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QUALITY CONTROL AND IN-VITRO STUDY RELEASE BEHAVIOUR OF EURYCOMANONE IN HERBAL PRODUCTS SUPPLEMENT IN CAPSULE FORM

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***ABSTRACT**--Controlled releasedelivery system of active ingredients has a significant effect on their pharmacological activity. In fact, it is considered as one of the most important quality control test to predict their bioavailability. In this study the in-vitro study release behaviour was carried out using dissolution testing apparatus USP type-11 and submit to an independent model methods using time point approach whereby the percentage drug releases at a given time were determined. Three brands (TA, TB, TC) of herbal product supplements from different manufacturers which consist of Eurycomanone as active ingredients were compared. Brand TS is a pure extract of Eurycomanone used as a benchmark. The parameters employed to compare in-vitro study release behaviour are different dissolution media namely 0.1 N HCl, 30% EtOH, acetate buffer (pH 4.8) and phosphate buffer pH 6.8 for each brands for 105 minutes. Samples were withdrawn at 15 minutes time interval and analysed for the Eurycomanone release by using HPLC technique. Content of the Eurycomanone for brand TA and brand TS analysed via HPLC were the highest.When submitted to in-vitro release study, the release rate of Eurycomanonein brands TA was the highest in both acidic and alkaline medium (0.1 M HCl and 0.1 M PBS medium)while the release rate of Eurycomanonein brands TB was the highest in the alkaline medium compared to acidic medium. Amongst the three sample, brand TC showed the lowest released rate of Eurycomanone in all medium.The highest percent of drug release at a given time (Y_{30}) for brand TA, brand TB and brand TC were 39.7% (0.1 M HCl), 33.5% (0.1 M PBS) and3.2% (0.1 % PBS), respectively. In this study, both results from in-vitro released study and HPLC testwere compliment and Eurycomanone used as chemical marker could be applied for quality control for the development of herbal products supplements in markets.*

***Keywords**-- quality control, independent model method, in-vitro study release behaviour, herbal products supplement*

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I INTRODUCTION

Drug release testing is an essential component of pharmaceutical growth and for regular tracking purposes. The profiles from the release rate study using dissolution test method were also used in an effort to successfully characterize the drug's *in-vitro*behaviour which must be carried out under precise conditions (Mandloi, et. al., 2009) in order to investigate the bioavailability of the active ingredients. It is a main analytical test that is used to detect physical modifications in an active ingredient and the product formulated. In addition, dissolution apparatus also used for quality control in the pharmaceutical industry. The particular method of dissolution used is determined by the features of the dosage form and the planned route of administration. The U.S. Pharmacopoeia (USP) Apparatus 1 (basket) and the USP Apparatus 2 (paddle) are normal dissolution test methods for strong dosage forms. Developing a dissolution procedure includes choosing the product-specific dissolution media, type of machinery and hydrodynamics which include agitation rate (Tadey, et. al., 2009).

Formulating herbal product supplement means mixing the medicinal herbs with other ingredients which called excipients according to a prescribed recipe. These ingredients have number of purposes, which might help them bind together, control the rate of release of the active compound and also improve the taste or mast the bitter taste of the ingredients (Mubarak et. al., 2012). It is known that the therapeutic effect is highly dependent on the bioavailability of the active compounds release when it is being digested. Therefore it is important to investigate the release of active ingredients when they interact with gastrointestinal fluid following oral administration. The evaluation of release control study give information on the solubility of active ingredients in herbal product supplement which is actually been absorb in the gastrointestinal (Basanth. et. al., 2019). Dissolution of a capsule or tablet involves its disintegration into smaller and smaller particles from which the active ingredient is released into more rapidly. In the pharmaceutical industry, one important aspect for the quality control of herbal product supplement is the determination of the chemical marker, which consists of a constituent (or groups of constituents) that is chemically defined and present in the plant product. These constituents may or may not be related to their pharmacological and/or therapeutic activity (Marques. et. al., 2019). There are different analytical techniques can be used to identify and quantify these substances, however, in this study a high performance liquid chromatography (HPLC) (Zollner., et. al., 2013) was used to determine the active compounds in *EurycomanoneLongifolia Sp*. namely Eurycomanone.

