Effects of Eurycoma Longifolia Jack (Tongkat Ali) Alcoholic Root Extract Against Oral Pathogens

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ABSTRACT

Introduction: Eurycoma longifolia jack (E.L) is a herbal medicinal plant of South-East Asian origin, popularly recognized as ‘Tongkat Ali’. The plant roots have been scientifically proven to have many biological effects including antibacterial activity however, no previous research to date has studied the effect on oral pathogens including cariogenic bacteria. This study was done to determine the antibacterial and antifungal activities of E.L. root extract against three common oral pathogens. Material and Methods: The microorganisms used were Candida albicans, Streptococcus mutans and Lactobacillus casei. E.L root was extracted using soxhlet method. Disc diffusion assay was conducted using extract concentration of 200 mg/ml. Nystatin and Ampicillin were used as positive control for fungal and bacterial tests respectively. Minimum Inhibitory Concentration (MIC) was done to determine the lowest inhibitory concentration of the extract on the microorganisms. Results: E.L extract inhibited the growth of Candida albicans and Streptococcus mutans at a concentration of 200 mg/ml with a zone of inhibition of 16.0 ± 3.0 mm and 70 ± 1.0 mm respectively. There was no antimicrobial effect of the extract on Lactobacillus casei. The MIC of E.L extract against both Candida albicans and Streptococcus mutans was 25 mg/ml. Conclusions: The results from this study revealed the potential use of Eurycoma longifolia jack as an antimicrobial agent which can be used to produce new oral care products.

Key words: Eurycoma longifolia jack, Antifungal, Candida albicans, Streptococcus mutans, Lactobacillus casei, Root extract.

INTRODUCTION

Herbal and traditional medicines have already been used since thousands of years ago and showed improvement in health. Currently researchers are exploring the medicinal benefits of herbal plants because they contain high amounts of biologically active compounds and produce less side effects and also to overcome the emergence of multi drug bacterial resistance.¹

Eurycoma longifolia jack is an herbal medicinal plant of South-East Asian origin, popularly recognized as ‘Tongkat Ali’. The plant roots have been traditionally used for its antimalarial, aphrodisiac, anti-diabetic, antimicrobial and antipyretic activities, which have also been proved scientifically.² Isolation of secondary metabolites from the root of E. longifolia jack showed the presence of eurycomanone, eurycomanol, eurycomalactone, cathine-6-one alkaloid, phenolic components, tannins, quassionoids, and triptertenes.³ The outcome of phytochemical screening of E.longifolia stated they are good sources of phytochemicals, both from their stem and root extracts.⁴

Ethanol extracts of E. longifolia Jack root displayed antibacterial activity against pathogenic Gram+ve and some Gram–ve bacteria. The results are new in documenting antibacterial activity of the root extract using alcohol and heat mediated extraction⁵ to determine the anticipated antimicrobial activity of E. longifolia jack root.

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.⁴ Streptococcus mutans are gram-positive cocci shaped bacteria. These facultative anaerobes are commonly found in the human oral cavity and is a major contributor of tooth decay. The result of decay can greatly affect the overall health of the individual. S. mutans is considered one of the primary causative agents of dental caries and an also be a source of infective endocarditis. The main virulence factors c associated with cariogenicity include adhesion, acidogenicity, and acid tolerance. Each of these properties works coordinately to alter dental plaque ecology.⁵

Previous researchers have documented the antibacterial effect of natural product on S.mutans such as extract of Myristica fragrans (nutmeg), widely cultivated for the spice and flavor of foods, possessed strong inhibitory activity against S. Mutans.⁶

In a study done by B.K. Choi et al., 2001 other natural products that is chitooligosaccharides that derived from the exoskeletons of crustaceans and arthropods has been used to study the antibacterial effect on S.mutans. The antibacterial activity of a chitooligosaccharide mixture was evaluated using two representative oral pathogens. Inactivation experiments performed in a laboratory medium
showed that A. actinomycetemcomitans was sensitive to the chitoooligosaccharides at a concentration of 0.1%, whereas the same concentration showed only a minor inhibitory effect on S. mutans.6

Lactobacillus casei is one of the many species of bacteria belonging in the genus Lactobacillus. It is a gram positive, rod shaped, anaerobic bacteria. According to Badet and Thebaut (2008), lactobacilli were the first microorganisms implicated in dental caries development and they appear during the first year of child's life. A strong correlation has been established between the lactobacillus count and caries whereby the higher the DMF index, the higher the number of children harbouring a high lactobacillus count.9

Natural products such as Bakuchiol (seeds of Pсорalea corylifolia) showed bactericidal effects against all bacteria tested, including S. mutans, Streptococcus sanguis, Lactobacillus acidophilus, Lactobacillus casei etc.10

The development of dental caries were mainly initiated by Streptococcus mutans and believed to be further enhanced by the presence of genus Lactobacillus.11 There are large numbers of cariogenic microorganisms that can be defined by their ability to colonize teeth, causing a marked reduction in pH in the presence of a sugar substrate and consequently inducing caries. Streptococcus mutans, Streptococcus sanguis, Lactobacillus casei and Actinomycesviscosus fulfill most of these criteria. However, Streptococcus mutans seems to be the most efficient cariogenic microorganism, rapidly inducing caries in germ free rodents.11

