Antibacterial activity of *Eurycoma longifolia* Jack

A Malaysian medicinal plant

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ABSTRACT

Objective: To evaluate methanolic, ethanolic, acetone and aqueous extracts from different parts of *Eurycoma longifolia (E. longifolia*) (leave, stem, and root) for antibacterial activity against Gram-positive and Gram-negative bacteria and to utilize the leaves and stem parts rather than the root, which is already used for male sexual enhancement in Malaysia.

Methods: The study took place in the Laboratory of Molecular Biology of Biotechnology Engineering Department, Malaysia between January 2005 and June 2006. Methanolic, ethanolic, acetone and aqueous extracts of leaves, stems and roots of *E. longifolia* were investigated for their antibacterial properties using Agar-well diffusion method.

Results: The alcoholic and acetone extracts of the leaves and stem extracts were active on both Gram-positive and Gram-negative bacteria except against 2 strains of Gram-negative bacteria (*Escherichia coli* and *Salmonella typh*i). The root extracts had no antibacterial activity against Gram-positive and Gram-negative bacteria tested. Aqueous leaves extract showed antibacterial activity against *Staphylococcus aureus* and *Serratia marscesens*.

Conclusion: The alcoholic and acetone extracts from leaves and stems of *E. longifolia* contain potent antibacterial agent(s). This plant can serve as a potential source of antibacterial compounds.

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Eurycoma longifolia (*E. longifolia*) Jack, known locally as Tongkat Ali originates from Southeast Asia including Indonesia, Malay Peninsula, Thailand, Laos, Cambodia and Vietnam.[1] All parts of *E. longifolia* plant, in particularly the roots have long been used medicinally. The bark of the roots is used in the Malay Peninsula to relieve fever, ulcers in the mouth, and intestinal worms.[2] It is also used traditionally as a blood coagulant for complication during childbirth and as an aphrodisiac.[3] At present, there are many studies on the chemical and biological activities of *E. longifolia*. For biological activities, it was reported that methanol, n-butanol and chlorophorm root extract except water extract of *E. longifolia* produced a significant cytotoxicity effect against KB, DU-145, RD, MCF-7, CaOV-3, and MBBK cell lines.[4] By using nuclear magnetic resonance (NMR) and mass spectral data, 65 compounds were isolated from the root extract of *E. longifolia* and screened for cytotoxicity, anti- HIV and anti-malarial activity. Eight of these compounds demonstrated strong cytotoxicity against human lung cancer (A-549) cell lines, 7 compounds exhibited strong cytotoxicity towards human breast cancer (MCF-7) cell lines while 2 displayed anti-malarial activity.[5] It was also shown that *E. longifolia* stem extract exhibits a highly positive nematicidal activity.[6] *Eurycoma longifolia* root extract has been shown to possess antipyretic, plant growth inhibition and a direct antiproliferative activity on MCF-7 cells by inducing apoptosis through the modulation of Bcl-2 protein (down regulation of the anti

apoptotic Bcl-2 protein).[7] In recent years, multiple resistances in human pathogenic microorganisms have developed due to the indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases. Screening of plant extract and plant product for antimicrobial activity has shown that higher plants represent a potential source of a new antimicrobial agents.[8,9] The present study was conducted to investigate antibacterial activity of leaves, stem and root of *E. longifolia* against pathogenic Gram-positive and Gram-negative bacteria.

Methods.

Bacterial cultures. Eight species of pathogenic bacteria such as 4 Gram-positive (*Bacillus subtilis, Staphylococcus aureus, Enterococcus feacalis*, and *Micrococcus leteus*) and 4 Gram-negative (*Escherichia coli, Salmonella typhi, Proteus vulgaris*, and *Serratia marcescens*) were used in this study. They were obtained from the Microbiology Laboratories of International Islamic University Malaysia (IIUM) and University of Kebangsaan Malaysia (UKM).

Plant material. *Eurycoma longifolia* was obtained from the garden of the International Islamic University Malaysia (IIUM).

Preparation of alcoholic and aqueous extracts. Plant materials collected were gently pounded using a mortar, oven dried, ground and stored in a cool place until required for use. Fifty milligrams of each plant sample was suspended separately in 1 ml of 85% ethanol, 1 ml of 85% methanol, 1 ml of 85% acetone and 1 ml of 100 mM phosphate buffer. The mixture was then shaken for 5 minutes using vortex apparatus. The homogenate was centrifuged at 8000 rpm for 10 min, and then the supernatant was filtered through 0.45µm sterile filters. The extracts were kept in -80ºC for later use.

Assay for antibacterial activity. The antibacterial activity of the extracts was evaluated using the agar well diffusion method. A 50µl of overnight broth culture ($3x10^9$ CFU/ml) of each test bacteria, 100 µl of glycerol and 4 ml of top agar (3:1 of Luria-Bertani (LB) broth and LB agar) were mixed and layered over the surface of LB agar contained in sterile Petri dishes. The Petri dishes were previously prepared with LB agar and were allowed to settle. Wells of 6 mm in diameter were made into the inoculated plate and then 50µl aliquots of the extract with concentration of 2.5 mg/ml (w/v) were poured into the wells. The plates were incubated for 24 hours at 37ºC. The antibacterial activity of the extracts was compared with reference antibiotics such as 5 mg/ml tetracycline and 5 mg/ml chloranphenicol. All the experiments were carried out in triplicates. The diameter of inhibition zones were measured after 24-hours of incubation. Negative control wells were filled with 50 µl of 85% ethanol, acetone and methanol and with 50 µl of 100 mM phosphate buffer pH 7.

