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Original Article

Eradication of Biofilm Formation by *Crocus sativus* Alcoholic Extract in *Streptococcus mutans* Clinical Isolates

Mahsa Jalili[1], Somayeh Sharifi[1], Khadijeh Abdal[2] and Fahimeh Daneshyar[3*]

[1]*Department of Microbiology, Faculty of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran*
[2]*Department of Oral and Maxillofacial Pathology, Faculty of Dentistry, Ilam University of Medical Sciences, Ilam, Iran*
[3]*Department of Pediatric Dentistry, School of Dentistry, Hamadan University of Medical Sciences, Hamadan, Iran*

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Abstract

Despite major advances in oral health in the past decades, tooth decay is one of the most common preventable diseases in the worldwide. Nowadays, *Streptococcus mutans* is discussed as one of the most important challenges in tooth decay. Also, medicinal herbs can be considered as an effective weapon against infectious diseases. The purpose of the current study was to investigate the biofilm formation in *S. mutans* clinical isolates and to evaluate the anti-biofilm properties of *Crocus sativus* against *S. mutans* clinical isolates. In this study, thirty dental plaque samples were collected. Then, identification of samples was performed by standard methods. Biofilm formation was evaluated on *S. mutans* clinical isolates. *C. sativus* alcoholic extract was applied as an anti-biofilm formation in this isolates. Our results demonstrated that a significant number of *S. mutans* samples were identified as biofilm producers. Then, *C. sativus* in 60 μg/ml, 30 μg/ml and 8 μg/ml were able to eradicated strong, moderate and week biofilm formation in this isolates, respectively. In addition, more extensive studies and *in vivo* research are needed to confirm the results of this study.

Keywords: Tooth Decay, *Streptococcus mutans*, Biofilm formation, *Crocus sativus*

Introduction

Despite major advances in oral health in the past decades, tooth decay is one of the most common preventable diseases in the worldwide [1]. Tooth decay is very common in developing countries. There are many factors involved in tooth decay such as nutrition, poor oral hygiene and the accumulation of bacteria causing dental plaques [2]. Also, among the microorganisms in the oral cavity, lactic acid producing bacteria such as *Lactobacillus*, *Bifidobacter* and virulent *Streptococcus* are of particular importance. So, *viridans streptococcus* group is more prone to oral flora and tooth decay. One of the important virulent is a *Streptococcus mutans* [3].

S. mutans was isolated from dental caries in 1924 [3]. *S. mutans* is recognized as a major cause of tooth decay in the mid-1960s [4]. *S. mutans* is capable of producing large amounts of organic acids (acidogenicity). *S. mutans* is ability to survive in acidic pH and the ability to synthesize external glucan from sucrose [5].

S. mutans plays an important role in establishment and accumulation of biofilm on the tooth surface. *S. mutans* biofilm plays key role in dental plaque formation and caries [6]. In addition, most infections caused by biofilm formation in bacteria are resistant to treatment. According to previous reports, bacterial resistance in biofilm formation state is 1000 times higher than planktonic state [7]. *S. mutans* is important role in dental caries, lip fissure, parotid gland inflammation and *etc*.

In the other hand, antibiotics, antimicrobial agents, and antimicrobial mouthwashes have some side effects besides being useful [8,9].

New drugs discovery can be a good choice for eradication of biofilm formation in bacteria [10]. Also, many studies have shown that different types of medical plants can be considered as an effective weapon against infectious diseases [11].

In addition, various reports mentioned *Crocus sativus* is

*Corresponding author: Department of Pediatric Dentistry, School of Dentistry, Hamadan University of Medical Sciences, Hamadan, Iran
Email Address: mahsajalili1993@gmail.com

an antibacterial and anti-biofilm properties [12,13].
Due to these reasons, in this study, biofilm formation in *S. mutans* was investigated and anti-biofilm properties of *C. sativus* were evaluated on *S. mutans* clinical isolates.

Method and Materials

Bacterial Collection and Identification

A total of thirty samples of dental swabs were prepared from the teeth surface of the patients (with patient satisfaction). In this study, patients were referred to the medical centers of Hamedan University of Medical Sciences. Then, isolation of samples with standard methods was performed.

Cell Culture and Toxicity Assay

The *C. sativus* ethanol extracts were applied to determine their cytotoxicity effect on a Vero cell line. MTT assay was performed and the absorbance of the transformed dye was measured at a 600 nm wavelength. MTT assay was done by MTT assay kit (Sigma, United States).

Biofilm Formation Assay and Analysis of biofilm formation

Briefly, 0.5 McFarland solutions of *S. mutans* was prepared using Muller Hinton-broth medium. Then, 200 µL of suspensions of *S. mutans* clinical isolate with broth media (BHI broth supplemented with Sucrose 2%) were inoculated in 96 well polystyrene plates and incubated at 37 °C for 48 hours and evaluation of biofilm formation. Broth media were used for negative control. Hence, all of the wells were stained with 200 µl of crystal violet. Also, Optical densitometry was measured at 630 nm by immunoassay reader. Finally, isolates were divided into three categories according to biofilm formation. These groups including biofilms with 75 percentage of the biomass of the positive control, moderately adherent biofilms with 25-75 percentage biomass or weak biofilms with 25 percentage of the biomass of the positive control.

