Original Article

A Study on Antioxidant Properties of Eurycoma Longifolia (Tongkat Ali)

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Abstract

In Malaysia, the use of herbal medicines is common. Among the famous herbal medicine is *Eurycoma longifolia* (*E. longifolia)* also locally called Tongkat Ali. As a results of numerous health benefits, the market value for *E. longifolia* products has grown stronger annually and has emerged as one of the most rapidly growing products in herbal market. The *E. longifolia* products are added in coffee and tea bags or in the supplementary form of capsules and tablets. Antioxidant properties of *E. longifolia* in its crude form in different solvents (aqueous and alcohol) have been widely reported but very few studies have looked into the antioxidant properties in the commercial *E. longifolia* products in capsule form. In this study, aqueous extract of commercial *E. longifolia* was tested for its antioxidant properties using total phenolic content (TPC) assay, 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and ferric reducing ability of plasma (FRAP) assay. In the TPC assay, it was found that the Gallic acid equivalent of the *E. longifolia* tested was 7.3229 mg GAE/g. Meanwhile for the FRAP assay, the ferrous sulphate equivalence (FSE) of *E. longifolia* tested was 1.095 mM FSE/g. At concentrations of 0.001 g/L, 0.01 g/L, 0.1 g/L, 1 g/L and 10 g/L, the radical scavenging activities of *E. longifolia* and ascorbic acid (positive control) were 38.90% and 46.79%, 59.25% and 73.66%, 33.07% and 49.32%, 39.46% and 88.43% 50.55% and 90.83% respectively. In conclusion, aqueous extract of commercial *E. longifolia* shows antioxidant properties comparable to previous studies on *E. longifolia* crude extracts.

Keywords: Eurycoma Longifolia, E. Longifolia, Tongkat Ali, Longjack, Antioxidant Properties, Ferric Reducing Ability of Plasma (FRAP) Assay, 2,2-diphenyl-1-picrylhydrazyl (DPPH) Assay, Total Phenolic Content (TPC) Assay.

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INTRODUCTION

Eurycoma longifolia (*E.longifolia*) has several names including Tongkat Ali in Malaysia and is commonly used to increase sexual virility amongst men. *E.longifolia* works by enhancing testosterone levels and acting as energy booster in men (Rehman et al., 2016). *E.longifolia* has been used as a herbal therapy in southeast Asian countries such as Malaysia, Singapore, Brunei, Indonesia, Cambodia and Thailand. It was proven to have phytoandrogenic effects in animal models and have been shown to improve virility in men (George and Henkel, 2014). Apart from the aphrodisiac effect, *E.longifolia* is also commonly used to treat many ailments including diarrhea, glandular swelling, bleeding, dropsy, persistent dry cough, chronic hypertension, and relief of osteogenic pain (Harun et al., 2015; Rehman et al., 2016).

Previous studies have shown that *E.longifolia* extracts possess antiulcer, cytotoxic, antimalarial, anti-pyretic anthelminthic, and anticancer activities (Ismail et al., 1999; Jagananth & Ng, 2000). Over 60 compounds have been identified from *E.longifolia* root extract (Kuo et al., 2004). Alkaloids and quassinoids groups are the major form of bioactive compounds, were reported to be active compound for its medicinal and therapeutics benefits (Kuo et al., 2004; Bhat & Karim, 2010). Quassinoid is of *Simaroubaceae* family that is responsible for the bitter taste in plants including *E.longifolia*. The major components of quassinoids in *E.longifolia* include eurycomanone, 13α (21)-epoxyeurycomanone, eurycomanol, eurycomanol-2-O-β-ɒ-dglucopyranoside, and 13,21-dihydroeurycomanone (Teh et al., 2011).

Previous studies conducted by the Forest Research Institute Malaysia (FRIM) and the Department of Science, University Kebangsaan Malaysia (UKM) found that *E.longifolia* extract have antioxidant activity. It was discovered that *E.longifolia* contains an antioxidant enzyme and SOD (*superoxide dismutase*) which could interfere with the reactions of free radicals that are harmful to the body system. These finding correlates well with the findings of several similar studies on *E.longifolia* conducted in Malaysia (Tambi and Imran, 2010).

