Ficus Deltoidea Leaves Extracts - Promising Therapeutic Agent for Oral Candidiasis and Denture Stomatitis

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Abstract. The aims of this study are to investigate antimicrobial and antibiofilm activities of *Ficus deltoidea* (*F. deltoidea*) leaves extract against *Candida albicans* (*C. albicans*). Methods: The antifungal activity was evaluated using minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC). The potential of *F. deltoidea* leaves extract as antibiofilm agent was evaluated using biofilm inhibition assay and biofilm eradication assay. Chlorhexidine 0.12% (CHX) was used as a positive control. Each experiment was performed in triplicate and repeated four times independently. All the data obtained were analysed using SPSS version 23. Statistical significance measured using Kruskal Wallis test and post-hoc Mann Whitney test which presented as means ±SD with P value <0.001. Results: *F. deltoidea* extract showed antifungal activity against *C. albicans* with MIC and MFC, 50mg/ml and 100mg/ml respectively. The extract exhibited inhibitory effect on *candida* biofilm formation (69.5%). The preformed biofilm was significantly dispersed by extract upon 5 minutes treatment with eradication percentages 66.63%. The biofilm inhibition and biofilm eradication percentages for positive control CHX 0.12% were 43.0% and 58.97% respectively. Conclusion: Results of this study suggest the potential of *F. deltoidea* leaves extract as preventative and therapeutic agents against *candida* biofilm-related infections such as oral candidiasis and denture stomatitis.

1. Introduction

Candida albicans (*C. albicans*) is a polymorphic opportunistic fungus that has the potential to cause life-threatening systemic infections. 75% of the population of *Candida* species including *C. albicans* are found present in the oral cavity of a healthy individual and generally remains latent [1]. However, this species has the ability to disseminate to non-commensal niches, resulting in hazardous colonization and invasive disease in an immunocompromised patient with the support of wide range of their virulence factors which includes recognition biomolecules (adhesins), morphogenesis (the reversible transition between unicellular yeast cells and filamentous, growth forms), secreted aspartyl proteases and phospholipases [2,3]. In an immunosuppressed individual, *C. albicans* can cause oral infections such as oral candidiasis and denture stomatitis.

Oral candidiasis is a common oral opportunistic infection caused by overgrowth of Candida species especially *C. albicans* [4]. The incidence of *C. albicans* isolated from the oral cavity has been reported to be 50%–65% of people who wear removable dentures [4]. Denture is one of the predisposing factors that may lead to transition of commensal to pathogenic Candida [5]. Globally, denture stomatitis is nominated as the top problem that arises for denture wearers. Candida-associated denture stomatitis is known to be one out of seven of the most common fungal infections caused by *C. albicans* involving oral mucosa [6]. Several studies have shown that 70% of individuals who wear removable complete dentures experience denture stomatitis [7]. This infection usually happens in elderly denture wearing population [7] and more frequently occur in women than men [8]. However, there is no discrimination based on racial predilection yet to be identified [9].

Various treatments have been suggested for relieving denture stomatitis and oral candidiasis. Basically, the treatment of denture stomatitis is done through denture cleaning procedures, discontinuation of nocturnal denture wearing, denture replacement or realignment, topical or systemic antifungal agents, microwave irradiation, disinfect solution such as chlorohexidine (CHX) and sodium hypochlorite in which patient needs to immerse the denture into the solution for overnight for optimal effect [10]. However, disinfection technique has minor side effects such as staining of the prosthesis and can cause reduce in appetite. Nowadays, the most common of antifungal agents have been suggested for relieving and treating denture stomatitis are nystatin, amphotericin B, miconazole, ketoconazole and fluconazole [11]. However, few issues in relation with the use of antifungal agents have been arisen due to fungal resistance and drug toxicity. The undesirable effects from the drugs that might happened are nausea, vomiting, hepatotoxic and nephrotoxic effects [12]. Miconazole gel associated with warfarin coagulation. There are also high recurrence rates of denture stomatitis

and recolonization with candida when stopping the antifungal treatments [12].

