *Escherichia coli*	Present	in 1g	CENLAB/WI/BIOTECH-TM/004 (Appendix XVI B, BP 2009 Harmonised Method)
4.	Detection of *Staphylococcus aureus*	Present	in 1g	CENLAB/WI/BIOTECH-TM/005 (Appendix XVI B, BP 2009 Harmonised Method)
5.	Detection of *Salmonella sp*	Present	in 1g	CENLAB/WI/BIOTECH-TM/006 (Appendix XVI B, BP 2009 Harmonised Method)
6.	Detection of *Pseudomonas aeruginosa*	Present	in 1g	CENLAB/WI/BIOTECH-TM/007 (Appendix XVI B, BP 2009 Harmonised Method)
7.	Detection of Bile Tolerant Gram Negative Bacteria	Present	In 1 g	CENLAB/WI/BIOTECH-TM/003 (Appendix XVI B, BP 2009 Harmonised Method)

The above analysis is based on the sample submitted by the customer.

FADHLULLAH RAHMAN
SCIENCE OFFICER

APPENDIX M

THE SURVEY FORM (ENGLISH VERSION)

INFORMATION SHEET

Background of Product

This document intended to provide concise yet sufficient information related to our study. This research is not a clinical trial on a pharmaceutical drug developed for the market but rather an herbal powdered Tongkat Ali root. The capsule containing herbal powdered root once obtained from indigenous person been tested for safety (microbial load, heavy metal and adulterated steroid contents). In completion of these tests the capsule found to contain permissible levels or none at all of the test substances. In additional, the capsule been authenticated to contain Tongkat Ali original root by determining the Eurycomanone content as well as presence of our very own in house marker. As such the participant of this study can be rest assured that this product is free from any chemicals, harmful chemicals, toxic materials as well as adequately recommended amount of Tongkat Ali powdered root per capsule. Further the untested capsule been sold for some 15 years to keen customers without any untoward testimonies of yet.

Procedure of Trial

You will be required to consent your participation and followed by answering the first part of our questionnaire. Next you will be given a complementary product containing 60 capsules to be taken twice daily after meal. The capsules are to be taken orally for an exact duration of 4 weeks. During that periods you will resume your daily routine as preferred and considered normal to you. You are kindly requested to observe any differences in your energy level; sexual drive and performance during this trial period for the purpose of answering question in Part C of our questionnaire upon completing your trial period of 4 weeks.

Caution

The product regarded safe for the general public however due to variation of tolerance of one individual to the other it is hereby cautioned if found with side effects to immediately restrained from continuing trial and seek help if necessary. In most instances the side effects are not prolonged and a return to normal expected upon discontinuing any herbal preparations.

Consent for participation in a research project

1.	I have read and understood the information about the project, as provided in the Information Sheet dated 8th Augusts 2017.	☐
2.	I have been given the opportunity to ask questions about the project and my participation.	☐
3.	I voluntarily agree to participate in the project.	☐
4.	I understand I can withdraw at any time without giving reasons and that I will not be penalized for withdrawing nor will I be questioned on why I have withdrawn.	☐
5.	The procedures regarding confidentiality have been clearly explained (e.g. use of names, pseudonyms, anonymization of data, etc.) to me.	☐
6.	If applicable, separate terms of consent for interviews, audio, video or other forms of data collection have been explained and provided to me.	☐
7.	The use of the data in research, publications, sharing and archiving has been explained to me.	☐
8.	I understand that other researchers will have access to this data only if they agree to preserve the confidentiality of the data and if they agree to the terms I have specified in this form.	☐
9.	Select only one of the following: • I would like my name used and understand what I have said or written as part of this study will be used in reports, publications and other research outputs so that anything I have contributed to this project can be recognized. • I do not want my name used in this project.	☐ ☐
10.	I, along with the Researcher, agree to sign and date this informed consent form.	☐

Participant:

_____ _____ _____

Name of Participant Signature Date

Researcher:

_____ _____ _____

Name of Researcher Signature Date

I agree to participate in a research project conducted by Dr Jaya Vejayan from Universiti Malaysia Pahang. The purpose of this document is to specify the terms of my participation in the project through being interviewed.