Eurycomalongifolia Sp. which also known as ‘Tongkat Ali’ is a home grown therapeutic plant found in South East Asia (Malaysia, Vietnam, Java and Sumatra, Thailand). Tongkatali decoction can be consume as tonic for enhancement in physical and mental vitality levels. The roots of TongkatAli regularly called “Malaysian ginseng”, are utilized as an adaptive and as a conventional “anti-aging” cure to assist more seasoned people adjust to the decreased vitality, temperament, and charisma that regularly comes with age. In cutting edge dietary supplements, TongkatAli can be found in an assortment of items aiming to move forward charisma and vitality, re-establish hormonal adjust (cortisol/testosterone levels) and improve both sports execution and weight loss (Talbott, et. al., 2013). Past research findings have appeared standardized tongkatalito invigorate discharge of free testosterone, make strides sex drive, diminish weariness, and progress well-being (Abubakar, et. al., 2017).

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As there is increasing demand of complementary and traditional herbal products supplements, the need to develop an appropriate guidelines and methods for the quality control of such products for their pharmaceutical dosage form is required. To our knowledge, there are none established studies reporting on bioavailability of TA using dissolution test and quality control data of commercialTongkat Ali with Eurycomanoneas a marker compound. Therefore, the aim of this study was to determine the *in-vitro*study release behaviour using a non-dependant method and quantification of Eurycomanoneas chemical markers for quality control purposes in commercial Tongkat Ali herbal product supplements.

Table 1: Ingredients of commercial brands of EurycomaLongifolia Sp.

Main Ingredients	**Brand**	**Other Ingredients**	**Appearance**	**Contents per capsule**
EurycomaLongifolia Sp. (TA)	TA	*Eurycomalongifolia extract (Radix), Smilax calophylla extract (Radix), Excipent (maltodextrine)*	Light yellow, dark brown precipitate.	500 mg (40 mg extract radix Tongkatali)
	TB	*Eurycomalongifolia extract (Radix)*	Light yellow, dark brown precipitate.	500 mg (100 mg extract radix Tongkat Ali)
	TC	*Radix EurycomaLongifolia Jack, Semen Pimpinella Anisum, Semen CuminumCyminum,Rhizome Zingiber Officinale, Sticepus Variegatus, Semen Piper Nigrum, Semen Coriandrum Sativum, Rhizome Alpine Galanga, Radix CurcumaeZadoaria, Garlic Oil Macerate*	Light yellow, dark brown precipitate.	500 mg (354 mg extract radix Tongkat Ali)

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II MATERIALS AND METHODS

2.1 Chemicals and Material

Chemicals

Phosphate dibasic heptahydrate, sodium phosphate, monobasic monohydrate, acid hydrochloric (HCl), natrium hydroxide (NaOH) and Eurycomanonestandardwere of analytical grade purchased from Sigma Alderich. Distilled water was obtained from a Mili-Q system (Milipore, Bedford, MA, USA).

Materials used

Commercial products were purchased from a local retail pharmacy in Taman Universiti, Skudai Johor. All of the products (TA, TB, TC) were in a capsules form. Brand TS was used as benchmark and contain pure TongkatAli. All the capsules were at least one year away from their expiration dates at the time of testing. Before subjecting to the dissolution testing each products was tested for contents of Eurycomanoneusing analytical method. The ingredients of each products were listed in Table I.

2.2 Instrumentation

The dissolution test was performed in a Labindia DS 8000 dissolution test system, in accordance with United States Pharmacopoeia (USP) general methods. The HPLC procedure utilized a Waters 2695 series HPLC equipped with auto sampler, PDA detector and Empower Software with C18 column, 5µ (150 x 4.60 mm) or equivalent. The column temperature was maintained at $25^{\circ}C$ from the very beginning of the procedure.

2.3 Chromatography Condition

The chromatographic separation was carried out using a mobile phase with phosphoric acid:water (3% phosphoric acid solution) as solvent A and acetonitrile as solvent B at a flow rate of 1.0/min. The solution was degassed using sonicator for 10-15 minutes prior to analysis. The injection volume was 20 µl and the detection wavelength was 272 nm. The HPLC was programmed to isocratic with 95% of Mobile Phase and 5% of Mobile Phase B.