Candida Albicans is an opportunistic fungus that causes infection (candidiasis) in areas including the mouth. Candida species are in fact the most common fungal pathogens isolated from the oral cavity and frequently cause superficial infections such as oral candidiasis and denture-associated erythematous stomatitis2 (Arzmi et al, 2014). According to Botelho et al (2007), the essential oil of Lippia sidoides and its major components, carvacrol and thymol exerted potent antimicrobial activity against Streptococcus mutans and Candida albicans it's likely useful to combat oral microbial growth.11

The main purpose of this study is to investigate the antimicrobial activity of Eurycoma longifolia jack ethanolic root extracts and their minimal inhibitory concentrations against three oral pathogens; Streptococcus mutans, Lactobacillus casei and Candida albicans.

MATERIALS AND METHODS

Eurycoma longifolia jack roots were extracted with alcohol using soxhlet apparatus. Three types of pathogenic s strains were used, Streptococcus mutans, Lactobacillus casei and Candida albicans. They are obtained from Hospital Universiti Kebangsaan Malaysia, HUKM. The pathogens were grown on deMan, Ragosa, Sharpe (MRS), Brain Heart Infusion (BHI) and Yeast extract Peptone Dextrose (YPD) agar and incubated at 37°C for 24 hours. Then, the turbidity of the suspension was verified by measuring the optical density at 600 nm (OD600) by the spectrophotometer. Proper dilutions were done to get an absorbance value of 0.008-0.10 which corresponds to 0.5 McFarland standards.

Agar disc diffusion assay

The Kirby-Bauer Disc Diffusion method was used to determine the anticipated antimicrobial activity of E. longifolia jack root. The pathogenic bacteria and fungi were swabbed onto the MRS, BHI and YPD agar respectively. Then, sterile filter paper discs were immersed in the working stocks of E. longifolia root extract at concentration of 200mg/ml the positive control antibiotic-containing disc (10 µg/disc ampicillin and 100 µg/disc nystatin) were 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. 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 37oC for 24 hours. After the incubation period, the zone of inhibition (mm) around each disc will be measured using ruler and compared with reference antibiotics used.

Broth microdilution test (MIC)

To determine the minimal inhibitory concentration (MIC), microdilution method was used in this study (Serrano et al. 2004). Serial double fold dilution were carried out in 96 well plate. The wells are filled with 180µl of YPD broth containing C. albicans in one well plate while the other plate is filled with 180 µl of BHI broth containing S.mutans. Then, 20µl of root extract were transferred to the first well. Three fold serial dilution was formed. The micro plate was incubated at 37°C for 24 hours. The MIC value was determined by comparing the turbidity of the mixture in test wells with blank wells. The test of all sample and control were performed in triplicates.

RESULTS

The ethanol extract showed positive antifungal and antibacterial properties. Table 1 shows that the extract inhibited C. albicans and S.mutans but not Lactobacillus casei. The positive control (Nystatin and Ampicilin) inhibited the growth of all microorganisms while the negative control (25% ethanol) did not inhibit any. When comparing the zone of inhibition between the test and the positive control, we found that for Candida albicans the zone of inhibition was comparable to the positive control; 16.0 ± 3.0 mm and 18.0 ± 1.00 mm respectively. However, when looking at the inhibition of S.mutans we found that the zone of inhibition is 7.0 ± 1.0 that is smaller than the positive control which was 31.0 ± 0.50 (Figure 1). Regarding the MIC results, Table 2 shows that the minimum inhibitory concentration of E. L. extract against Candida albicans and Streptococcus mutans were both found to be at 25 mg/ml as concentrations lower than this did not inhibit the growth.

DISCUSSION

Bacterial and fungal infections are becoming a serious problem health. Infections from resistant bacteria are now too common and certain antimicrobial even ineffective to some pathogen.15 World Health Organization (WHO) defined antimicrobial resistances the ability of a microorganism (like bacteria, viruses, and some parasites) to stop an antimicrobial (such as antibiotics, antivirals and antimalarial) from working against it. As a result, standard treatments become ineffective, infections persist and may spread.

Table 1: Antibacterial activity profile of ethanol extract of Eurycoma longifolia Jack roots.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Zone of inhibition (mm) (Mean ± SD)</th>
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<tbody>
<tr>
<td>Eurycoma Longifolia Jack</td>
<td></td>
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<tr>
<td>Positive Control</td>
<td></td>
</tr>
<tr>
<td>Negative control</td>
<td></td>
</tr>
<tr>
<td>Ethanol extract</td>
<td></td>
</tr>
<tr>
<td>Candida albicans</td>
<td>16.0 ± 3.0</td>
</tr>
<tr>
<td>Streptococcus mutans</td>
<td>7.0 ± 1.0</td>
</tr>
<tr>
<td>Lactobacillus casei</td>
<td>-</td>
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</tbody>
</table>

Note: + = positive inhibition
- = negative inhibition
glucan, using glucosyl transferase. De novo synthesis of water-insoluble glucan is essential for the adherence of *S.mutans* and other oral microorganisms to the tooth surface. Varying *Lactobacillus* species have also been consistently associated with caries and are thought to be important secondary pathogens in dental caries.

