Preliminary studies on the extraction of Glycospanonins in Tongkat Ali extract

S Abirame , K Sivakumar[1], L S Chua[2], M R Sarmidi
[1]Department of Chemical Engineering, Faculty of Engineering, *Universiti Malaysia Sabah, Kota Kinabalu*, Sabah, Malaysia
[2]Institute of Bioproduct Development, Faculty of Chemical Engineering, *Universiti Technology Malaysia*, Skudai, Johor, Malaysia

E-mail: [1]shiva@ums.edu.my, [2]lschua@ibd.utm.my

Abstract. *Eurycoma longifolia,* locally known as Tongkat Ali, is a famous medicinal plant in the family of Simaroubaceae and well known for its aphrodisiac properties from its water extract. The root of *E. longifolia* is used to extract wide range bioactive components of Tongkat Ali. Previous works standardised Tongkat Ali extracts by measuring the concentration of eurycomanone, a quassinoid marker chemical, within the overall extract. There is a newer Malaysian standard that specifies that Tongkat Ali can be standardised to glycosaponin, thus it is desired to determine how extraction parameters such as particle size, extraction temperature, and solvent type affects the glycosaponin content in the extract. The overall study is aimed to determine how the extraction parameters affect the glycosaponin amount in extract. This paper presents the preliminary work where in this study the effect of particle size on overall extract and glycosaponin quantification method development is presented. A reflux extraction method was used to extract Tongkat Ali with a particle size of 0.5 mm, 1.0 mm and 2.0 mm of raw material to study effect of particle size on overall extract. Water and methanol were the two types of solvent used for extraction to study the quantity of glycosaponin.

1. Introduction

Eurycoma longifolia is found in primary and secondary, evergreen and mixed deciduous forest in Burma, Indochina, Thailand, Malaysia, Sumatra, Borneo and the Philippines [1]. *Eurycoma longifolia* Jack (EL) or commercially known as Tongkat Ali in Malaysia, Pasak bumi in Indonesian, Piak and Tung saw in Thailand, and Cay ba binh in Vietnam is a famous medicinal plant in the family of Simaroubaceae. There are three other plant species also known locally as Tongkat Ali which literally means “Stick of a man” and “Ali” of which referring to its aphrodisiac property. The three plant species are *Entomophthora apiculata*, *Polyathia bullata*, and *Goniothalamus sp*. However, EL is the most widely used species for its therapeutic activities [2].

EL is a shrub tree that grows up to 10 metres in height, with long leaves that are green in colour. The leaves are pinnate in shape as the leaflets are arranged in pairs. The flowers of this tree are dioecious, whereas its ovoid-shaped fruits will turn to dark brown colour when they are ripe. A wide

[1,2] To whom any correspondence should be addressed.

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range of chemical compounds have been isolated, especially from the root of EL, which include eurycomanone, eurycomanol, eurycomalactone, canthine-6-one alkaloid, 9-hydroxycanthin-6-one, 14,15!-dihydroxyklaineanone, phenolic components, tannins, quanissoids, and triterpenes [2]. Quassinoids form the major bioactive constituents in this plant and are mainly responsible for its bitter taste [3].

E. longifolia Jack, from the Simaroubaceae family and identified locally as 'Tongkat Ali and Pasakbumi' has been commonly prescribed in traditional medicine as a febrifuge and a remedy for dysentery, glandular swelling, intermittent fever (malaria) [4]. Traditionally, people will boil the Tongkat Ali root chip and drink the decoction [5]. The roots of tongkat ali, often called “Malaysian ginseng”, are used as an adaptogen and as a traditional “anti-aging” remedy to help older individuals adapt to the reduced energy, mood, and libido that often comes with age [6].

