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Antioxidant and Anti-inflammatory Activity of Eurycoma Longifolia Jack, A Traditional Medicinal Plant in Malaysia

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ABSTRACT

Tongkat Ali (*Eurycoma longifolia* Jack, family, Simaroubaceae) is traditionally used in Malaysia as health supplement for hypertension, diarrhea, aches, persistent fever, malaria, sexual insufficiency, dysentery, and glandular swelling. In this study, hydroalcoholic extract of *Eurycoma longifolia* Jack was studied for its antioxidant and in-vitro anti-inflammatory properties. The antioxidant activity (free radical scavenging) was evaluated to determine the total antioxidant capacity of extract *Eurycoma longifolia* Jack. The DPPH assay showed significant antioxidant activity in all concentrations (10, 25, 50,100 and 250 µg/ml). The antioxidant property of the extract was compared with the values of ascorbic acid, a standard antioxidant. Human RBC (HRBC) stbilization method was utilized to evaluate the in-vitro anti-inflammatory activity of the extract. The extract showed a significant anti-inflammtory activity in all the concentrations tested (25, 50,100, 250, 500 and 1000 µg/ml) and the activity was increased in a concentration dependent manner.

KEY WORDS: Antioxidants; DPPH; Free radicals; Antiinflammatory; Eurycoma longifolia.

Introduction

The chemical species that have an unpaired electron is known as free radicals. Free radicals show a variety of reactivity from relatively low (oxygen molecule) to very high (hydroxyl radical, (OH·) (Packer, 1994). Highly reactive free radicals are produced by exogenous chemicals and endogenous metabolic processes, especially oxygen derived radicals, which oxidize biomolecules, leading to cell death and tissue damage. Many human diseases like cancer, emphysema, cirrhosis, arteriosclerosis, and arthritis have been correlated with oxidative damage (Halliwell and Gutteridge, 1984). Almost all organisms are well protected against free radical damage by enzymes such as superoxide dismutase, catalase and by antioxidant compounds such as ascorbic acid, tocopherols, and gluthatione (Niki et al., 1994). When the mechanism of antioxidant protection becomes unbalanced, polymorphonuclear leukocytes, macrophages and peroxisomes are stimulated, the result may be the above-mentioned diseases and accelerated aging. However, antioxidant supplements or foods containing antioxidants may be used to help the human body reduce oxidative damage (Halliwell and Gutteridge, 1984; Mau et al., 2011; Gülçin et al., 2002).

Antioxidants are the substances that when present in low concentrations compared to those of an oxidisable substrate significantly delay or prevent the oxidation of that substance (Halliwell and Gutteridge 1989). Inflammatory diseases including rheumatic diseases are a major cause of morbidity of the working force throughout world (Chattergee and Pal, 1984). Inflammation is considered as a primary physiologic defense mechanism that helps body to protect itself against infection, burns, toxic chemicals, allergens or other noxious stimuli. An uncontrolled and persistent inflammation may act as an etiologic factor for many of these chronic illnesses (Kumar et al., 2004). Present drugs for the management of pain and inflammation are either narcotics (e.g. opioids), non-narcotics (e.g. salicylates), and corticosteroids (e.g. hydrocortisone). All of these drugs have side effects. On the contrary, many medicines of plant origin had been used since long time with little side and adverse effects (Besra et al., 1996).

Eurycoma longifolia jack is a common herbal shrub or small tree found in primary and secondary forests of Malaysia. Water decoction of its root is a well-known folklore medicine to enhance sexuality, fertility and anti aging (Ang and Sim, 1997; Ang and Sim, 1998a; Ang and Sim, 1998b). The plant contains quassinoid alkaloid (Miyake et al., 2009) with properties curing malaria, allergies, fevers and tumors (Jiwanjinda et al., 2002; Hamzah and Yusof, 2003; Kuo et al., 2004). It also contains tannins, high molecular weight polysaccharides, glycoproteins and mucopolysaccharides. The glycoprotein components exert anti-cancer, pro-fertility, aphrodisiac and anti-aging properties based on animal studies. Recent human trials have proven its testosterone enhancing effect, its anti-stress and anti-aging effect with normalizing growth hormone and anti-oxidant

activity (Tambi and Kamarul, 2005; Tambi, 2006). The plant is reported to be anxiolytic (Ang and Cheang 1999) and also antibacterial (Farouk and Benafri, 2007).

In the present study, we investigated the anti-inflammatory and antioxidant properties of the hydroalcohlic root extract of *Eurycoma longifolia* jack.

Materials and Methods

Plant Material and Chemicals

The roots of *Eurycoma longifolia* jack were collected from Jabatan Pertanian Bumbung Lima (Agriculture department Malaysia) Penang, Malaysia. The roots were washed under running tap water followed by washing with distilled water to remove the surface debris. DPPH (Diphenyl picryl hydrazine) was purchased from Merck and ascorbic acid was purchased from R&M- Saintifik Jaya, Malaysia, Human RBC (10% solution) and Alsever's solution were prepared in the laboratory.

