ANTIFUNGAL ACTIVITY OF EURYCOMA LONGIFOLIA JACK (TONGKAT ALI) ROOT EXTRACT

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Abstract

Eurycoma longifolia jack (Tongkat Ali) has been known to possess many important biological properties and its uses are gradually expanding . To test the presence of antifungal activity of the ethanolic Eurycoma longifolia Jack (E.L) root extract. E.L. roots were extracted using ethanol . Two types of fungal strains were used; *Candida albicans* and *Aspergillus fumigatus*. Disc diffusion assay and Minimum inhibitory concentration (MIC) tests were used to determine the antifungal activity. The extract was prepared into three concentrations of 50, 100 and 150 mg/ml, Nystatin was used as positive control. The ethanolic extract of E. longifolia Jack root showed positive antifungal activity against *C.albicans* and *A.fumigatus*. The antifungal activity, measured through the zone of growth inhibition on the agar disc diffusion assay, against *A.fumigatus* was recorded to be 3.78±0.63, 6.11±1.45, 9.89±0.74mm, while for *C.albicans* was 4.44±0.5, 6.56±0.50, 8.44±0.90 mm for the extract concentrations of 50, 100 and 150 mg/ml respectively. The results of MIC showed the inhibition of visible growth of *A.fumigatus* and *C.albicans* at the minimum concentration of 12.5 and 25 mg/ml respectively. E.L root extract possesses potent antifungal properties and has the potential to be used as a medicinal product.

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Introduction

Nowadays, fungal infection is becoming a global issue and one of the life threatening diseases. There is an increasing incidence of invasive and fatal fungal infections worldwide due to many factors one of which is the increasing number of immunocompromised patients[1].

Although there are many available antifungal medications, however antifungal resistance is growing and making treatment difficult and complicated[2]. Another issue is the potentially harmful side effects and toxicity of these antifungal treatments that may require discontinuation of the treatment[3]. From these facts we can anticipate the great importance of discovering new antifungal agents from natural resources that can have much less toxicity and side effects than the synthetic ones.

Eurycoma longifolia jack (E.L.) also known as Tongkat Ali, can be found in Malaysia, Cambodia, Sumatra and Borneo. It belongs to family Simaroubacae. E.L root is known to have many medical properties such as aphrodisiac[4] anticancer[5] and antimalarial[6].

The root extract contains many active metabolites such as eurycomanone, eurycomanol, eurycomalactone, cathine-6-one alkaloid, phenolic components, tannins, quassionoids, and tripertenes[7]. Ethanolic E.L root extracts have been reported to possess antibacterial activity against Gram positive and Gram negative bacteria[8,9].

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Research regarding the antifungal activity of the root extract is very limited and so the objective of our study is to investigate the presence of antifungal activity of ethanolic E.L. root extract against two medically important pathogenic fungi; Candida albicans and Aspergillus fumigatus.

Material and Methods

Plant materials

Eurycoma longifolia Jack roots were purchased from a certified supplier in Malaysia. The roots were dried in the oven at 40^{o}C until constant weight was obtained. After that, the dried plant materials were grinded into powder using a dry grinder and 35.7404 gm was obtained. The powdered root was extracted using the Soxhlet method. Six liters of absolute ethanol were used to dissolve powdered root. The active compounds of the sample were extracted with absolute ethanol around its boiling point at 60- 65 ºC using Soxhlet extraction method. It was done for approximately 24 hours.

Then, the extracts were put in a rotary evaporator at 60 °C and 175 mbar pressure and vacuum. This was done to evaporate the solvents and to obtain the crude extracts. The weight of final extract was 11.8 g and the percentage yield of ethanol extract of E. longifolia Jack was 33%. The extract obtained was placed in universal bottle and stored in freezer at -4°C until further test conducted.

The working stocks used for antifungal testing were prepared at a concentration of 50 mg/ml, 100 mg/ml and 150 mg/ml by dissolving 100 mg, 200 mg and 300 mg of the crude extracts into 2 ml of 25% ethanol respectively. Nystatin discs were used as positive control and 25%ethanol was used as negative control for antifungal testing.

Fungal strains

Two types of pathogenic fungal stains were used, Candida albicans and Aspergillus fumigatus. They were obtained from Hospital Universiti Kebangsaan Malaysia, HUKM. The fungi were grown on the Saboroud dextrose broth (SDB) and Saboroud 4% dextrose agar (SDA) and incubated at 37 ^{o}C for 48 hrs.

