

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

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## History

- Submission Date: 24-07-2019;
- Review completed: 19-08-2019;
- Accepted Date: 19-08-2019.

DOI : 10.5530/pj.2019.11.201

## Article Available online

<http://www.phcogj.com/v11/i6>

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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  $16.0 \pm 3.0$  mm and  $7.0 \pm 1.0$  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.<sup>1</sup>

*Eurycoma longifolia* jack is an herbal medicinal plant of South-East Asian origin, popularly recognized as 'Tongkat Ali.' The plant parts have been traditionally used for its antimalarial, aphrodisiac, anti-diabetic, antimicrobial and anti-pyretic activities, which have also been proved scientifically.<sup>2</sup> 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 triperthenes.<sup>3</sup> The outcome of phytochemical screening of *E.longifolia* stated they are good sources of phytochemicals, both from their stem and root extracts.<sup>4</sup>

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 extraction<sup>5</sup> 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.<sup>4</sup>

*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 associated with cariogenicity include adhesion, acidogenicity, and acid tolerance. Each of these properties works coordinately to alter dental plaque ecology.<sup>6</sup>

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*.<sup>7</sup>

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

**Cite this article:** Alloha IB, Aziz NALB, Faisal GG, Abdllah Z, Arzmi H. Effects of *Eurycoma Longifolia* Jack (Tongkat Ali) Alcoholic Root Extract Against Oral Pathogens. Pharmacog J. 2019;11(6):1299-302.

showed that *A. actinomycetemcomitans* was sensitive to the chitooligosaccharides at a concentration of 0.1%, whereas the same concentration showed only a minor inhibitory effect on *S. mutans*.<sup>8</sup>

*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 Thebaud (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.<sup>9</sup>

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

The development of dental caries were mainly initiated by *Streptococcus mutans* and believed to be further enhanced by the presence of genus *Lactobacillus*.<sup>11</sup> 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 *Actinomyces viscosus* fulfill most of these criteria. However, *Streptococcus mutans* seems to be the most efficient cariogenic microorganism, rapidly inducing caries in germ free rodents.

*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 stomatitis<sup>12</sup> (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.<sup>13</sup>

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 was extracted with alcohol using Soxhlet apparatus. Three types of pathogenic 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, Rogosa, 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 (OD<sub>600</sub>) 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 37°C 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 Ampicillin) 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.<sup>15</sup> World Health Organization (WHO) defined antimicrobial resistance as the ability of a microorganism (like bacteria, viruses, and some parasites) to stop an antimicrobial (such as antibiotics, antivirals and antimalarials) from working against it. As a result, standard treatments become ineffective, infections persist and may spread

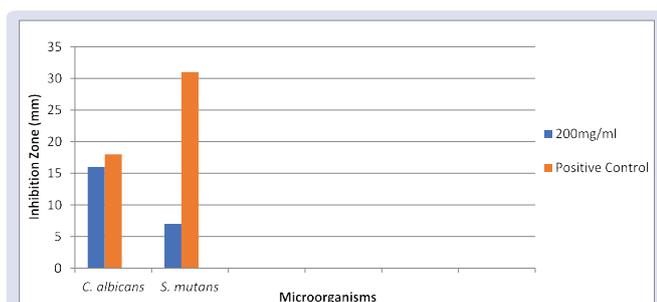
**Table 1: Antibacterial activity profile of ethanol extract of *Eurycoma longifolia* Jack roots.**

	Zone of inhibition (mm) (Mean ± SD)			
	<b>Eurycoma Longifolia Jack ethanol extract</b>	<b>Positive Control</b>	<b>Negative control</b>	
Bacteria	200 mg/ml	Ampicillin 10 µg/disc	Nystatin 100 µg/disc	25% Ethanol
<i>Candida albicans</i>	16.0 ± 3.0	-	18.0 ± 1.00	-
<i>Streptococcus mutans</i>	7.0 ± 1.0	31.0 ± 0.50	-	-
<i>Lactobacillus casei</i>	-	-	-	-

Note:

+ = positive inhibition

- = negative inhibition



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

**Table 2:** Table of minimum inhibitory concentration (MIC) on *Candida albicans* and *Streptococcus mutans*.

Concentration of Tongkat Ali extract (mg/ml)	Minimum Inhibitory Concentration (MIC)	
	<i>Candida Albicans</i>	<i>Streptococcus Mutans</i>
250	+	+
125	+	+
50	+	+
25	+	+
12.5	-	-
6.25	-	-
3.125	-	-
1.560	-	-

to others. This study was done to determine the antibacterial and antifungal activities of *Eurycoma longifolia* Jack root extract against three common oral pathogens. The pathogenic microorganisms used in this experiment are *Candida albicans*, *Streptococcus mutans* and *Lactobacillus casei*. Disc diffusion assay and Minimum Inhibitory Concentration (MIC) were used to determine the inhibition zone and turbidity of suspension due to effect of the extract to the pathogenic microorganisms.

In this study, we wanted to determine the antimicrobial activity of *Eurycoma longifolia* jack root extract on alcoholic based extract as previous studies carried out by Tzar *et al.* (2011) on aqueous root extract, it exhibit antifungal activity against other fungi of *Candida* species.<sup>16</sup>

According to Bhat and Karim (2010), the chemical composition of the *E. Longifolia* particularly from the roots include canthin-6-one alkaloids,  $\beta$ -carboline alkaloids, quassinoids, quassinoid diterpenoids, Eurycomaoside, tirucallane-type triterpenes, squalene derivatives, biphenylneolignans, eurycolactone, laurycolactone, and eurycomalactone.<sup>2</sup> Ethanolic *E. Longifolia* root extracts have been reported to possess antibacterial activity against Gram positive and Gram negative bacteria.<sup>5</sup>

The oral microflora is a complex ecosystem which contains a wide variety of microbial species. In our present studies, the three microorganisms were among the common microorganisms that caused oral diseases. *Candida* has been identified as the common member of the oral microflora and estimated to be present in approximately 40-60% of the general population. It can be present either as transient or permanent colonizer in the oral cavity.<sup>17</sup> It is also recognised as an opportunistic microorganism that has the ability to cause oral diseases, such as oral candidiasis.<sup>18</sup>

*S. mutans* plays an essential role in the pathogenesis of dental caries in which they can initiate plaque formation by their ability to synthesize extracellular polysaccharides from sucrose, mainly water-insoluble

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.<sup>10</sup> Various *Lactobacillus* species have also been consistently associated with caries and are thought to be important secondary pathogens in dental caries.<sup>19</sup>

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 Karpiński 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.<sup>20</sup> 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.<sup>21</sup> 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 zone of 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 *Streptococcus mutans* was  $7.0 \pm 1.0$  mm while the inhibition zone value for *Candida albicans* was  $16.0 \pm 3.0$  mm. However, all inhibition zone values recorded were lower than 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.<sup>22</sup> Next, study was been done by Abd-ElAzim Farouk (2008) also indicated that only leaves part of *Eurycoma longifolia* jack shows the antibacterial activity.<sup>23</sup> 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.<sup>16</sup>

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.<sup>24</sup> 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

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 *Stertococcus 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.

## ACKNOWLEDGEMENTS

We would like to express our gratitude International Islamic University Malaysia for funding this project under P-RIGS grant number: 18-030-0030.

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**Cite this article:** Alloha IB, Aziz NALB, Faisal GG, Abdllah Z, Arzmi H. Effects of *Eurycoma Longifolia* Jack (Tongkat Ali) Alcoholic Root Extract Against Oral Pathogens. Pharmacog J. 2019;11(6):1299-302.