The Antibacterial or Antifungal Effects of Eurycoma longifolia Root Extract

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Citation

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Abstract

Background:
Eurycoma longifolia Jack (E. long) has anticancer and antimalarial properties. Even though there are various claims of the ability of E. long to treat infections, there was only one study reported on its antibacterial effects, while there was no study on its antifungal effects. Therefore, we have conducted a study on the antibacterial and antifungal effects of using the current and most relevant clinical strains of bacteria and fungi. Methods: A macrobroth dilution method was used in this study. Three types of fungi (Candida albicans, Candida glabrata and Candida krusei) were tested against an aqueous extract of E. long root at concentrations of 10, 5, 2.5, 1.25 and 0.625 mg/mL. Six types of bacteria (methicillin-resistant Staphylococcus aureus, Enterococcus faecium, extended-spectrum beta lactamase-producing Klebsiella pneumoniae, group-1 beta lactamase-producing Pseudomonas aeruginosa, multidrug-resistant Acinetobacter baumanii and Salmonella typhi) were tested against an aqueous extract of E. long root at concentrations of 50, 25, 12.5, 6.25 dan 3.125 mg/mL. The minimum inhibitory concentrations were determined by looking at the clarity of the final solutions. Results: There was no reduction in turbidity in all test tubes containing various concentrations of E. long root extract. Conclusion:
E. long root extract did not show any antibacterial or antifungal effect at concentrations of equal to or less than 50 mg/mL and 10 mg/mL, respectively.

INTRODUCTION

Eurycoma longifolia Jack or more commonly known among Malaysian locals as 'Tongkat Ali' is an herb frequently used in Malay traditional medicine. It is also known as 'paiak' or 'tung saw' in Thailand, 'pasak bumi' in Indonesia dan 'cay ba bihn' in Vietnam[1]. The herb that belongs to family Simaroubaceae may grow up to 10 metres high and about 10 centimetres in diameter. The pinnate leaves are spirally arranged while the brownish fruits are ovoid in shape[2]. Several parts of E. long including the root, bark, leaves and fruits have been used traditionally to treat various diseases such as urinary tract infection, cancer, hypertension, indigestion, fever and itchiness. The most commonly used part of E. long is the root because it is said to have many active substances. Indeed, most E. long extracts that are available in the market are root extracts.

A study has shown that E. long was able to inhibit Plasmodium falciparum activity up to 80%[3]. E. long acted as an antimalarial agent by inhibiting ribosomal protein synthesis of Plasmodium falciparum[4]. However, E. long is more popularly known as an aphrodisiac agent for men[5]. From literature search, there were many studies done on the effects of E. long as aphrodisiac[6], antimalarial[7] and anticancer agents[8]. Nevertheless, studies on the effects of E. long as an antibacterial or antifungal agent are very scarce despite various claims being made of its effectiveness to treat many infections.

E. long extract was said to contain many potent, active substances to treat many diseases. Quassinoids active substances such as eurycomanone, 13, 21-dihydroeurycomanone and 13-alpha-epoxyeurycomanone[9] have been shown to have antimalarial effects and were also thought to have antibacterial and antifungal activities. However, there was only one report by Farouk and Benafri[10] who concluded that only the stem and leaf extracts of E. long have shown some antibacterial activities against several types of Gram-positive and Gram-negative bacteria. The root extract did not show any antibacterial effects. However, the bacterial strains used in the study did not have significant clinical importance. Therefore, it is more relevant and

meaningful to test E. long against bacterial strains that cause difficult-to-treat infections. In addition, there is also a need to study the antifungal effects of E. long as fungal infection is fast becoming an emerging infection especially among immunocompromised patients. To our knowledge, there was no published study on the antifungal effects of E. long. We prefer to use the aqueous root extract as this part was considered to contain many biochemical compounds with medicinal values and the standardized extract from the root is available[11]. Most importantly, these studies should be performed according to strict microbiological standards to ensure reliability and reproducibility of the results.

