Morus alba L.: Creating Miles of Smiles

Nurhayati Mohamad Zain[1], *Indah Mohd Amin*[1], *Vivi Noryati Ahmad* [1], *Nur Haleesa Mohd Hairi*[1], *and Noor Izzyani Mahadzir*[1]

[1]Faculty of Dentisry, Sg. Buloh Kampus, Universiti Teknologi MARA, Malaysia

Objectives: The aims of the study are to determine the antimicrobial and antibiofilm activities of *M. alba* leaves extract against *Streptococcus mutans* (*S.mutans*). **Method:** The antimicrobial activity was evaluated using minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The antibiofilm potential of extract was evaluated using biofilm inhibition assay and biofilm eradication assay. Oradex mouthwash with 0.12% CHX was used as a positive control. All experiments were performed in triplicate and repeated four times independently. Data were analysed using SPSS software version 23. A Kruskal-Wallis test followed by post-hoc Mann-Whitney U test was applied and level of significance was set at $P < 0.001$. **Results:** *Morus alba* (*M.alba*) leaves extract showed antimicrobial activity against *S.mutans* with MIC and MBC, 25mg/ml and 50mg/ml respectively. The adherence of bacteria on extract treated surface (0.5x MIC) was significantly reduced with adherence inhibition percentage of 72.5% compared to positive control CHX 0.12% (63%). At 0.5x MIC concentration, the extract also disrupted preformed biofilms with eradication percentage of 52.87%. **Conclusion:** The results suggest that *M. alba* leaves extract represents an untapped source of local plant with antibiofilm activity against *S.mutans* that could be a resource in the development of therapeutic natural products in managing dental caries.

1. Introduction

Dental caries (tooth decay) is a transmissible bacterial disease process caused by the acid released from the acidogenic bacteria which enters the enamel and dentine and dissolving the mineral [1]. Dental caries attacks all ages including school-age children and adults. In Malaysia, the prevalence of dental caries among 5 and 6-year-old children was reported to be 76.2% and 74.5%, respectively [2,3]. Higher caries prevalence (90.3%) was also recorded among adult in Malaysia from the age of 15 and above [4]. It is also the main cause of oral pain and tooth loss, which will lead to difficulty in chewing, speech problems, general health disorder and affects the quality of life [5]. The existence of dental caries is mainly associated with oral pathogens, especially cariogenic bacteria [6].

The oral cavity contains a wide variety of oral bacteria, but only a few specific species of bacteria are believed to cause dental caries which are *Streptococcus mutans*, *Lactobacillus acidophilus*, *Actinomyces viscosus* and *Nocardia sp.* [7]. The major role in prognosis and development of the oral disease is played by *Streptococcus mutans*. Thus, preventive therapy for dental caries will be the elimination of *Streptococcus mutans* [8]. The ability of *Streptococcus mutans* to produce a large quantity of glucans as well as acid, exceeding salivary buffering capacities, which gives the bacteria an advantage to outcompete noncariogenic commensal species at low pH environment [9]. Therefore, in our study, we used *S. mutans* because it is one of the most important pathogens involved in the formation of dental plaque and its prognosis to dental caries [8].

Nowadays, researchers are keen to investigate the therapeutic agents originated from a natural source such as plant extract or plant active compounds which give the same effects as the commercial drugs but with lesser side effects that are safer for the user. Medicinal plants still play an important role in some emerging and developing countries and are the major components of all indigenous or alternative system of medicine [10].

Morus is a genus of flowering plants in the family Moraceae. Its dried leaves have been consumed as herb-tea beverage and food supplements [11]. It was reported that *M.alba* leaves were chewed for toothache treatment to prevent further destruction or cavitation of the tooth [12]. Various pharmacology activities were reported for *M.alba* leaves extract such as antidiabetic activity against diabetes mellitus [13]. *M. alba* also provides potential antimicrobial activity against pathogenic microorganisms [14]. In addition, subacute toxicity and genotoxicity studies reveal that *M.alba* is significantly not toxic and considered safe [15]. The antibiofilm potentials of *M.alba* leaves were reported in few studies[16,17]. However, in all the studies, the extract was incubated with the bacteria for 18-24 h which do not fit the clinical application. In the clinical setting, the drugs (oral rinse/gel/paste) are only exposed to the teeth/oral cavity in a short period during the treatment/application. Thus our objectives in this study are to determine antibiofilm potential (initial adherence inhibition and biofilm eradication) of *M.alba* leaves extract against *S.mutans* in short exposure treatment.

2. Methods

2.1 Preparation of Extract

M.alba leaves extract was prepared following the methodology proposed by Sánchez et al. 2010, with minor modifications [18].

2.2 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC).

The MIC and MBC tests were performed in BHI broth (BHIB) via broth microdilution techniques according to Clinical and Laboratory Standards Institute (CLSI, 2012) [19].

2.3 Determination of Biofilm Inhibition

The plant extract at subMIC concentrations was evaluated for their inhibition potential against cell attachments according to the method described by Bazargani et al., 2016 [20]. The percentage of biofilm formation inhibition was calculated using the following formula :

Inhibition (%) =	$OD_{negative\ control}$-OD_{test}/ $OD_{negative\ control}$	X 100

2.4 Determination Eradication of Biofilm

The eradication of biofilm formation of the extract was performed according to methods described by Bazargani et al., 2016 [20]. Percentage of biofilm eradication was calculated by using the following equation.