Previous studies have reported the antioxidant properties of *E. longifolia* in its crude form in different solvents (aqueous and alcohol) but very few studies have looked into the antioxidant properties of the commercially available *E. longifolia* in capsule form. In this study, aqueous extract of commercial *E. longifolia* was tested for its antioxidant properties using total phenolic content (TPC) assay, 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and ferric reducing ability of plasma (FRAP) assay.

MATERIALS AND METHOD

Preparations of aqueous extract of Eurycoma longifolia

The extract of *E. longifolia* used in this study was obtained from a commercial product; Nu-Prep Lelaki (Batch number: NE 140313) as shown in Figure 2.1. The product was manufactured by Phytes Biotech Sdn Bhd. This product was purchased from Guardian, Shaftsbury, Cyberjaya. Each 350 mg capsule contained 100 mg of *E. longifolia* extract. To prepare the aqueous extract of *E. longifolia*, the vegetative capsules were first cut to obtain the root extracts of *E. longifolia* powders. Due to the hygroscopic effect of the powder, the aqueous extracts of *E. longifolia* were freshly prepared prior to the antioxidant assays.

Figure 0.1: Commercial product containing E. longifolia extract, Nu-prep Lelaki

Total phenolic content assay

Total phenolic content (TPC) assay of *E. longifolia* was done based on the method described by Hossain & Shah (2011). The sample to be tested was prepared by dissolving 10 mg of powder of *E. longifolia* in 100 ml of distilled water. The 10% Folin-Ciocalteu reagent were prepared by diluting 1 ml of pure Folin-Ciocalteu reagent in 9 ml of distilled water. Sodium carbonate solution was prepared by mixing 0.15 g of sodium carbonate powder in 10 ml of distilled water.

This assay was done by mixing 0.2 ml of the sample with 0.2 ml of the 10% Folin-Ciocalteu and was left in a dark room away from light for 4 minutes. After 4 minutes, 1 ml of 15% sodium carbonate solution were added into the solution and was left to stand for 30 minutes at room temperature, away from light. The mixture was then transferred into a cuvette and the absorbance was read using a UV/Vis spectrophotometer at 760 nm. The readings were done in triplicate. The concentrations of the total phenolic content of *E. longifolia* in this study was calculated as gallic acid equivalent (GAE) by using an equation obtained from a standard curve using Gallic acid of different concentrations.

Ferric reducing ability of plasma (FRAP) assay

Ferric Reducing Ability of Plasma (FRAP) analysis was adapted based on the method described by Benzie & Strain (1996) with a slight modification. Prior to this assay, the FRAP reagent was freshly prepared. To make the FRAP reagent, acetate buffer (Reagent A) with a concentration of 300 mM and pH of 3.6 was prepared by adding 1.6 mL of glacial acetic acid to 0.31 g of sodium acetate trihydrate and was made up to 100 mL using distilled water in a volumetric flask. The pH of the solution was tested using a pH meter (SevenEasy, Mettler Toledo). The 2,4,6-Tripyridyl-s-Triazine (TPTZ) solution (Reagent B) was prepared by adding 0.031 g of TPTZ into 10 ml of 40 mM of HCl and was dissolved in a water bath with a temperature of 50°C. Ferric chloride solution (Reagent C) was prepared by dissolving 0.054 g of ferric chloride in 10 ml of distilled water. Reagent A, B and C was then finally mixed with the ratio of 10:1:1 to make the FRAP reagent.

The mixed FRAP reagent was incubated in a water bath for at least 10 minutes at 37°C. While the FRAP reagent was still warm, 100 µl of the extract of *E. longifolia* was added with 3 ml of FRAP reagent. The mixture was then transferred into cuvettes and the absorbance of the mixture was taken after 4 minutes at 593 nm with a UV/Vis spectrophotometer. For each sample, the readings were done in triplicate. The antioxidant potential of the *E. longifolia* extract was determined using a standard curve plotted against concentration of ferrous sulphate.

2,2-diphenyl-1-picrylhydrazyl assay

The determination of the radical scavenging activity of *E. longifolia* was done using the DPPH assay based on the method outlined by Limei *et al*. (2007) with a slight modification. Five different concentrations of *E. longifolia* (10, 25, 50, 100 and 250 µg/ml) were prepared. To prepare the 0.1 mM of DPPH solution, 1.9 mg of DPPH were dissolved in methanol and the volume was made up to 100 ml with methanol. The solution was kept in aluminium wrapped bottle to prevent exposure of the solution to light.