PART A GENERAL DEMOGRAPHIC QUESTIONS

1. Name: _____
2. Date of Birth: _____
3. Which gender do you belong to?
   - Male
   - Female
4. Which race do you belong to?
   - Malay
   - Chinese
   - Indian
   - Other : _____
5. Which religion do you belong to?
   - Islam
   - Buddhism
   - Christian
   - Other : _____
6. Which age group do you belong to?
   - 18-25
   - 26-35
   - 36-45
   - 46 and above
7. Have you tried men's energy pills before? If no, please proceed to question 9.
   - Yes

- o No

8. Which of these brands of men's energy pills have you tried? (You may have multiple answers)
   - o Nu-Prep
   - o Long jack Orang Kampung
   - o Vigor Jamu for Men
   - o Tongkat Ali Plus Petani
   - o Yusmira Tongkat Ali
   - o Other (specify): ____

9. (For respondent who answered "No" in question 7) Why have you not tried men's energy pills? (You may have more than one answer)
   - o Too expensive
   - o Never have need for it
   - o Never heard of it before
   - o Not effective
   - o Tried other than men's energy pills. Specify: _____.

PART B QUESTIONS PRIOR TO PRODUCT TRIAL

The following questions ask about how completely you experience or were able to do certain things before trying out our product.

Questions	Very dissatisfied	Dissatisfied	Neither satisfied nor dissatisfied	Satisfied	Very satisfied
1.How satisfied are you with your energy?	1	2	3	4	5
2.How satisfied are you with your ability to perform your daily living activities?	1	2	3	4	5
3.How satisfied are you with your capacity for work?	1	2	3	4	5
4.How satisfied are you with yourself?	1	2	3	4	5

5.How satisfied are you with your personal relationships?	1	2	3	4	5
6.How satisfied are you with your sex life?	1	2	3	4	5

Section 1 (questions for pre-trial):

PART C QUESTIONS AFTER PRODUCT TRIAL

The following questions ask about how completely you experience or were able to do certain things after 4 weeks' trial period of our product.

Section 1 (questions for post-trial):

Questions	Very dissatisfied	Dissatisfied	Neither satisfied nor dissatisfied	Satisfied	Very satisfied
1.How satisfied are you with your energy?	1	2	3	4	5
2.How satisfied are you with your ability to perform your daily living activities?	1	2	3	4	5
3.How satisfied are you with your capacity for work?	1	2	3	4	5
4.How satisfied are you with yourself?	1	2	3	4	5
5.How satisfied are you with your personal relationships?	1	2	3	4	5

6.How satisfied are you with your sex life?	1	2	3	4	5

Section 2 (Overall questions for post-trial):

Questions	Very dissatisfied	Dissatisfied	Neither satisfied nor dissatisfied	Satisfied	Very satisfied
1. How satisfied or dissatisfied are you with the ability of the product to enhance the sexual activity?					
2. How satisfied or dissatisfied are you with the amount of time it takes the product to start working?					
3. How convenient or inconvenient is it to take the product as instructed?					
4. Overall, how confident are you that taking this product is a good thing for you?					
5. How confident that you will recommend this product to your friends?					

APPENDIX N

THE SURVEY FORM (MALAY VERSION)

MAKLUMAT

Latar Belakang Produk

Dokumen ini bertujuan untuk menyediakan maklumat ringkas dan mencukupi yang berkaitan dengan kajian kami. Penyelidikan ini bukan percubaan klinikal pada ubat farmaseutikal yang dibangunkan untuk pasaran tetapi sebaliknya tumbuhan herba Tongkat Ali serbuk herba. Kapsul yang mengandungi akar serbuk herba yang diperolehi daripada orang asli telah diuji untuk keselamatan (beban mikroba, logam berat dan kandungan steroid yang dikandung). Setelah selesai ujian ini, kapsul yang didapati mengandungi tahap yang dibenarkan atau tidak ada pada semua bahan ujian. Di samping itu, kapsul telah disahkan mengandungi akar asal Tongkat Ali dengan menentukan kandungan Eurycomanone serta kehadiran penanda(marker) kami sendiri. Oleh itu peserta kajian ini boleh yakin bahawa produk ini bebas dari sebarang bahan kimia, bahan kimia berbahaya, bahan toksik, serta mengikuti jumlah yang dicadangkan untuk serbuk akar Tongkat Ali bagi setiap kapsul. Selanjutnya kapsul yang belum diuji telah dijual selama 15 tahun untuk pelanggan yang berminat tanpa sebarang masalah yang tidak diingini.