Adjustment of Microorganism Number and Inoculums Preparation

Direct Colony suspension method was used to prepare inoculation suspension [10, 11]. Barium Sulfate (0.5 McFarland) of standard suspension was prepared by adding 0.5 part of 0.048M $BaCl_2$ to 99.5 parts of 0.18M H_2SO_4 and agitated vigorously until a homogenous suspension was obtained. The turbidity of the suspension was verified by measuring the optical density at 600 nm (OD_{600}) by spectrophotometer. Proper dilutions were done to get an absorbance value of 0.008-0.10 which corresponds to 0.5 McFarland standards. Under aseptic condition, two colonies isolated by ignition-sterilized inoculation loop from 48 hours cultivated agar plates of each fungus were suspended separately in 20 ml pre-warmed (37^{o}C) Saboroud dextrose broth (SDB) and kept in screw-cap bottles and incubated at 37^{o}C. During the incubation period, aliquots of 1 ml were taken from the culture at hourly intervals and optical density OD_{530} for fungal suspension was measured using spectrophotometer. Finally, the resultant broth suspensions contained 10^4 CFU/ml which was used for all experiments performed. Suspensions were always agitated thoroughly before OD measurement and inoculation.

Agar Disc Diffusion Assay

The Kirby-Bauer Disc Diffusion method was chosen to determine the anticipated antifungal activity of the extract[10]. The pathogenic fungi were swabbed onto the SD agar and were incubated for 48 hours at 37^{0}C. Then, filter paper discs were immersed in the working stocks at three different concentrations of 50,100and 150mg/ml and the positive control antifungal-containing disc (5 µg/disc Nystatin) was used. For negative control, we used discs immersed in 25% ethanol. The test discs were laid down on the inoculated agar plates using sterile forceps with gentle pressing to ensure a good adherence to the agar surface. Each plate contained two test discs of the same concentration, one positive control and one negative control disc. The discs were distributed to be at least 15mm from the edge of the plate and no closer than 24 mm from center to center.

Finally, the plates were inverted upside downward and incubated at 37 ^{o}C for 48 hours. The test was done in triplicates to test each

concentration of the extract. After the incubation period, the zone of inhibition (mm) around each disc was measured using ruler and compared with the positive and negative control in each plate.

Broth Microdilution test (MIC):

Microdilution method [12]was used in this study to identify the inhibition of fungal growth with the minimal concentration of root extract. Serial double-fold dilutions were carried out in sterile 96-well plate. First, all wells to use were filled with 180 µl of Saboroud dextrose broth containing microorganisms. Then, 20 µl of sample extract were transferred to the first well and mixed. Three fold serial dilution was performed by transferring 100 µl of the mixture in the first well into the next consecutive wells, until the end of the row. The last well, 100 µl of the mixture were discharged, so the total volume of each well was 100 µl. The micro plate was incubated at 37 ^{o}C for 48 hours. The MIC value was determined by comparing the turbidity of the mixture in test wells with blank wells. To decrease the occurrence of bias the test was assessed by two examiners who were blinded to the type of mixture. The test of all sample and control were performed in triplicates.

Statistical analysis

Statistical package for social science program, version 20.0 (SPSS 20.0) was used to calculate the mean and standard deviation of the measurements of the inhibition zones.

Results

The ethanol extract showed positive antifungal properties. Table 1 shows that the extract inhibited both fungal types and the zone of inhibition was greater with higher concentration of the extract. The positive control (Nystatin) inhibited the growth of both fungal types while the negative control (25% ethanol) did not inhibit both. When comparing the zone of inhibition between the test and the positive control, we found that for Candida Albicans the zone of inhibition at the highest test concentration of 150mg/ml was lower than the positive control; 8.44 ± 0.50 and 23.89 ± 0.89mm respectively. However, when looking at the inhibition of Aspergillus fumigatus we found that the lowest test concentration of 50mg/ml produced a zone of inhibition of 3.78 ± 0.63mm that is very close to the positive control which was 4.00 ± 0.82mm (fig.1).

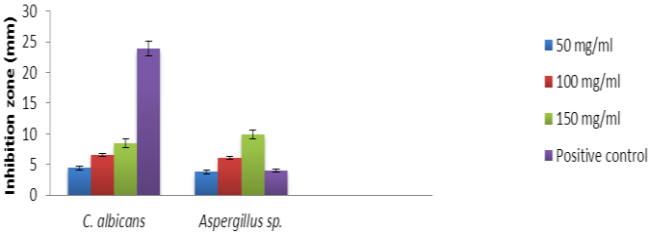

Figure 1. Bar chart showing the zone of inhibition (mm) against different test concentrations and positive control.