MATERIALS AND METHODS

Powdered-form of aqueous E. long root extract (Eurycoma longifolia Jack) was obtained commercially (Phytes Biotek Sdn. Bhd., Shah Alam, Selangor, Malaysia). It was extracted from the root of the plant using a patented high pressure water extraction (US Patent No: US7,132,117 B2). The powder was dissolved in sterile distilled water to make a concentration of 100 mg/mL (w/v). The cloudy brown solution was then filtered with a membrane filter sized 0.45 µm (Supor® Membrane (Acrodisc®) Syringe Filter, Michigan, USA) to produce a clear and sterile solution. Double dilutions were performed to achieve solutions with various concentrations (100, 50, 25, 12.5 and 6.25 mg/mL, approximately 1 mL in each test tube).

FUNGAL INOCULUM PREPARATION AND INOCULATION

The method was adapted from M27-A3 document by the Clinical and Laboratory Standards Institute (CLSI)[12]. The fungi used included Candida albicans (ATCC 10231), Candida glabrata (ATCC 64677) and a clinical isolate, Candida krusei (MB08022508). All three fungi were cultured onto Sabouraud Dextrose agar (SDA) (Oxoid, Hampshire, England) at 35°C in air for 24 hours. Several colonies were dissolved in 5 ml sterile distilled water. The suspension was vortexed and its turbidity adjusted visually to 0.5 McFarland (BioMerieux, France). This created a stock solution that contained approximately 1 - 5 x 10^6 yeast cells per ml. A working solution was created by diluting the stock solution by 1:100 and followed by 1:20 dilution using brain-heart infusion broth (BHIB) (Oxoid, Hampshire, England). The final solution contained approximately 5.0 x 10^2 to 2.5 x 10^3 yeast cells per ml.

For inoculation, 900 µL of the working solution was pipetted into six glass test tubes. 100 µl of E. long extract solutions with five different concentrations (100, 50, 25, 12.5 and 6.25 mg/mL) were mixed with the first five glass test tubes. 100 µl of sterile distilled water was mixed with the sixth test tube, which acted as a positive control. Hence, the concentrations of E. long extract solutions had been diluted by 1:10 to 10, 5, 2.5, 1.25 and 0.625 mg/ml, respectively. Similarly, the inoculum density had been diluted by 10%. The test on each fungal isolate was done in triplicates. Negative control tubes were prepared by using uninoculated E. long solution 100 mg/mL (negative control 1) and uninoculated BHIB solution (negative control 2).

BACTERIAL INOCULUM PREPARATION AND INOCULATION

The method was adapted from M07-A8 document by the CLSI[13]. Six bacterial strains used were methicillin-resistant Staphylococcus aureus (MRSA) (UK 10002287), Enterococcus faecium (MB 10014383), extended-spectrum beta-lactamase (ESBL)-producing Klebsiella pneumonia (UK 10002259), group-1 beta-lactamase (G1BL)-producing Pseudomonas aeruginosa (MB 10015448), multidrug-resistant (MDR) Acinetobacter baumanii (MB 10011927) and Salmonella typhi (UKM 201031820). Several bacterial colonies were dissolved in Mueller-Hinton broth (MHB) (Oxoid, Hampshire, England). The turbidity was adjusted to 0.5 McFarland (BioMerieux, France). A 1:150 dilution was performed with another MHB solution to create a working solution that contained approximately 1 x 10^6 colony-forming units (CFU) per ml.

For inoculation step, 400 µl of the working solution were pipetted into six glass test tubes. 400 µl of E. long extract solution of different concentrations (100, 50, 25, 12.5 and 6.25 mg/ml) were mixed into the first five test tubes. 400 µl of sterile distilled water were mixed with the sixth test tube, which acted as a positive control. Hence, the concentrations of E. long extract solutions had been diluted by 1:2 to 50, 25, 12.5, 6.25 and 3.125 mg/ml, respectively. Similarly, the inoculum density had been diluted by 50%. The test on each bacterial isolate was done in triplicates. Negative control tubes were prepared by using uninoculated E. long solution 100 mg/ml (negative control 1) and uninoculated MHB solution (negative control 2).