Extraction of Plant Material

Exactly 500 g of dried roots were weighed and powdered. Extraction of *Eurycoma longifolia* jack was carried out by using simple maceration technique: 100 g of powder was extracted using 1000 ml of hydroalcohol (70%) in a conical flask and kept for 24 hours (Mahendra et al., 2010). The solution was shaken in heavy watery shaker for 72 hours. Then the solution was filtered using muslin cloth and further with filter paper. The resultant filtrate was evaporated in a rotary evaporator under reduced pressure to obtain a dry residue. The obtained crude extract of *Eurycoma longifolia* jack (CEEL) was used for the studies. The yield of crude extract was noted and stored in desiccator for a maximum of 3 days; later preserved in a deep freezer for further use. The percentage yield of crude extract of *Eurycoma longifolia* Jack was found to be 7.85% w/w.

Diphenyl Picryl Hydrazine (DPPH) Scavenging Assay

The free radical scavenging activity was followed by the DPPH method (Limei et al., 2007). About 0.1mM solution of DPPH in ethanol was prepared and 1.0 ml of this solution was added to 3.0 ml of extract solution in ethanol at different concentration (10, 25,50,100 and 250 µg/ml). The assay was carried out in triplicate. The decrease in absorbance on addition of test samples was used to calculate the antiradical activity, as expressed by the percentage inhibition (% PI) of DPPH radical Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH radical (% inhibition) was calculated using the following equation:

DPPH Scavenged (%) = (Ac – At / Ac) × 100

where Ac and At are the absorbencies of the control and of the test sample after 30 min, measured at 518 nm, respectively. From a plot of concentration against % PI, a linear regression analysis was performed to determine the IC_{50} (extract concentration resulting in a 50% inhibition) value for sample and standard ascorbic acid.

Human Red Blood Cell Membrane (HRBC) Stabilization Method

The human red blood cell membrane stabilization method was used for this study (Gandhisan et al., 1991). The blood was collected from healthy human volunteer who have not taken any NSAIDS for 2 weeks prior to the experiment and mixed with equal volume of Alsever solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% NaCl) and centrifuged at 3,000 rpm. The packed cells were washed with isosaline and a 10% suspension was made. Various concentrations of extracts were prepared (25,50,100, 250, 500 and 1000 µg/ml) using distilled water and to each concentration 1 ml of phosphate buffer, 2 ml hyposaline and 0.5 ml of HRBC suspension were added. It is incubated at 37ºC for 30 min and centrifuged at 3,000 rpm for 20 min. The haemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm. Diclofenac (50 mcg/ml) was used as reference standard and a control was prepared omitting the extracts.

Statistic Analysis

The data collected for antioxidant activity was analyzed for statistical significance. The means of treatments showing significant difference ($p<0.05$) were analyzed by one-way ANOVA test.

Results

Antioxidant Activity (DPPH Assay)

The antioxidant activity of different concentration of CEEL was determined. The result showed that the extract possesses antioxidant activity. The free radical scavenging effect is increased with the concentration. The percentage inhibition of lowest concentration (10 µg/ml) CEEL is 20.392 ± 0.186 and 61.132 ± 0.113 for highest concentration (250 µg/ml). The IC_{50} value for CEEL was determined by linear regression and was found to be 169.56 µg/ ml). The antioxidant activity of different concentrations of the standard ascorbic acid solution was calculated. Ascorbic acid exhibits a significant ($p<0.05$) antioxidant property in all concentrations. The lowest concentration (10 µg/ml) showed about 31.84 ± 0.914 percentage inhibition whereas 93.40 ± 0.665 percentage for highest concentration (250 µg/ml). The IC_{50} value for ascorbic acid was determined by linear regression and was found to be 34.37µg/ml. The IC_{50} value of CEEL indicates that it possesses antioxidant activity ($p<0.05$) when compared with the antioxidant activity of ascorbic acid. The antioxidant effect of all concentrations of CEEL is less than the antioxidant effect of corresponding concentration of ascorbic acid (Figure. 1).

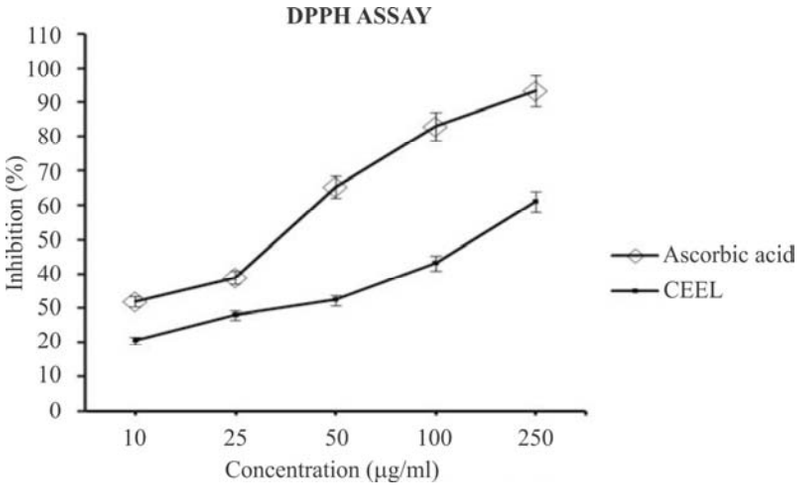

Fig. 1 Antioxidant effect of different concentrations of CEEL and ascorbic acid.