Studies have provided evidence in support of the claim that a particular compound, eurycomanone from Longjack root can increase testosterone levels in rats. A recent study conducted in humans involved supplementation of 76 of 320 patients suffering from late-onset hypogonadism with a 200 mg serving of a standardized water-soluble extract of Tongkat Ali for 1 month. Data from this study indicate that treatment of late-onset hypogonadism patients with Tongkat Ali extract significantly elevated serum testosterone concentrations. Following treatment, 90.8% of the subjects exhibited normal testosterone values. Another study found that in 14 men who participated in an intensive 8-week weight training program, the seven men who were supplemented with 100 mg/day of aqueous Longjack root extract experienced a 5 % increase in lean mass, a decrease in fat mass, and an average of 1.8 cm increase in arm circumference while those in the placebo group did not. The findings of a later study indicated increased muscle strength and larger quadriceps muscles [7].

Extraction, as the term used in pharmaceutical context, describes the separation of medicinally active components of plant or animal tissues from the inactive or inert components by using selective solvents in standard extraction procedures. Standardization of extraction procedures contributes significantly to the final quality of the herbal drug [8]. The production of standardised extracts, extracts that have a specific measurable amount of bioactive component is an important issue in the production of herbal medicine. Varying processing conditions of extracts can be utilised in order to produce standardised herbal extracts [9]. In this preliminary work, the effects of varying particle size, solvent and temperature on the quantity of glycosaponins and overall extract of Tongkat Ali extracted on a laboratory scale was studied.

A study conducted by Norhidayah et al. [5] use the eurycomanone level to determine the quality of Tongkat Ali product because eurycomanone which is a quassinoid is the major compound in Tongkat Ali. Hence it can be a good indicator on the level of Tongkat Ali in a product. Malaysian Standard (2011) states that eurycomanone level in Tongkat Ali freeze dried extract should be around 0.8-1.5 w/v (%) [5]. However, there is now a Malaysian standard which specifies that Tongkat Ali can be standardised to glycosaponin. According to a study conduct by Talbott et al. [6], hot-water root extracts standardized for known bioactive components such as 1% of eurycomanone, 22% of protein, 30% of polysaccharides, 35% of glycosaponin and verified to be extremely safe at high doses and for long-term consumption [6]. Moreover, there is another study stated in addition to eurycomanone, other active compounds that should be standardized for commercial purposes include the total protein, total polysaccharides and total saponins. Saponins have received much attention recently due to their health benefits such as cholesterol lowering and anticancer properties [10].

Hence, the objective of the overall study is to determine how the extraction parameters affect the glycosaponin extract yield. This paper presents the preliminary work. Therefore, throughout this study we expect to study the effect of particle size on overall extract and also to develop glycosaponin quantification method.

2. Methodology

2.1 Overall Extraction Method

Extraction of Tongkat Ali was studied at a fixed solvent to solid ratio of 10:1 (wt/wt) of Water:Tongkat Ali. 400 ml of deionized water was boiled to 90 °C and maintain at that temperature before adding in the Tongkat Ali by a magnetic stirrer. 40g of 0.5 mm Tongkat Ali particles was added and mixed well. 2ml of the extract was sampled at 5, 10, 15, 30, 60, 90, and 120 minutes. At the end of extraction, the final volume of liquid extract left was recorded. The wet Tongkat Ali particles were weighed. These procedures were repeated by using 1.0 mm and 2.0 mm of Tongkat Ali particles. Each sample of the extract was analysed using UV Vis Spectrophotometry at 350nm wavelength. A calibration curve was plotted.

2.2 Glycosaponins in extracts

2.2.1 Gravimetry method.

The glycosaponin is extracted from sample with methanol and deionized water. Acetone is added drop wisely to completely precipitate the glycosaponin.

Materials

Deionized water, analytical grade of methanol and acetone were used.

Method

1 g of extract was weighed and dissolved in 50 ml of methanol. The mixture was reflux on warm water bath at 50 °C for one hour and filtered. The filtrate was collected. Total methanolic extract was concentrated to 10 ml using rotary evaporator. 10 ml of methanolic extract concentrate was added dropwise to 50 ml of acetone in a pre-weighed beaker in order to precipitate the saponins. The precipitate was dried in oven to constant weight. Amount of glycosaponins was calculated using the formula:

$$\text{Glycosaponins, \%} = \frac{\text{weight of beaker - weight of empty beaker}}{\text{sample weight}} \times 100$$

These procedures were repeated for deionized water extraction.