INCUBATION AND RESULT INTERPRETATION

All test tubes were incubated in air at 35°C for 24 hours. After 24 hours, the turbidity in each test tube was compared visually with the positive control tube, against a background of three parallel, horizontal black lines. The degree of

turbidity was recorded as (-) clear, (+) turbid but the black lines were still visible or (++) too turbid to see the black lines. Minimum inhibitory concentration (MIC) was taken as the minimum E. long extract concentration required to inhibit any microbial growth (i.e. clear test tube).

RESULTS

All test tubes that contained mixtures of E. long extract and fungal isolates of Candida albicans, Candida glabrata and Candida krusei (tubes B, C, D, E and F) did not show any reduction in turbidity compared to the positive control (tube G). The negative controls (tubes A and H) remained clear. The MICs for all fungal strains tested were >10 mg/mL (Table 1).

All test tubes that contained mixtures of E. long extract and bacterial isolates of Staphylococcus aureus, Enterococcus faecium, Klebsiella pneumonia, Pseudomonas aeruginosa, Acinetobacter baumanii and Salmonella typhi (tubes B, C, D, E and F) did not show any reduction in turbidity compared to the positive control (tube G). The negative controls (tubes A and H) remained clear. The MICs for all bacterial strains tested were >50 mg/mL (Table 2). An example of the findings for MDR-Acinetobacter baumanii is shown in Figure 1.

Figure 1

Figure 1. root extract test on MDR-. A = uninoculated 50 mg/mL (negative control 1); B-F = inoculated (50, 25, 12.5, 6.25, 3.25 mg/mL); G = bacterial suspension without (positive control); H = uninoculated Mueller-Hinton broth (negative control 2

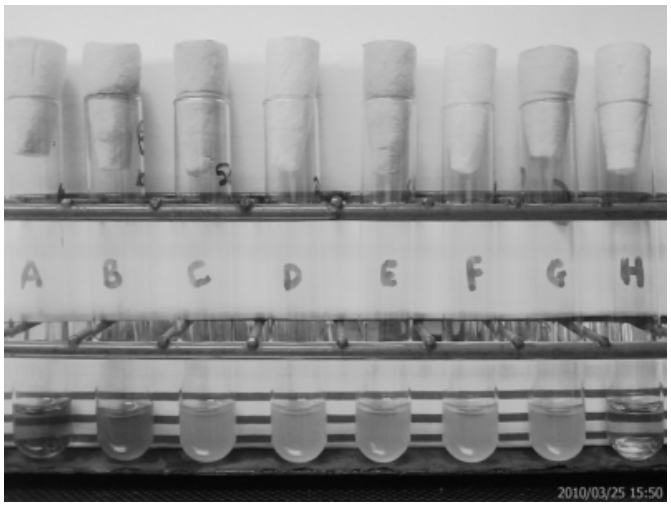

Figure 2

Table 1. Turbidity and minimum inhibitory concentration (MIC) for fungal isolates.

Isolate	*E. long* concentrations (mg/mL)						MIC (mg/mL)
	0	0.625	1.25	2.5	5	10	
Candida albicans	++	++	++	++	++	++	>10
Candida glabrata	++	++	++	++	++	++	>10
Candida krusei	++	++	++	++	++	++	>10

++ very turbid; + turbid; - clear

Figure 3

Table 2. Turbidity and minimum inhibitory concentration (MIC) for bacterial isolates.