2.4 Eurycomanoneacid standard

Eurycomanone(1000ppm) stock solution was prepared by dissolving 1.0 mg of standard Eurycomanoneacid into 1 mL of 50% Methanol. The stock solution was filtered using syringe nylon filter (0.45 µm) into vial. A series of minimum four different concentrations of standard Eurycomanonewas prepared: 20 ppm, 50 ppm, 80 ppm, and 200 ppm. Concentration unit was set in ppm (mg/l or µg/ml)

2.5 Dissolution Conditions

Dissolution testing was performed in compliance with USP29 using the USP-2 (paddle) rotating at 75 rpm. The media used were 500 ml of different dissolution media (30% Ethanol, 0.1 M Hydrochloric Acid, Acetate Buffer pH 4.9, Phosphate Buffer pH 7.6 at 37 ± 0.5^{0}C. The dissolution vessels were covered to minimize

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evaporation and manual sampling of aliquots of 1 ml were withdrawn at 15, 30, 45, 60, 75, 90 and 105 min and at the same time, the replacement of the same volume of the medium was done for constant maintenance of the volume. Manual sampling was done by using a syringe and was transferred into vials for HPLC through a nylon membrane filter, 0.45µm.

Preparation of various dissolution media

In conducting dissolution test, four dissolution medium namely 30% ethanol (I), 0.1 M hydrochloric acid (II), Acetate buffer pH 4.9 (IV) and Phosphate buffer pH 7.6 (III) were prepared. 3 L of 30% ethanol was prepared by adding 900 ml of ethanol into 2.1 L of distilled water. 3 L of 0.1 M Hydrochloric acid was prepared by adding 300 ml of 1 M Hydrochloric acid into 2.7 L of distilled water. The 3 L of buffer solution with required pH was prepared using buffers booklet [14]. To prepare Acetate Buffer (pH 4.6), 915 ml of 0.1 M Acetic acid and 585 ml of 0.1 M Sodium Acetate was added into 1 L of distilled water. The pH was adjust by adding 0.1 M Sodium Hydroxide and 0.1M Hydrochloric acid in order to increase or decrease the pH. If the pH is stable at 4.6, the distilled water was added into the buffer up to 3 L. To prepare Phosphate Buffer (pH 6.8), 765ml of 0.1 M Sodium Phosphate Monobasic and 735 ml of 0.1 M Sodium Phosphate Dibasic was added into 1 L of distilled water. The pH was adjust by adding 0.1 M Sodium Hydroxide and 0.1 M Hydrochloric acid to (if pH is not 6.8) in order to increase or decrease the pH level as needed, respectively. If the pH is stable at 6.8, add distilled water into the buffer up to 3 L.

2.6 Dissolution Profiles

2.6.1 Model Independent Using Time Point Approach

A simple model independent using Time Point approach is the percentage of active ingredient released at a given time. Main application of this model is to distinguish the specific dissolution parameters.

III RESULT AND DISCUSSION

3.1 *In-vitro*study release behaviour

*In-vitro*study release testing is a fundamental analytical method which allows quality assurance of solid pharmaceutical forms for oral administration. The study release behaviour of Eurycomanonefor the three brands in different media solutionvia the paddle method at 75 rpm are shown in Figure 1. The overall results showed that the highest released rate of Eurycomanonewas observed from brands TA compared to brands TB and TC respectively. It is also observed that at the first 15 to 30 minutes the released rate of Eurycomanonefor all brands was the highest and increased linearly then remained stable at the time of 40 minutes. It was observed that the release behaviour of the three commercially available brands employed in this study demonstrated similar release patterns.

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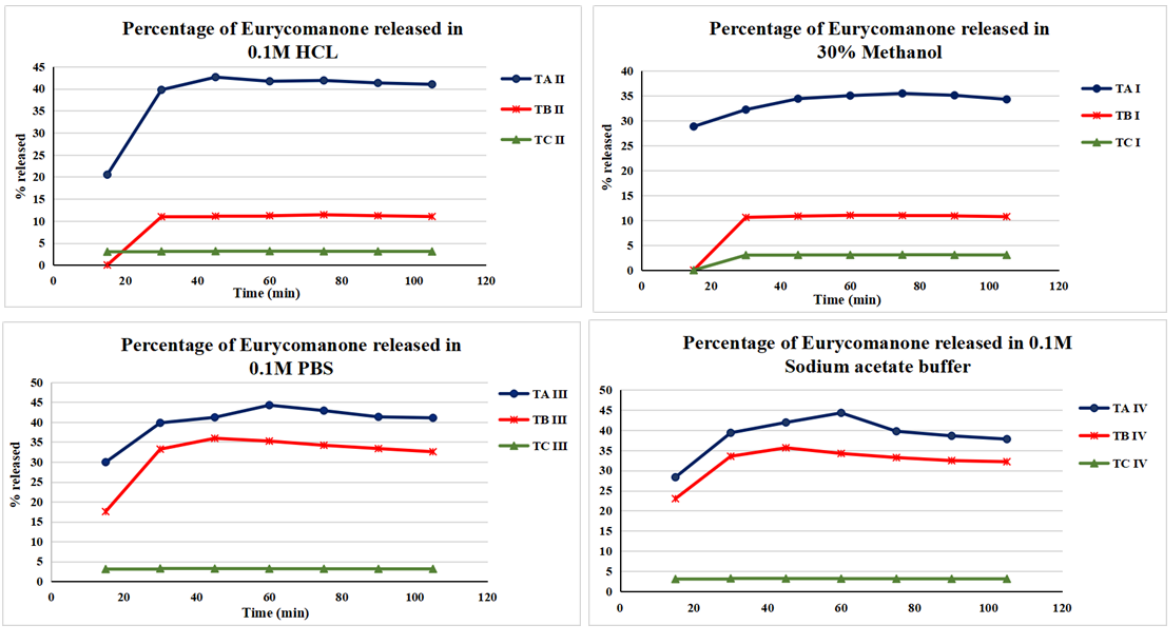