Nowadays, herbal medicine has gain popularity and it is considered as a therapeutic agent [13]. *Ficus deltoidea* (*F. deltoidea*) is considered one of the herbal plants that is widely used, especially by the Malay population. It is a green shrub that falls under *Moraceae* family and usually known as mas cotek [13]. Traditionally, the fruits are chewed to relieve toothache [13]. Until now, many scientific researches on this plant has been conducted which it possesses variety of pharmacological activities. These include anti-inflammatory activity [14]; anti-nociceptive activity [15]; anti-diabetic activity and anti-oxidant activity [16]. Meanwhile, there is a study conducted reveals that *F. deltoidea* effectively accelerates oral ulcer healing process and significantly found no toxic effect [13]. In spite of these evidences, the effect of *F. deltoidea* on antifungal activity has lack scientifically documented. Thus, the aims of this study are to investigate antimicrobial and antibiofilm activities of *F. deltoidea* leaves extract against *C. albicans*.

2. Material and Methods

2.1 Preparation of the extract

F. deltoidea leaves extract was prepared following the methodology proposed by Sánchez et al. 2010 [17], with minor modifications.

2.2 Determination of minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC)

The MIC analysis was performed in BHI broth (BHIB) via broth micro-dilution techniques according to Clinical and Laboratory Standards Institute [18].

Minimum Inhibition Control (MIC)	Minimum Fungicidal Control (MFC)
50 mg/ml	100 mg/ml

2.3 Determination of biofilm inhibition - inhibition of initial candida cell attachment. illustrations

The plant extract at subMIC concentrations were evaluated for their inhibition potential against cell attachments (antiadhesion test) according to method described by Bazargani et al., 2016 [19]. The percentage of biofilm formation inhibition was calculated using the following formula:

Biofilm inhibition %=	$OD_{\text{negative control}}$-$OD_{\text{test}}$/ $OD_{\text{negative control}}$	X 100

2.4 Eradication of biofilm

The eradication of biofilm formation of the extract was performed according to methods described by Bazargani et al., 2016 [19]. Percentage eradication was calculated by using the following equation:

Biofilm eradication %=	1- $OD_{\text{negative control}}$-$OD_{\text{test}}$/ $OD_{\text{negative control}}$	X 100

3. Results

3.1 Minimum inhibitory and minimum fungicidal concentration (MFC)

Minimum Inhibition Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of *F. deltoidea* leaves extract on *C. albicans* determined using the broth microdilution method outlined by the Clinical and Laboratory Standard Institute (CLSI) with minor modifications (CLSI, 2009). From the result, *F. deltoidea* leaves extract showed antifungal activity against *C. albicans* with MIC and MFC, 50mg/ml and 100mg/ml respectively.

3.2 Biofilm eradication assay

Based on the biofilm eradication result as shown in Figure 3, the effect of *F. deltoidea* leaves extract had significantly ($p<0.001$) increasing the percentage of biofilm eradication, as compared to CHX 0.12%. Percentage of biofilm eradication by *F. deltoidea* leaves extract with concentration of 50mg/ml was 66.63 ± 0.32%, 25 mg/ml was 21.13 ± 0.84%, 12.5mg/ml ± 0.62% whereas for biofilm treated with CHX 0.12% was 58.97 ± 0.53%.

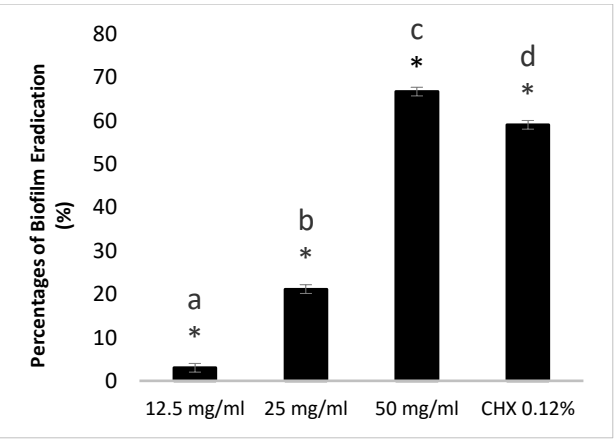

Fig. 1. The effect of *C. albicans* eradication after treated with *F. deltoidea* and CHX 0.12%. The overall percentage of biofilm eradication obtained from 4 sets of experiments in triplicates (n=12) is presented in a bar graph. (*) indicates significant differences in mean percentages were compared to the untreated control ($p < 0.001$) according to the non-parametric Kruskal Wallis test with Mann Whitney. Different letters indicate statistically significant differences between groups.