Isolate	*E. long* concentrations (mg/mL)						MIC (mg/mL)
	0	3.125	6.25	12.5	25	50	
MRSA	++	++	++	++	++	++	>50
Enterococcus faecium	++	++	++	++	++	++	>50
ESBL *Klebsiella pneumonia*	++	++	++	++	++	++	>50
G1BL *Pseudomonas aeruginosa*	++	++	++	++	++	++	>50
MDR *Acinetobacter baumanii*	++	++	++	++	++	++	>50
Salmonella typhi	++	++	++	++	++	++	>50

++ very turbid; + turbid; - clear; MRSA, methicillin-resistant *Staphylococcus aureus*; ESBL, extended-spectrum beta-lactamase; G1BL, group-1 beta-lactamase; MDR, multidrug-resistant.

DISCUSSION

The search for new antibiotics is badly needed to overcome problems posed by emerging resistant bacteria. For the past few decades, new antibiotic discoveries have become rare events. This has been attributed to strict approval and low returns on investment by pharmaceutical companies[14]. For the past 20 years, the number of new antibiotics in the market has plummeted to half[15,16]. At the same time, the incidence of antibiotic resistance organisms is increasing, such as MRSA[17] and Pseudomonas aeruginosa[18]. These two bacteria were included in our study.

Many methods and resources can be used to discover new antibiotics. It is very important for researchers to follow certain standardized guidelines in antimicrobial susceptibility testing to ensure the quality and reproducibility of an experiment. We had chosen to follow internationally accepted standards set by CLSI[12,13]. Use of non-standardized methods will make it difficult to compare results among different experiments.

One of the reasons for the slow progress in new antibiotic discovery is the lack of study on natural products[19]. E. long is a traditional herb popular for its aphrodisiac effects but studies have shown that it also possesses anticancer, antimalarial and antibacterial activities. E. long extract contains an active substance, quassinoid, which is thought to be responsible for these effects. Based on a single report of antibacterial effect of E. long by Farouk and Benafri[10], we

conducted a study on the antimicrobial effects of E. long on clinically important bacterial and fungal isolates. The six bacterial strains used in this study (viz. MRSA, Enterococcus faecium, ESBL-producing Klebsiella pneumoniae, G1BL-producing Pseudomonas aeruginosa, MDR-Acinetobacter baumanii and Salmonella typhi) often showed high levels of resistance to current antibiotic therapy whether in Malaysia or other countries. However, our study had shown that E. long root extract did not have any significant antibacterial effects to these six bacterial isolates. This finding is in agreement with the study by Farouk and Benafri who reported antibacterial effects were only observed from the leaf and stem, but not the root extracts of Tongkat Ali[10].

Our study was the first on antifungal effects of E. long extract. Three different species of Candida were used viz. Candida albicans, Candida glabrata and Candida krusei. Candida albicans is the most common cause of fungal infections in humans. Studies have shown that Candida albicans was one of the three main species causing fungaemia[20,21]. Candida glabrata and Candida krusei were selected because of their tendency to develop resistance to fluconazole, a commonly used antifungal drug. These three fungi often cause opportunistic infections in immunocompromised patients. E. long was hoped to be an alternative remedy for these patients. Unfortunately, E. long extract did not show any antifungal activities at the concentrations tested.

There were several limitations of our study. First, was the use of crude extract of E. long. This could have effectively reduced the concentrations of truly active antimicrobial compounds. Second, the choice of organisms tested. We had chosen to test E. long extracts against clinically resistant microorganisms. The E. long extract could potentially show some antimicrobial activities against clinically susceptible microorganisms. However, testing against 'normally' susceptible microorganisms would defeat the purpose of finding an alternative therapy for clinically-resistant strains.

CONCLUSIONS

E. long root extract did not show any antibacterial or antifungal activities at concentrations of 50 mg/mL or less and 10 mg/mL or less, respectively. Further studies can be done on extracts from different parts of E. long or with higher concentrations of extracts. However, the high concentration of E. long to be used in future studies must be reasonable to achieve if it is to be commercially produced as a health supplement.

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