Firstly, 1 ml of the DPPH solution was added to 3 ml of each different concentrations of the *E. longifolia* extracts solution. The mixture was left to stand at room temperature in a dark chamber for 30 minutes. After 30 minutes, absorbance was measured at 514 nm using a UV/Vis spectrophotometer and the readings were done in triplicate. The radical scavenging activity (RSA) was calculated by using Equation in Figure 2.2.

$$\text{DPPH Scavenged (\%)} = \frac{(Absorbance\ of\ control - Absorbance\ of\ sample)}{Absorbance\ of\ control} \times 100$$

Figure 2.2

Statistical Analysis

Statistical analysis was done by using Statistic Package for Social Sciences Programmes (SPSS) version 20.0. The results for the mean ROW and mean ulcer score in this study was analysed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. Significant difference between means was determine at 95% significant correlation ($p<0.05$). The results for antioxidant assays of *E. longifolia* were compared with previous studies on the sample or on the respective assays.

Results & Discussion

Total phenolic content (TPC) assay

Figure 3.1 shows the standard curve for total phenolic content assay. The gallic acid solutions conformed to Beer's law with a regression co-efficient (R^2) of 0.9901. The plot had a slope (m) of 0.0255 and intercept of 0.1234. The equation of standard curve is y = 0.0255x + 0.1234 and was used to calculate the Gallic acid equivalent (GAE) of *E. longifolia* tested. Using the equation y = 0.0255x + 0.1234, it was found that the Gallic acid equivalent of the *E. longifolia* tested was 7.3229 mg GAE/g.

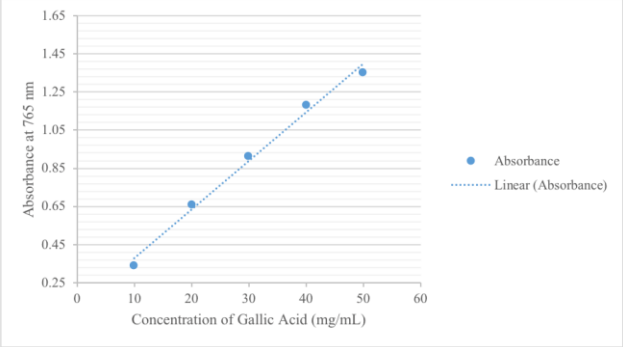

Figure 0.1: Graph of concentration of Gallic acid (g/L) against absorption at 765 nm

The total phenolic content for *E. longifolia* used in this study were estimated by Folin-Ciocalteu method using gallic acid as standard. Abdelhady (2011) reported that there is a strong relationship between the total phenolic content with the antioxidant activity. This is due to the fact that phenols possess strong scavenging ability for free radicals. Based on a study by Khanam *et al*. (2015), it was reported that from a phytochemical screening, the root extracts of *E. longifolia* contains phenolic compounds. This result is consistent with the finding in this present study which showed that *E. longifolia* contained phenol contents. Khanam *et al*. (2015) reported that root extract of *E. longifolia* contains flavonoid which belongs to a polyphenolic compound. This compound was reported to possess antioxidant effect. Abdelhady (2011) also stated that phenolic compound are responsible for a majority of the antioxidant properties in plants. Therefore, the phenolic content of *E. longifolia* used in this present study may have contributed to the antioxidant activity in this study.

Ferric reducing ability of plasma (FRAP) assay

Figure 3.2 shows the standard curve for FRAP assay. The ferrous sulphate solutions conformed to Beer's law with a regression co-efficient (R^2) of 0.8566. The plot had a slope (m) of 1.0162 and intercept of 0.0978. From the linear line plotted, the equation of y = 1.0162x + 0.0978 was used to calculate the ferrous sulphate equivalence (FSE) of *E. longifolia* tested. It was found that the FSE for *E. longifolia* tested was 1.095 mM FSE/g *E. longifolia*.

Ferric reducing ability plasma assay is a non-enzymatic antioxidant assay. In this assay, the antioxidants contained in the samples acts as reductants in a redox-linked colorimetric reaction. The reducing power of the antioxidants translates to the value obtained in this assay.