2.2.2 Spectrophotometric method.

Spectrophotometric method also known as vanillin-sulfuric acid assay is the most commonly selected spectrophotometric method in plant saponin quantification. Extracts were mixed with vanilin (8% w/v) and sulphuric acid (77% w/v) and incubated at 60°C water bath for 10 minutes.

Preparation for spectrophotometric method:

1. Preparation of reagent solution

Vanilin solution with a concentration of 80g/L was prepared by dissolving 0.8g vanilin in 10mL ethanol. 72% sulphuric acid was prepared by dissolving 18.567mL of 97% of sulphuric acid in 6.44mL of water.

2. Preparation of extracts

0.12535 g of desiccated sample is dissolved in 30 mL methanol.

3. Determination of absorption wavelength absorption wavelength was determined based on Cheok *et al*., 2014 procedure. Blank was prepared by mix 1.0 mL vanillin solution, 1.0 mL of water and 8.0 mL of 72 % sulphuric acid. 1.0 mL of vanilin solution and 1.0 mL extract were mixed, 8.0 mL of 72% sulphuric acid was added. Absorption wavelength was determined using UV visible spectrophotometry.

3. Results and Discussions

3.1 Overall Extract

Figure 1 illustrates the effect of raw material particle sizes on the extraction of eurycomanone. Smaller particles (0.5 mm) shows higher absorbance unit compared to larger particles (2.0 mm) which indicate eurycomanone concentration increases as particle size decreases. This is consistent with mass transfer theory.

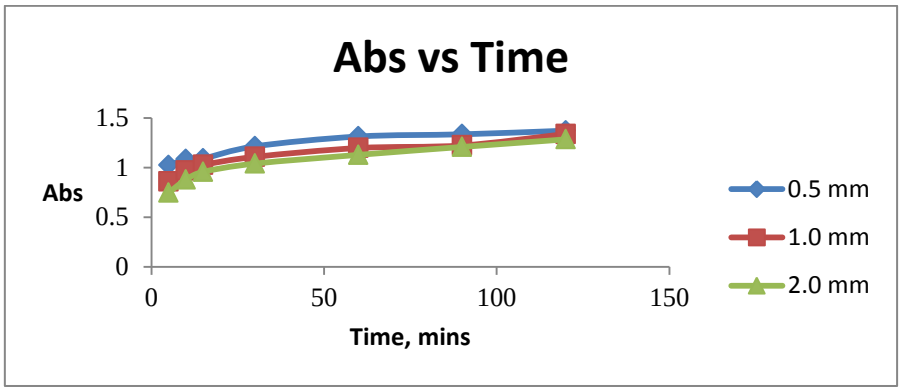


Figure 1. UV-Vis spectrophotometry value for different particle size

Raw material particle size influences the extraction rate by the increase in the total mass transfer area per unit volume when the particle size is reduced. The length of diffusion pathways will also decrease when the particle size decreases, resulting in the increase of mass transfer rate for the process [3]. In this study, three different sizes of raw materials of Tongkat Ali particles were considered. The range of particle size for Tongkat Ali used were from 0.5 mm, 1.0 mm, 2.0 mm.

3.2 Glycosaponin Extract

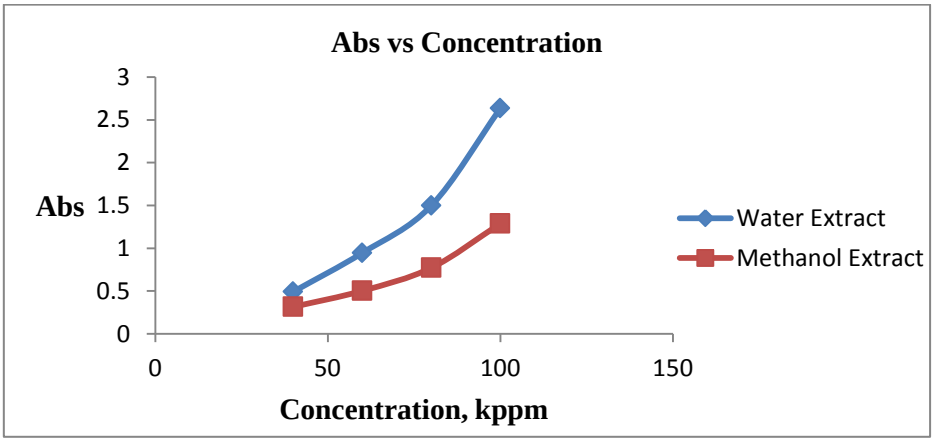


Figure 2. Colorimetric assay for glycosaponins for water and methanol extract.