Figure 1:*In-vitro* study behaviour of the three brands in a specific media solution containing Eurycomanone using paddle method at 75 rpm and 500 mL of certain media, at 37˚C ± 0.5˚C (n=3)

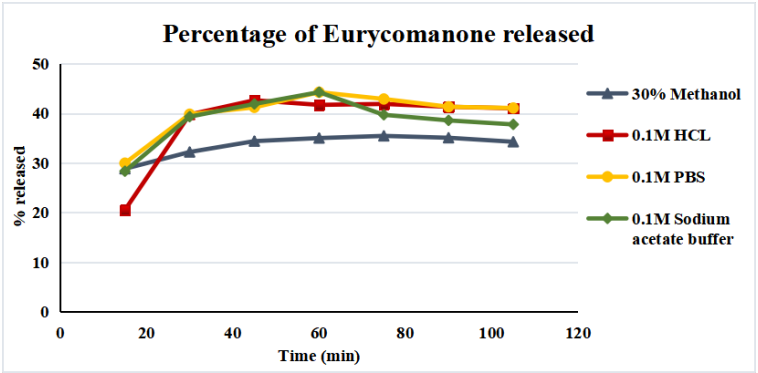

Figure 2:*In-vitro* study release behaviour of Eurycomanone in Tongkat Ali capsules (brand TA) using paddle method at 75 rpm and 500 mL of various media, at 37˚C ± 0.5˚C (n=3)

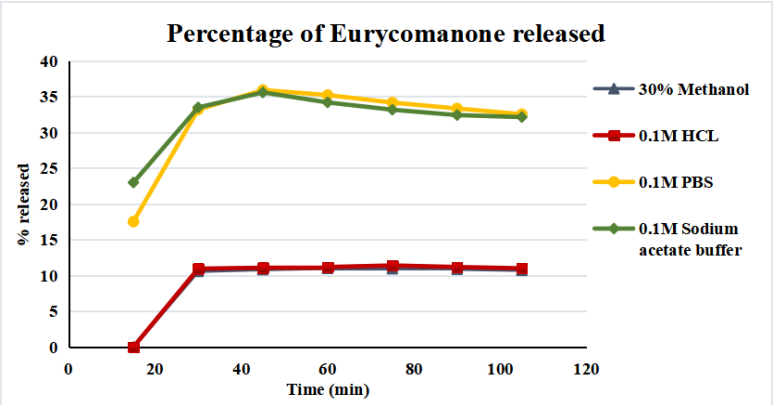

Figure 3:*In-vitro* study release behaviour of Eurycomanone in Tongkat Ali capsules (brand TB) using paddle

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method at 75 rpm and 500 mL of various media, at 37˚C ± 0.5˚C (n=3)

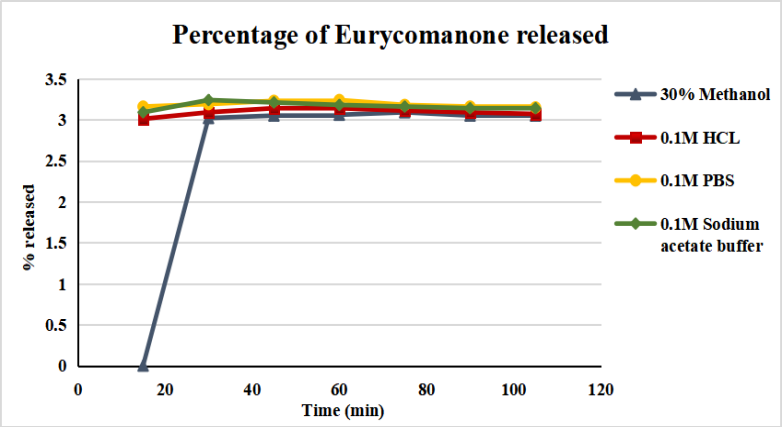

Figure 4:*In-vitro* study release behaviour of Eurycomanone in Tongkat Ali capsules (brand TC) using paddle method at 75 rpm and 500 mL of various media, at 37˚C ± 0.5˚C (n=3)