n = 3 Values are expressed as Mean ±SEM

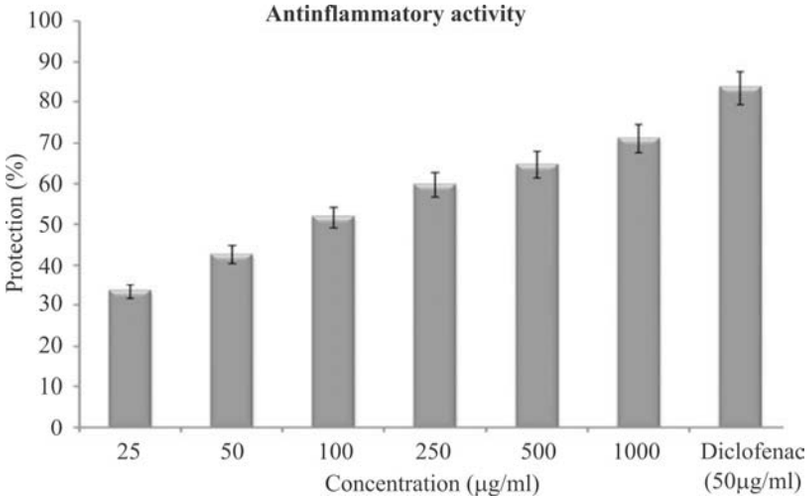

Fig. 2 Percentage protection of (anti-inflammatory) CEEL and diclofenac.

n = 3 Values are expressed as Mean ±SEM

Anti-inflammatory Activity (HRBC Stabilization Method)

The hydroalcoholic extracts of the roots of *Eurycoma longifolia* Jack were studied for in vitro anti-inflammatory activity by HRBC membrane stabilization method. All the concentrations of extract studied showed significant ($p>0.05$) anti-inflammatory activity in a concentration dependent manner. Extract at a concentration of 1000 µg/ml showed 70.968 ± 0.931% protection of HRBC in hypotonic solution. All the results were compared with standard diclofenac (50 µg/ml), which showed 83.333 ± 2.343% protection (Figure. 2).

Discussion

The beneficial health effects from the consumption of diet rich in fruits and vegetables are mainly due to the presence of antioxidants such as polyphenols, carotenoids and anthocyanins. Due to their susceptibility to oxidation, erythrocytes have been used as a cellular model to investigate oxidative damage in biomembranes. Many methods are now followed to estimate the antioxidant activity of plant extracts. The stable DPPH radical model is widely used, relatively quick method for the evaluation of free radical scavenging activity. The effect of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen donating ability (Baumann et al., 1979). The decrease in absorbance of DPPH caused by antioxidant was due to the scavenging of the radical by hydrogen donation. This extract also showed a similar decrease in absorbance which is shown as an increase in percentage inhibition.

Erythrocytes are considered as prime targets for free radical attack owing to the presence of both high membrane concentration of polyunsaturated fatty acids (PUFA) and the O_2 transport associated with redox active hemoglobin molecule, which are potent promoters of reactive O_2 species. The erythrocyte membrane is analogous to the lysosomal membrane (Chou, 1997) and its stabilization implies that the extract may as well stabilize lysosomal membranes. As the RBC membrane

is stabilized by the extract, the hemoglobin content of the supernatant was reduced and hence the absorbance. As stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil (Murugasan et al., 1981) and since RBC membrane is similar to lysosomal membrane in its constitution RBC membrane stabilization can be considered as a factor for anti-inflammatory activity.

The plant, *Eurycoma longifolia.* Jack is reported to contain quassinoids, squalene derivatives and beta carboline alkaoids (Rashid et al., 2009). The quassinoids originally found to be plant growth inhibitors were later proved as antitumour, antischistozomal and antiplasmodial. The beta carboline alkaloids are also reported to be cytotoxic and antimalarial (Ping-Chung et al., 2003).

Conclusions

This study has demonstrated the antioxidant and anti-inflammatory effects of crude extracts of *Eurycoma longifolia.* Jack leaves. The extracts showed significant antioxidant activity in DPPH assay and protected the RBC membrane from the hemolytic effect of hypotonic saline. These observations lead to the conclusion that the plant is having antioxidant and anti-inflammatory potential. Identification and isolation of the active principle of this plant may in future yield a natural antioxidant and anti-inflammatory agent.

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