Eradication (%)	= 1 -	$OD_{negative\ control}$-OD_{test}/ $OD_{negative\ control}$

3. Results

3.1. Minimum Inhibitory Concentration and Minimum Bactericidal Concentration

The antimicrobial activity of *M. alba* leaves extract against *S. mutans* showed minimum inhibitory concentration (MIC) of 25mg/mL and minimum bactericidal concentration (MBC) of 50mg/mL.

3.1 Biofilm Inhibition Assay

Figure 1 shows, the adherence inhibition percentage of *S.mutans* on extract- treated surface . Percentage of biofilm inhibition by *M. alba* extracts is 72.5% whereas for biofilm treated with CHX 0.12% is 63%.

3.2 Biofilm Eradication Assay

Based on the result as shown in Figure 2, the biofilm eradication activities of *M. alba* leaves extract against *S. mutans* biofilm upon 5 minutes treatment was concentration- dependent. The percentage increased from 40.7% at concentration 6.25 mg/ml to 52.87% at 12.5 mg/ml. Exposure to Oradex mouthwash (CHX) had exhibited 50.54% biofilm eradication.

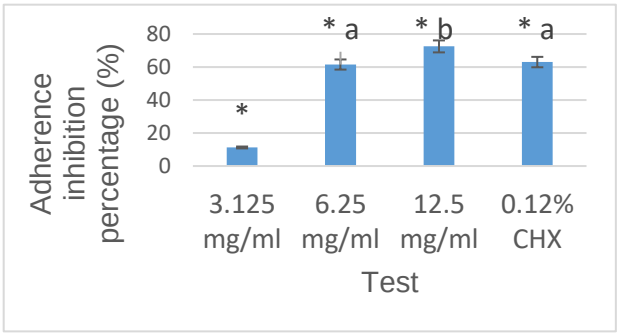

Figure 1: Percentage of biofilm inhibition after treated with *M. alba* extracts at 3.125 mg/ml, 6.25 g/ml, 12.5 mg/ml concentrations and CHX 0.12%. The overall percentage of biofilm obtained from 4 sets experiments in triplicate (n=12) is presented in a bar graph. * indicates significant differences 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 group.

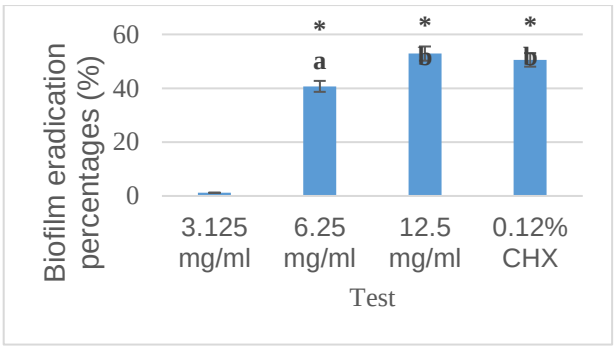

Figure 2: Percentage of biofilm eradication after treated with *M. alba* extracts at 3.125 mg/ml, 6.25 mg/ml, 12.5 mg/ml concentrations and CHX 0.12%. The overall percentage of biofilm obtained from 4 sets experiments in triplicate (n=12) is presented in a bar graph. * indicates significant differences 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. Discussions

In our study, the antimicrobial activity (MIC and MBC) of *M. alba* leaf extract was tested against *S. mutans.* The *M. alba* leaves extract was found to exhibit antibacterial activity against *S. mutans.* with MBC 50mg/mL, and MIC was 25mg/mL. Our finding indicated that the *M. alba* leaves extracts exhibited antimicrobial activity and in agreement with previous study that also showed the similar extract inhibited the growth of pathogenic bacteria [17] and fungal [21]. Sharma et al, (2016) also

reported the antimicrobial effectiveness of *M. alba* leaves extract against *S.mutans* with MIC of 2.5mg/10mL [12].

Another study was conducted where it provided evidence that the *M. alba* leaves extracts also showed an anticariogenic potential based on bacteriostatic effects and biofilm of *S. mutans* [16]. However their study was different from our study because the extract was incubated with the bacteria for 24 hours. In our study the the base of wells (represent tooth) was exposed to the extract for 5 minutes to mimic the use of oral rinse followed by addition of bacterial culture.

A number of previous studies reported that *M. alba* contains beneficial phytocompound such as chalcomoracin, moracin C, 1-deoxynojirimycin (DNJ), morin, kuwanon G and oxyresveratrol [14, 16, 22-24]. Inhibition of biofilm formation can be explained by the presence of 1-deoxynojirimycin (DNJ) in *M. alba*, which is previously reported by Islam et al, 2008 that DNJ could be a promising compound for targeting biofilms of *S.mutans* [16].Another study also shows the efficacy of morin isolated from *M. alba* as it depicts antibacterial activities by using paper disc diffusion method. It was found to be useful for managing populations of oral bacteria although growth inhibiting for morin was slightly lower than that of chlorhexidine [23].

Our findings are in agreement with other published works which shows that *M. alba* can inhibit and eradicate biofilm of *S. mutans*. This study highlight that *M. alba* demonstrate antibiofilm properties which might be an adjunctive for another antibiofilm therapy.

5. Conclusion

As conclusion, the result suggests that *M. alba* leaves extract represents an untapped source of the local plant with antimicrobial and antibiofilm activity against *S.mutans* that could be a resource in the development of therapeutic natural products in managing dental caries.

6. References

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