Prosedur Percubaan

Anda akan diminta untuk memberi persetujuan mengenai penyertaan anda dan diikuti dengan menjawab bahagian pertama soal selidik kami. Seterusnya anda akan diberi produk yang mengandungi 60 kapsul yang akan diambil dua kali sehari selepas makan. Kapsul perlu diambil secara rutin untuk tempoh 4 minggu(sebulan). Semasa tempoh itu, anda akan meneruskan rutin harian anda seperti biasa dan normal. Anda diminta untuk melihat sebarang perbezaan dalam tahap tenaga anda dan prestasi seksual semasa tempoh percubaan ini untuk tujuan menjawab soalan di Bahagian C soal selidik kami setelah melengkapkan tempoh percubaan anda selama 4 minggu.

Langkah keselamatan

Produk ini dianggap selamat untuk orang ramai namun disebabkan oleh variasi toleransi satu individu kepada yang lain ia dengan ini diingatkan jika didapati mengalami sebarang kesan sampingan, anda digalakkan untuk terus berhenti daripada percubaan ini dan meminta bantuan jika perlu. Dalam kebanyakan kes, kesan sampingan tidak berpanjangan dan dijangkakan kembali normal berhenti mengambil sebarang herba produk.

BAHAGIAN A: SOALAN DEMOGRAFIK

1. Nama: _____
2. Tarikh lahir: _____
3. Jantina :_____
4. Umur: _____
5. Bangsa
   - Melayu
   - Cina
   - India
   - Lain-lain _____
6. Agama
   - Islam
   - Buddha
   - Kristian
   - Lain-lain: _____
7. Adakah anda pernah mencuba makan pil tenaga lelaki? Jika tidak, sila jawab soalan no. 9
   - Ya
   - Tidak
8. Berdasarkan pilihan jawapan produk manakah anda pernah cuba? (Anda boleh menjawab melebihi satu)
   - Nu-Prep
   - Longjack Orang Kampung
   - Vigor Jamu for Men
   - Tongkat Ali Plus Petani
   - Yusmira Tongkat Ali
   - Lain-lain:_____
9. (Kepada yang menjawab tidak pada soalan no. 7) Kenapa tidak pernah mencuba? (Anda boleh menjawab melebihi satu)
   - Terlalu mahal
   - Tidak pernah memerlukan
   - Tidak pernah dengar
   - Tidak berkesan
   - Mencuba produk yang bukan untuk tenaga lelaki _____.

BAHAGIAN B: SOALAN BERKAITAN PERCUBAAN PRODUK (PRA-PERCUBAAN)

Soalan-soalan berikut bersoalkan sejauh mana anda mengalami atau dapat melakukan perkara-perkara tertentu sebelum mencuba produk kami.

Bahagian 1

Soalan	Sangat tidak berpuas hati	Tidak berpuas hati	Neutral	Berpuas hati	Sangat berpuas hati
1. Adakah anda berpuas hati dengan tenaga anda?	1	2	3	4	5
2. Adakah anda berpuas hati dengan kemampuan anda dalam melaksana aktiviti harian?	1	2	3	4	5
3. Adakah anda berpuas hati dengan kemampuan anda untuk bekerja?	1	2	3	4	5
4. Adakah anda berpuas hati dengan diri anda sendiri?	1	2	3	4	5
5. Adakah anda berpuas hati dengan hubungan peribadi anda?	1	2	3	4	5
6. Adakah anda berpuas hati dengan kehidupan seks anda?	1	2	3	4	5

BAHAGIAN C: SOALAN BERKAITAN PERCUBAAN PRODUK (SELEPAS-PERCUBAAN)

Soalan-soalan berikut bersoalkan sejauh mana anda mengalami atau dapat melakukan perkara-perkara tertentu selepas mencuba produk kami.

Bahagian 1

Soalan	Sangat tidak berpuas hati	Tidak berpuas hati	Neutral	Berpuas hati	Sangat berpuas hati
1. Adakah anda berpuas hati dengan tenaga anda?	1	2	3	4	5
2. Adakah anda berpuas hati dengan kemampuan anda dalam melaksana aktiviti harian?	1	2	3	4	5
3. Adakah anda berpuas hati dengan kemampuan anda untuk bekerja?	1	2	3	4	5
4. Adakah anda berpuas hati dengan diri anda sendiri?	1	2	3	4	5
5. Adakah anda berpuas hati dengan hubungan peribadi anda?	1	2	3	4	5
6. Adakah anda berpuas hati dengan kehidupan seks anda?	1	2	3	4	5

Bahagian 3 - Rumusan:

Soalan	Sangat tidak berpuas hati	Tidak berpuas hati	Neutral	Berpuas hati	Sangat berpuas hati
1. Adakah anda berpuas hati dengan kemampuan produk untuk meningkatkan aktiviti seksual?					
2. Adakah anda berpuas hati dengan jumlah masa yang diperlukan untuk memulakan produk?					
3. Adakah anda merasa mudah untuk mengambil produk seperti yang diarahkan?					
4. Secara keseluruhan, adakah anda yakin bahawa mengambil produk ini adalah satu perkara yang baik untuk anda?					
5. Bagaimana yakin bahawa anda akan mengesyorkan produk ini kepada rakan-rakan anda?					

APPENDIX O

THE CONSENT FORM

Consent for participation in a research project

1.	I have read and understood the information about the project, as provided in the Information Sheet dated 8th Augusts 2017.	☑
2.	I have been given the opportunity to ask questions about the project and my participation.	☑
3.	I voluntarily agree to participate in the project.	☑
4.	I understand I can withdraw at any time without giving reasons and that I will not be penalised for withdrawing nor will I be questioned on why I have withdrawn.	☑
5.	The procedures regarding confidentiality have been clearly explained (e.g. use of names, pseudonyms, anonymisation of data, etc.) to me.	☑
6.	If applicable, separate terms of consent for interviews, audio, video or other forms of data collection have been explained and provided to me.	☑
7.	The use of the data in research, publications, sharing and archiving has been explained to me.	☑
8.	I understand that other researchers will have access to this data only if they agree to preserve the confidentiality of the data and if they agree to the terms I have specified in this form.	☑
9.	Select only one of the following: • I would like my name used and understand what I have said or written as part of this study will be used in reports, publications and other research outputs so that anything I have contributed to this project can be recognised. • I do not want my name used in this project.	☑ ☐
10.	I, along with the Researcher, agree to sign and date this informed consent form.	☑

Participant:

Ku Mohamad Aminuddin		11.08.2017
Name of Participant	Signature	Date

Researcher:

NURSYAZANA ROSDI		11/08/2017
Name of Researcher	Signature	Date

APPENDIX P

OUTPUT OF THE PROJECT

- **Exhibitions:**
  - Bahtera 2018 (Bumipreneurs of tomorrow), 24-25th Feb 2018 at Sultan Ahmad Shah International Convention Centre, Hotel Zenith, Kuantan.
  - Engineer's day exhibition held 2nd May 2018 at UMP, Gambang
  - CITREX 2018 exhibition organised by UMP with silver award
  - 2nd Advanced Innovation of Engineering Exhibition 2017 (AINEX 2017) organised by Automative Engineering Centre (AEC), Universiti Malaysia Pahang held in Astaka, Universiti Malaysia Pahang (Gambang campus) on 3rd May 2017, gold medal
- **ISI journal (Current Science) manuscript accepted, Title:** Marker useful to authenticate *Eurycoma longifolia* (Tongkat Ali) contained aphrodisiac herbal products.
- **Perpustakaan Wadah UMP article, Title:** Tongkat Ali: Pusaka Bumi di Persada Dunia
- **Undergraduate:**
  - Nursyazana binti Rosdi (SB14040) with thesis title: PRELIMINARY DEVELOPMENT OF TONGKAT ALI CAPSULE BY FOLLOWING THE MALAYSIA STANDARD AND NATIONAL PHARMACEUTICAL REGULATIONS AGENCY
  - Fatin Hasnina Hafiz (SB13035) with thesis title: UMP STANDARDIZE TONGKAT ALI CAPSULE
- **Internship:**
  - Nursyazana binti Rosdi (IC950606065490): period of internship 5/3/2018 – 10/8/2018
- **Master students**
  - YASMIN AMIRAH BINTI CHE YAHAYA (MKT18002) with master on-going, proposal of thesis titled: DEVELOPMENT OF A PROTEIN MARKER AND ITS UTILIZATION IN DERIVING A TONGKAT ALI HERBAL PROTOTYPE PARTIALLY COMPLYING TO DRUG REGULATORY AGENCY
- **Entrepreneur company set up by Fatin Hasnina Hafiz:**
  - Nina Square Enterprise (CA0246106-A)

- **Letter of intent**
    - Between researcher and Fatin Hasnina Hafiz
- **Consultancy**
    - Between Nina Square Sdn Bhd and Dr Jaya Vejayan