The formation of dental caries is multifactorial as it required all factors to be involved for the caries to be formed. One of the main factors is involvement of bacteria. According to Karpinski and Szkaradkiewicz (2013), it is believed that bacteria of the species *Streptococcus mutans* is the main factor that initiates caries and very important factor of enamel decay. This is due to the ability of the *S. mutans* to metabolise a number of fermentation products such as lactate, acetate, formate and ethanol that contribute to the acidogenic potential of the local environment. Hence, induce the demineralization of enamel and lead to caries formation.

The antibacterial and antifungal activity of ethanol-based extract of root *Eurycoma longifolia jack* against three selected microorganisms are summarized in Table 1. Our study showed positive results as there were inhibition zones for the bacterial and fungal growth. Ethanol extracts of *Eurycoma longifolia jack* root displayed positive antibacterial effect on *Streptococcus mutans* and positive antifungal effect on *Candida albicans*. However, it showed negative results on *Lactobacillus casei*. The positive controls showed maximal inhibition zone against these microorganisms, while negative control (25% ethanol) did not show any inhibition zone against the microorganisms.

At 200 mg/ml concentration of *Eurycoma longifolia jack* root extracts, the inhibition zones value for *S.mutans* was 7.0 ± 1.0 mm while the inhibition zone value for *C.albicans* was 16.0 ± 3.0 mm. However, all inhibition zone values recorded were lower that positive control values. There is no zone of inhibition noted on *Lactobacillus casei* at same root extract concentration.

Thus, from the result, *Eurycoma longifolia jack* root extracts showed an inhibitory effect to *Streptococcus mutans* and *Candida albicans*. In order to measure the strength of the exact inhibitory concentration, further test was performed according to positive result in disc diffusion assay. We selected the positive results with zone of inhibition and performed the MIC test with ethanol extract. The results showed that the minimum inhibitory concentration of the extracts towards *C.albicans* and *S.mutans* were at the same concentration (25 mg/ml). The result is supported by the study done by previous researchers (Faisal GG et al., 2016) where the MIC obtained against *C. Albicans* was also at 25 mg/ml.

The study was done on antifungal and antibacterial properties by Farouk and Benafri (2007) showed that the antibacterial activity only occurs in leaves and stems, while roots part of *Eurycoma longifolia* does not possess to suppress the bacterial activity. Next, study was been done by Abd-ElAziem Farouk (2008) also indicated that only leaves part of *Eurycoma longifolia jack* shows the antibacterial activity. The active compound on the leaves and stems has high antibacterial activity that can be used as one of the treatment of infection. According to M Tzar et al. (2010) also showed that there was no antifungal and antibacterial activity on root of *Eurycoma longifolia* at equal to or less than 10 mg/ml and 50 mg/ml of concentrations. There were a few study claimed that *Eurycoma longifolia* shows antibacterial activity, and only one study about antifungal activity. But, there was no inhibition on the pathogenic microbial.

However, the recent study was done by Danial et al., (2013) reported that there were inhibition on the pathogenic bacterial from the crude extract of root of *Eurycoma longifolia jack*. So, this conclude that many factors contribute for plants to show its therapeutic properties, for example the mode of processing of samples to obtain the crude extract.

### Table 1: Table of minimum inhibitory concentration (MIC) on *Candida albicans* and *Streptococcus mutans*.

<table>
<thead>
<tr>
<th>Concentration of <em>Tongkat Ali</em> extract (mg/ml)</th>
<th>Minimum Inhibitory Concentration (MIC)</th>
</tr>
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<tbody>
<tr>
<td>250</td>
<td>+</td>
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<tr>
<td>125</td>
<td>+</td>
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<tr>
<td>50</td>
<td>+</td>
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<td>25</td>
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<tr>
<td>12.5</td>
<td>-</td>
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<td>6.25</td>
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<td>3.125</td>
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<tr>
<td>1.560</td>
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*Figure 1:* Bar chart showing the zone of inhibition (mm) against different test concentrations and positive control.
and the right handling before, after and during the experiment was conducted. Thus, our results were supported by this study stated that *Eurycoma longifolia jack* poses antibacterial and antifungal properties.

**CONCLUSION**

Our study showed that ethanol based *Eurycoma longifolia jack* root extract has antibacterial and antifungal properties on *Streptococcus mutans* and *Candida albicans*. Thus, the results from this study have revealed the potential of *Eurycoma longifolia jack* as antimicrobial agents. As a recommendation from our study, Tongkat Ali can be considered to be in part of mouthwash or toothpaste content which might result in new pharmaceutical products in the market.

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**REFERENCES**