The study release behaviour of Tongkat Ali for the three brands showed in Figure 2, Figure 3 and Figure 4 respectively. In general, the result showed variation in the behaviour of Eurycomanonefor all brands when submitted to the different media. It was observed that therelease of Eurycomanoneas in brand TA and brand TC were not affected by the different media used (Figure 2 and Figure 4, respectively), while the release of Eurycomanone as in brand TB showed two different behaviour (Figure 3). Based on Figure 3, therelease of Eurycomanone were the highest in alkaline media compared to the acidic media. This suggest the dissolution process was affecting by the dissolution medium and by the solubility of the products itself. pH of the medium also might affected the release rate of Eurycomanone. Based on US Pharmacopoeia active ingredients solubility and solution stability are important properties to be considered when selecting the dissolution medium. In addition, the method of manufacture may also influence the *in-vitro* release behaviour. Apart from that, differences in composition can sometimes affect the extent of absorption, which has been previously reported (Chandrasekaran, 2011).

Table 2: Percentage of Eurycomanonerelease (Y_{30}) at 30 minutes

Sample	**TA**	**TB**	**TC**
	Y_{30} (%)	**Y_{30} (%)**	**Y_{30} (%)**
0.1 M HCL	39.8	10.9	3.1
30% Methanol	32.2	10.6	3.0
0.1 M PBS (pH 7.6)	39.8	33.2	3.2
Acetate buffer (pH 6.9)	39.3	33.5	3.2

The percentage of Eurycomanonerelease at 30 minutes of the three brands TA, TB and TC showed in Table 2. Brand TA showed the highest release of Eurycomanone for all the medium used while brand TC showed the lowest released of Eurycomanone for all the medium used at 30 minutes after dissolution test were performed. Apparently, Eurycomanone dissolved faster in the alkaline buffer media compared to the acidic media despite the

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lowest percentage release of Eurycomanone observed in brands TC. Since the formulation of those three brands were varied (brand TA consists of 40 mg extract Tongkat Ali powder, brand TB consists of 100 mg of Tongkat Ali powder and brands TC consists of 354 mg Tongkat Ali powder) this suggested that the released of active ingredient in the brands were significantly affected by the formulation.

3.2 Eurycomanone as chemical marker for quality control using HPLC

For determination of Eurycomanonein the dissolution aliquots, the standard Eurycomanonecurve was constructed. The curve showed high degree of correlation with coefficient of determination ($r^2 = 0.9917$). The chromatogram for each of the brands were obtained and calculated based on the standard curve plotted of Eurycomanone. Table 3 shows the quantification of Eurycomanonefrom Tongkat Ali in a pure extract and from thebrands purchased. Apparently, brand TS contain the highest Eurycomanone than brand TA; 0.40 %w/w and 0.30 %w/w respectively. As discussed earlier brand TS is the pure Tongkat Ali powder thus the Eurycomanone compound is the highest compared to brand TA which contain a filler in its formulation; meanwhile brand TB and brand TC contain the lowest Eurycomanonedue to the excipients.

Table 3: Determination of Eurycomanonein Tongkat Ali pure extract and commercial brands.

Commercial Tongkat Ali	%w/w
Brand TA	0.30
Brand TB	0.08
Brand TC	0.07
Brand TS	0.40

IV CONCLUSION

This study found variation in the release of Eurycomanone in the products tested which is commercially available in market. Therefore it is suggested that the release rate of Eurycomanone obtained from the analysed brands were found to be quite different from each other, thus demonstrating that the result determined are highly dependent on formulation. The average percentage of Eurycomanonein brands TB and brands TC were lower than in brand TA due to the amount of excipients in brand TA is the least and also affect the release rate of Eurycomanone itself. In conclusion, each of the Tongkat Ali commercial brands consist of Eurycomanoneas active ingredient however the efficiency of release of the Eurycomanoneare depends on the type of media used, pH and the formulationamount of ingredients of the actual bioactive formulated. The study of commercially available brands highlights the importance of quality control and is recommended as a support in the development of finished herbal products supplements.

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