Zone of inhibition (mm) (Mean ± SD)					
	Ethanol extract			Positive Control	Negative control
Fungai	50 mg/ml	100 mg/ml	150 mg/ml	Nystatin	25% Ethanol
C. albicans	4.44 ± 0.50	6.56 ± 0.50	8.44 ± 0.50	23.89 ± 0.89 -	-
Aspergillus fumigatus.	3.78 ± 0.63	6.11 ± 1.45	9.89 ± 0.74	4.00 ± 0.82	-

Table 1. Antifungal activity of E.L. root extract according to the zone of inhibition in the disc diffusion assay.

Regarding the MIC results, table 2 shows that the minimum inhibitory concentration of E. L. extract against Candida albicans was found to be 25mg/ml as concentrations lower than this did not inhibit the growth and the suspension remained turbid. However, the MIC for Aspergillus fumigatus was lower as it was found to be 12.5mg/ml.

Minimum Inhibitory Concentration (MIC)		
Concentration of plant extract (mg/ml)	*Candida albicans*	*Aspergillus fumigatus*
200	+	+
100	+	+
50	+	+
25	+	+
12.5	-	+
6.25	-	-
3.125	-	-
1.560	-	-

+ = clear
- = turbid

Table 2. Minimum inhibitory concentration (MIC) of E.L. root extract against fungal strains

Discussion

In our present study we chose two medically important types of fungi that are responsible for many types of infections. Candida albicans is the causative agent for opportunistic oral and genital infections as well as the more serious systemic fungal infection in immunocompromised patients with a high rate of morbidity and mortality [13, 14]. Aspergillus fumigatus causes a wide range of infections (Aspergillosis) in immune competent and immune deficient patients [15] ranging from chronic pulmonary Aspergillosis and Aspergilloma in patients with normal immune system but have an underlying disease such as tuberculosis to more severe disseminated fungal infection in patients with immune deficiency that can affect the heart and liver and may lead to death [16, 17].

In our present study we investigated the antifungal activity of the ethanolic E.L. root extract as alcoholic extracts contain higher amounts of active metabolites than aqueous extracts which can contribute to having higher antibacterial and antifungal activity than aqueous extracts[18]. Studies on the antimicrobial effect of E.L. are very limited and each of the few studies available uses a different method of extraction and also different parts of the E.L. plant. One study used the aqueous E.L. root extract and reported negative antifungal and antibacterial activity[19]. Another study that was done by Danial et al., (2013), used sonication method for root extraction and reported high antibacterial activity of E.L. root extract but negative antifungal activity[9]. The most recent study in 2015 by Khanam et al also used the alcoholic extraction but their procedure involved only soaking the plant material in alcohol and not boiling it, their results showed negative antifungal activity of the E.L. root extract[20].

Our study showed positive results as there was inhibition of fungal growth displayed by the zone of inhibition in the disc diffusion assay. The extract was effective against both fungal types and displayed potent antifungal activity with all test concentrations. To our knowledge, our findings are novel since we did not find any other published work reporting antifungal activity of the root extract.

The reason behind obtaining potent antifungal activity may be due to the different extraction method used as we applied heat to the alcohol solution and brought it up to the boiling point which maybe the cause for extraction of more potent phytochemicals from the roots.

The extract showed different antifungal potency towards the two different fungal types. It's potency against Candida albicans was less than Aspergillus fumigatus which was also proved by the MIC test where by the minimum inhibitory concentration for A.fumigatus is lower than that for C.albicans. The difference in the susceptibility to the root extract may be due to the structural and biological differences between yeasts and molds[21].

The safety of E.L. root extract consumption has been confirmed by many studies[22, 23] which means that this extract has a strong potential to be developed into an antifungal agent that can be used topically and systematically.

Conclusion

The results from this study have revealed the potential of E. longifolia Jack root extract as an antifungal agent further studies are needed to establish the in vivo antifungal activity.

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Declaration of Interest

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References

1. Loeffler, Juergen, and David A. Stevens. Antifungal drug resistance.*Clinical Infectious Diseases* 36.Supplement 1, 2003 ;S31-S41.
2. Pfaller MA. Antifungal drug resistance: mechanisms, epidemiology, and consequences for treatment. The American journal of medicine. 2012;125(1):S3-13.
3. Laniado-Laborín R, Cabrales-Vargas MN. Amphotericin B: side effects and toxicity. Revista iberoamericana de micología. 2009 ;26(4):223-7.
4. Ang, H., T. Ngai and T. Tan. Effects of Jack on sexual qualities in middle aged male rats. Phytomedicine. 2003; 10: 590–593.
5. Bhat, Rajeev, and A. A. Karim. Tongkat Ali (Eurycoma longifolia Jack): A review on its ethnobotany and pharmacological importance.Fitoterapia . 2010;7; 669-679.