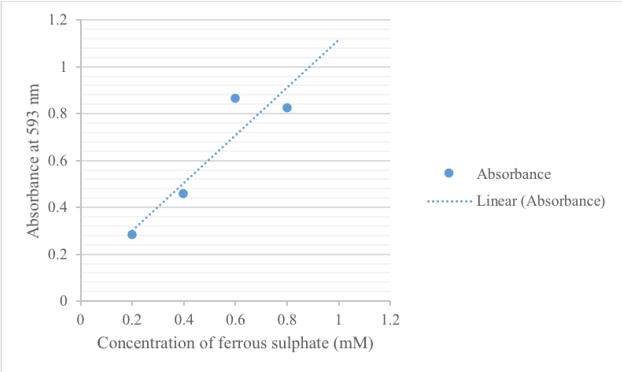

Figure 0.2: Graph of concentration of ferrous sulphate against absorption at 593nm

Previous study done on the extract of a *Diplazium esculentum* in different solvents showed that the FSE result ranged between 0.229-7.6 mM/dry weight of extract (Kaushik *et al*., 2012). A study by Halvorsen *et al*. (2002) shows that berries have high antioxidant properties and the FSE was found to be in the range of 9.78-40.59 mM/g (Halvorsen *et al*., 2006). The range for freeze dried sample of *Garcinia atrovirdis* using aqueous extraction was between 0.109 - 0.434 mM/g which was lower when compared to other solvents used in the study (Rabeta & Faraniza, 2013). Result obtained in this study showed that *E. longifolia* possess antioxidant properties although the value was lower when compared to other plants in previous studies.

2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

Figure 3.3 shows the percentage of radical scavenging activities of different concentrations of *E. longifolia* and ascorbic acid (standard). At the lowest concentration (0.001 g/L), the radical scavenging activities of *E. longifolia* and ascorbic acid were 38.90% and 46.79% respectively. At the concentration of 0.01 g/L, the radical scavenging activities of *E. longifolia* and ascorbic acid were 59.25% and 73.66% respectively. At the concentration of 0.1 g/L, the radical scavenging activities of *E. longifolia* and ascorbic acid were 33.07% and 49.32% respectively. The radical scavenging activities of *E. longifolia* and ascorbic acid at the

concentration of 1 g/L were 39.46% and 88.43% respectively. At the highest concentration (10 g/L), the radical scavenging activities of *E. longifolia* and ascorbic acid were 50.55% and 90.83% respectively.

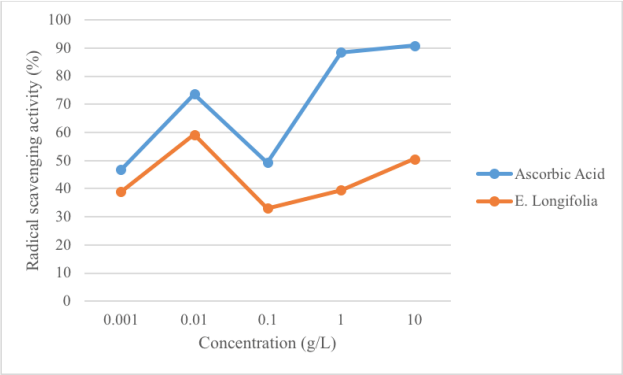

Figure 0.3: Free radical scavenging activities of different concentrations of E. longifolia compared to ascorbic acid

The DPPH assay method is based on the reduction of DPPH which is a stable free radical. A study done by Varghese *et al.* (2013) used the crude extract of *E. longifolia* leaves. When compared, the radical scavenging activities at the concentration of 10 g/L for both the crude extract of *E. longifolia* leaves and the *E. longifolia* tested in this study was 20.39% and 50.56% respectively. It is shown that the *E. longifolia* root tested in this study had a higher radical scavenging activity. High scavenging activity shows high antioxidant properties.

Limitation of Study

During the conduct of the research, there were some unavoidable limitations faced. The room that houses the spectrophotometer was exposed by sunlight, thus, it might affect the DPPH result. Ideally, the test should be run in a dark room and away from sunlight. However, due to unavoidable circumstances, preventive measure were taken.

Conclusion

Using FRAP, DPPH and TPC assays, it was shown that *E. longifolia* extract has antioxidant properties consistent with previous studies and findings. Nevertheless, more research should be done in order to study the exact mechanism of action for antioxidant properties of *E. longifolia*.

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