3.3 Biofilm inhibition assay

Based on the biofilm inhibition result as shown in Figure 4, the effect of *F. deltoidea* leaves extract had significantly ($p<0.001$) increasing the percentage of biofilm inhibition, as compared to Chlorhexidine 0.12%. Percentage of biofilm inhibition by *F. deltoidea* leaves extract with concentration of 50mg/ml was 69.5 ± 0.93%, 25 mg/ml was 51.52 ± 1.11%, 12.5mg/ml 1.39 ± 0.82% whereas for biofilm treated with CHX 0.12% was 43 ± 0.71%.

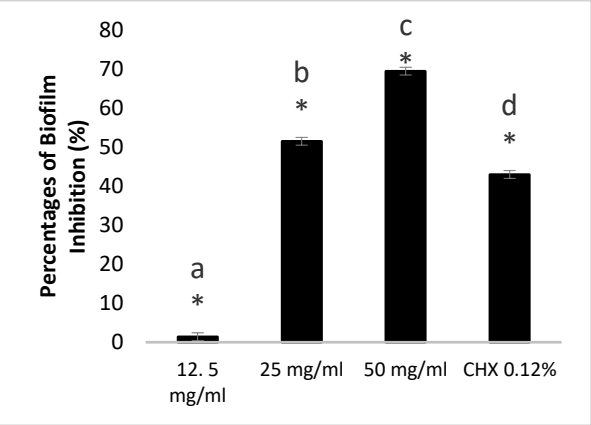

Fig. 2. Figure shows the effect of *C. albicans* inhibition after treated with *F. deltoidea* and CHX 0.12%. The overall percentage of biofilm eradication obtained from 4 sets of experiments in triplicates (n=12) is presented in a bar graph. (*) indicates significant differences in mean percentages were compared to the untreated control ($p < 0.001$) according to the non-parametric Kruskal Wallis test with Mann Whitney. Different letters indicate statistically significant differences between groups.

4. Discussion

In this study, the antifungal activity (MIC and MFC) of methanolic leaves extract of *F. deltoidea* was tested against *C.albicans.* The antibiofilm activity of *F.deltoidea* leaves extract was dose- dependent. The leaves extract was found to exhibit antifungal activity against *C. albicans* with a concentration of 50mg/mL for MIC and 100 mg/ml for MFC. The biofilm inhibition assay shows that 69.5% of *C. albicans* biofilm is dispersed by the extract at the concentration of 50mg/ml verifying a significantly higher as compared to control, CHX 0.12% with 43% of inhibition activity. Meanwhile for the eradication activity at the concentration of 50mg/ml, the *C. albicans* biofilm is disrupt by the *F. deltoidea* leaves extract with percentage of 66.63% and for the positive control, CHX 0.12% showing only 58.97%. Our study result is in agreement with AbdSamah et al.2012 [20]. The results obtained prove that *F. deltoidea* has an antibiofilm activity against *C. albicans* biofilm and has potential to be used in managing denture stomatitis and oral candidiasis.

F. deltoidea usage in traditional treatment can be clarified scientifically by studying the presence of its phytochemical constituents. A number of previous studies reported that inhibition of biofilm formation could be explained by the presence of tannins, phlobatannins, saponins, flavonoids, steroids, terpenoids, cardiac glycosides, alkaloids, anthraquinones and polyphenols [21]. Thus, further is required to identify the specific phytocompounds that exhibit the antifungal properties *F. deltoidea* leaves extract has a significant effect towards *C. albicans* activity in which it has greater anti-fungal activity compared to CHX 0.12%. This preliminary study provides a basis or platform for further research regarding the therapeutic effect of *F. deltoidea* as an anti-fungal agent.

5. Conclusion

Our preliminary study has successfully identified the antifungal activity of *F. deltoidea* leaves extract against *C. albicans*. It's shows that the methanolic leaves extract has the potential as an antifungal agent. These findings may help us to do further research on investigating the mechanism of action of *F. deltoidea* on biofilm of Candida via acrylic resin and also studying on the underlying inhibition mechanism at the molecular level. It is believed that *F. deltoidea* has a great potential as an alternative treatment of *C. albicans* infection in the oral cavity.

6. References

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