6. Chan, Kit-Lam, Chee-Yan Choo, Noor Rain Abdullah, and Zakiah Ismail. Antiplasmodial studies of Eurycoma longifolia Jack using the lactate dehydrogenase assay ofPlasmodium falciparum. Journal of ethnopharmacology.2004;2; 223-227.
7. Nadia Effendy, N. M., Mohamed, N., Muhammad, N., Mohamad, I. N., & Shuid, A. N. Eurycoma longifolia : Medicinal Plant in the Prevention and Treatment of Male Osteoporosis due to Androgen Deficiency. Evidence-based Complementary and Alternative Medicine . 2012: Article ID 125761, 9 pages
8. Ghasak Ghazi Faisal, Ghazi Faisal Najmuldeen, Siti Mastura Zakaria . In vitro antibacterial activity of Eurycoma longifolia jack (Tongkat ali) root extract. The International Medical Journal Malaysia. 2015; 14(1): 77-81.
9. Danial, Saghal, Sundarasekar, S. A. Antibacterial Studies on In Vivo Plant Parts of Medicianally Important *Eurycoma Longifolia* (Tongkat Ali). Pak. J. Bot. 2013;45: 1693–1700
10. Coyle, M.B. Manual of antimicrobial susceptibility testing. USA: American Soceity for Microbiology, 2005.
11. Goldman, E., & Green, L. H. Practical handbook of microbiology 2nd edn, USA: CRC Press,2009.
12. Serrano, M. C., Ramírez, M., Morilla, D., Valverde, A., Chávez, M., Claro, R., Almeida, C. A comparative study of the disc diffusion method with the broth microdilution and Etest methods for voriconazole susceptibility testing of Aspergillus spp . Journal of Antimicrobial Chemotherapy, 2004;53(5) :739–742.
13. Ryan KJ, Ray CG (editors) . Sherris Medical Microbiology (4th ed.). McGraw Hill.2004 ISBN 0-8385-8529-9.
14. Enfert C; Hube B (editors) Candida: Comparative and Functional Genomics. Caister Academic Press. 2007. ISBN 978-1-904455-13-4.
15. Smith, N; Denning, D.W. Underlying conditions in chronic pulmonary aspergillosis including simple aspergilloma. European Respiratory Journal. 2011; 37 (4): 865–872
16. Guinea J, Torres-Narbona M, Gijón P, Muñoz P, Pozo F, Peláez T, de Miguel J, Bouza E . Pulmonary aspergillosis in patients with chronic obstructive pulmonary disease: incidence, risk factors, and outcome. Clin Microbiol Infect . 2010; 16 (7): 870–7.
17. Garcia-Vidal, C; Upton A; Kirby KA; Marr KA . Epidemiology of invasive mold infections in allogeneic stem cell transplant recipients: Biological risk factors for infection according to time after transplantation. Clin Infect Dis . 2008; 47 (8): 1041–50.
18. Johnson, D. B., Shringi, B. N., Kumar, D., Sai, N., & Chalichem, S. Screening of Antimicrobial Activity of Alcoholic & Aqueous Extract of Some Indigenous Plants. Indo-Global Journal of Pharmaceutical Sciencess. 2011; 1(2) :186–193.
19. M Tzar, Y Hamidah, S Hartini, M Marianayati,& A Nazrun. The Antibacterial or Antifungal Effects of *Eurycoma longifolia* Root Extract. The Internet Journal of Herbal and Plant Medicine. 2010; 2(1)
20. Khanam, Zakia, Chew Shwu Wen, and Irshad Ul Haq Bhat. Phytochemical screening and antimicrobial activity of root and stem extracts of wild Eurycoma longifolia Jack (Tongkat Ali). Journal of King Saud University-Science. 2015;27.1:23-30.
21. Saral, Rein. Candida and Aspergillus infections in immunocompromised patients: an overview. Review of Infectious Diseases. 1991;13.3: 487-492.
22. Bhat, Rajeev, and A. A. Karim. Tongkat Ali (Eurycoma longifolia Jack): a review on its ethnobotany and pharmacological importance. Fitoterapia. 2010; 81.7 :669-679.
23. Li, Ching-Hao, et al. Evaluation of acute 13-week subchronic toxicity and genotoxicity of the powdered root of Tongkat Ali (Eurycoma longifolia Jack).Evidence-Based Complementary and Alternative Medicine 2013; 2013.