Semi-quantification of Biofilm Biomass

In this study, we used the methodology defined by Mowat *et al* [14].

Anti-biofilm Properties Determination of *C. sativus*

The bacterial suspension was inoculated in 96 microplates. Different concentrations of *C. sativus* (1-100 µg/ml) were performed. Finally, biofilm formation assay was applied.

Statistical Analysis

In this study, statistical analysis was performed by using SPSS (version 24). Also, significant differences were evaluated with using analysis software between the control group and the groups exposed to different concentrations of the *C. sativus* extract. So, IC50 of *C.sativus* extract against Vero cell line was obtained by prism 6. In addition, all experiments were performed in triplicate.

Result

Dental Swabs Sampling

The results of this study showed, the number of *S. mutans* identified in this research was 66.63% in males and 33.37% in females. The mean patient age was mentioned in Table 1.

Table 1 Results the mean age of patients

Frequency percentage	Age average
16.66	7-10
16.66	11-20
23.33	21-30
26.66	31-40
16.69	41-50

Biofilm Formation in *S. mutans*

Initially, the bacteria were confirmed by phenotypic methods. Furthermore, we discovered biofilm formations as a significant factor in *S. mutans* clinical isolates; then, we discovered several clinical isolates with a strong biofilm structure (n=8). In high number of *S. mutans* clinical isolates, moderate biofilm formation was also significant (n=12).

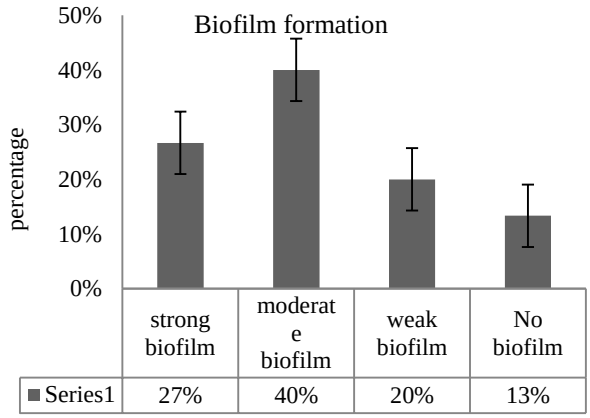

Fig. 1 The biofilm formation in *Streptococcus mutans* Clinical isolates.

Nevertheless, there was also *S. mutans* isolate with a weak biofilm formation (n=6). In addition, strains with no biofilm were very low and negligible (n=4). These results were summarized in Fig 1.

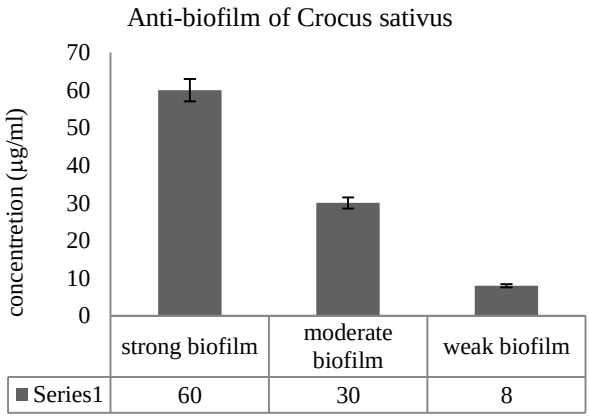

Fig. 2 Anti-biofilm properties of *Crocus sativus* in *S. mutans* clinical isolates.

Cytotoxicity assays were performed on ethanolic seed extract of *C. sativus* and the result was demonstrated IC50 of *C. sativus* is 100 µg / ml.

Different concentration (1-100 µg/ml) of *C.sativus* was performed for strain with ability of biofilm formation. Finally, *C. sativus* in 60 µg/ml, 30 µg/ml and 8 µg/ml were able to eradicate strong, moderate and week biofilm formation in *C. sativus*, respectively (Fig 2).

Discussion and Conclusion

Nowadays, Dental caries is a multifactorial infectious disease in developing countries. The primary cause of dental caries is dental plaque which is a complex biofilm [15]. Biofilm formation in bacteria can create serious challenges in the fight against infectious diseases. Also, the biofilm formation can harmful to human health [16]. In fact, one of the main mechanisms of survival of bacteria in different environments is the ability to biofilms formation [17]. In addition, bacteria capable of producing biofilms can escape the host immune system and thus cause chronic infections [18]. One of the contributing factors to chronic infections is the formation of biofilm structure in bacteria [19]. *S. mutans* is the most important etiologic agents in the development of biofilm formation for survival and persistence in dental plaque [20]. Medicinal plants extracts have a long history of use for treat various diseases [21]. Though, valid scientific research confirmed the properties of *C. sativus*. In addition, the essential oil of this plant has antibacterial effects [22]. In this study, our data demonstrated that biofilm formations as a significant factor in *S. mutans* clinical isolates. Furthermore, our results declared that medicinal plants can be used as a suitable candidate for the treatment of biofilm formation caused by *S. mutans*. However, it seems that studies in vivo and broader studies in this context were necessary.

Conflict of Interest

The author has no conflicts of interest.

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