Effect of *E. longifolia aqueous* leaves extract on the growth of *Staphylococcus aureus*. The growth of *Staphylococcus aureus* in the presence of 1 ml of *E. longifolia* aqueous leaves extract with concentration of 100 mg/ml (w/v) was monitored by measuring its absorbance at 420 nm. A reference antibiotic was

Table 1- Antibacterial activity of leaves and stem extracts of *Eurycoma longifolia* (Inhibition zone mm).

Sub-heading	Gram-positive				Gram-negative			
	Staphylococcus aureus	*Micrococcus leteus*	*Enterococcus feacalis*	*Bacillus subtilis*	*Staphylococcus aureus*	*Proteus vulgaris*	*Escherichia coli*	*Salmonella typhi*
Leaves								
Ethanol	13	12	0	15	18	11	0	0
Acetone	13	13	17	13	15	11	0	0
Methanol	13	11	11	12	15	10	0	0
Aqueous	13	0	0	13	13	0	0	0
Stem								
Ethanol	10	13	10	15	15	11	0	0
Acetone	10	11	9	11	19	12	0	0
Methanol	10	11	11	0	14	12	0	0
Aqueous	0	0	0	0	0	0	0	0
Roots								
Ethanol	0	0	0	0	0	0	0	0
Acetone	0	0	0	0	0	0	0	0
Methanol	0	0	0	0	0	0	0	0
Aqueous	0	0	0	0	0	0	0	0
Tetracycline 5mg/ml	32	14	13	14	6	12	30	30
Chloramphenicol 5mg/ml	0	0	15	10	10	14	20	30

The numbers presented in this table represent the mean of 3 trials.

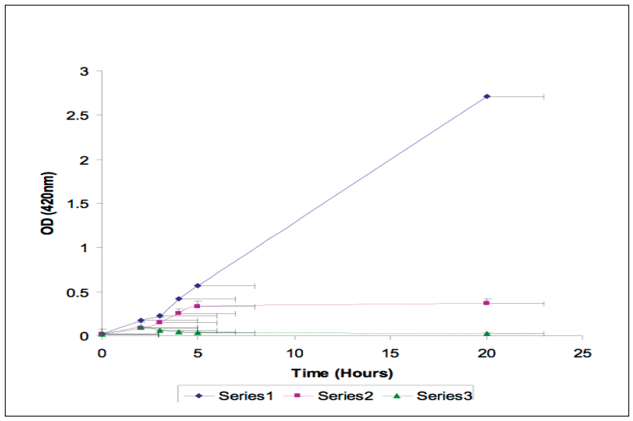

Figure 1 - Effect of aqueous leaves extract on the growth of *Staphylococcus aureus*.

used for comparison. The control group consisted of extract free broth inoculated with 50µl of *Staphylococcus aureus*.[10]

Results. The results of the antibacterial activity of the different parts of *E. longifolia* as well as the reference antibiotics against the test bacteria are presented in **Table 1**. The diameters of the inhibition zone were in the range of 9 to 18 mm. As shown in **Table 1**, the alcoholic extracts of the leaves and stem of *E. longifolia* were active against both Gram positive and Gram negative bacteria (except *Escherichia coli* and *Salmonella typhi*). The root extracts had no antibacterial activity against both Gram positive and Gram-negative bacteria. The aqueous leaf extracts were active against *Bacillus subtilis* and *Serratia marcescens*. The leaf extracts exhibited stronger antibacterial activity than the stem extracts. The negative controls constituting the solvents showed negative antibacterial activity.

Discussion. The results of this study have shown that the alcoholic extracts of the leaves and stem of *E. longifolia*possesses antibacterial activity. The extracts were active on both groups of bacteria indicating that they have a broad spectrum of antibacterial activity. Although the alcoholic extracts exhibited broad spectrum of activity, not all the Gram-negative bacteria tested were sensitive to the extracts. *Escherichia coli* and *Salmonella typhi* found to be resistant in this study have also been reported to be resistant to many plant extracts.[11,12] Generally Gram-negative bacteria are more resistant than Gram-positive bacteria.[13] The effect of 1 ml of aqueous leaves extract with concentration of 100 mg/ml (w/v) on the growth of *Staphylococcus aureus* is presented in **Figure 1**. This concentration inhibited the growth by 82.8% when compared with the control. It was reported that plant extracts that are active at concentration 100mg/ml (w/v) could be considered to have good antibacterial potency level.[11] A 1 ml of 100 µg/ml of streptomycin inhibited the bacterial growth by 99% when compared with the control.

In conclusion, more screening tests need to be carried out to select and purify the most active fraction.

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