Figure 2 illustrates the absorbance unit over the concentration of saponins. The spectrophotometric's gives a total saponin value in the quantification of saponins from plant materials. The spectrophotometric technique is simple, fast and inexpensive method to operate. Total saponins also known as vanillin-sulfuric acid assay, is the most commonly selected spectrophotometric method in plant saponin quantification. The basic principle of this method is the reaction of oxidized triterpene saponins with vanillin where sulfuric acid is used as oxidant [11].

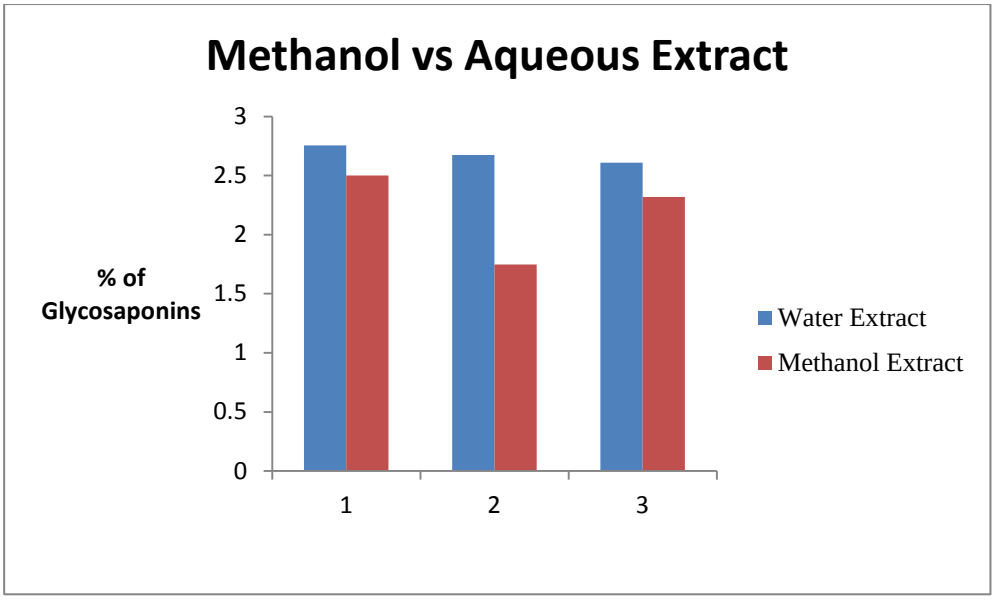


Figure 3. Glycosaponins percentage for water and methanol extract.

Figure 3 illustrates the effect of solvent used on the extraction yields of eurycomanone. From the graph, water extraction yield larger amount of glycosaponins than methanol extraction in every replicate due to their more solubility in water. The presence of water soluble glycone, more soluble in water, plays an important role in the extraction of glycosaponins [12]. Saponins are complex compounds characterized by their structure containing steroidal or triterpernoid aglycone (sapogenin), which linked to one or more oligosaccharide moieties by glycosidic linkage. Saponins are also show strong surface-active properties due to its amphiphilic nature. For example, the presence of a lipid-soluble aglycone and water-soluble sugar chain(s) in their structure [10].

4. Conclusion

The results concluded that smaller particle sizes are better for extraction processes. This is attributed to the larger total surface area presented by smaller particles for extraction. Furthermore, solvent penetration path length decreases when the particle size decreases as the specific surface area of the raw material increases thus influences solubility. There are two solvents for glycosaponins quantification was used, water and methanol. Water is a better solvent for glycosaponin extraction compared to methanol as water extraction produce more glycosaponins than methanolic extraction

5.0 Reference

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