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RESEARCH ARTICLE

Evaluation of Biofilm Formation and Anti-biofilm Properties of *Peganum Harmala* and *Crocus Sativus* in *Shigella Flexneri* Clinical Isolates

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Abstract:

Background:

Biofilm formation causes many serious problems in the treatment of bacterial infections. In addition, chronic infections due to biofilm formation can pose a huge burden to the health care systems. Also, many bacteria are biofilm producers as an important strategy for pathogenicity. Furthermore, the traditional use of herbal medicines such as *Peganum harmala* and *Crocus sativus* in Iran is interesting.

Objective:

The purpose of the current study was to investigate the biofilm formation in *Shigella flexneri* clinical isolates and to evaluate the anti-biofilm properties of *P. harmala* and *C. sativus* on *Shigella flexneri* clinical isolates.

Methods:

For the study purpose, Thirty *S.flexneri* clinical isolates were collected from Ahvaz, Iran. Then, the collected bacteria were subjected to biofilm formation assay. Afterward, *P. harmala* and *C. sativus* were applied as an anti-biofilm formation in *S. flexneri*.

Results & Conclusion:

Our results demonstrated that a significant number of samples were identified as strong biofilm producers. Then, *P. harmala* and *C . sativus* in a concentration of 30µg/ml and 60µg/ml were able to eradicate a strong biofilm formation in *S. flexneri,* respectively. In addition, it seems that more extensive studies and *in vivo* research should be done to confirm their properties.

Keywords: Biofilm formation, *Shigella flexneri*, *Peganum harmala*, Crocus sativus, *S. flexneri*, *P. harmala*.

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1. INTRODUCTION

Despite, there are many virulence factors in bacteria; the role of biofilm is notable [1]. The biofilm structure has many complications on human health and environment [2].In addition , numerous chronic infectious diseases are caused by biofilm formation in bacteria [3]. Many bacteria are biofilm producers, among them some true pathogens are more considerable for their biofilm formation [4,5].

Though, biofilm formation was investigated in different species of bacteria, including *Pseudomonas aeruginosa, Enterobacteriaceae etc* [6], *Shigella* species is considered in some countries to have the ability to produce biofilm [7].

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However, *S. flexneri* may also be one of the biofilm producers other than *Shigella* species. Many studies have investigated the intracellular survival and virulence factor in *S. flexneri*; but there is a big gap in the pathogenesis and intracellular survival of this bacterium [8].

Certainly, biofilm formation is a mechanism in bacteria, which can promote bacterial resistance [9]. Furthermore, the use of antibiotic treatments against the biofilm structure in bacteria is one of the main challenges in medical science [10]. New drug discovery can be a good choice for the eradication of biofilm formation. Also, many studies have shown that different types of medicinal plants can be considered as an effective weapon against infectious diseases [11]. In the meantime, *P. harmala* is originally a native Asian plant. This plant belongs to the Nitrariaceae family. *P. harmala* is traditionally

used to treat many infectious diseases [12]. Besides that, *Crocus sativus* is a native plant of Iran and used in traditional medicine in this country. The essential oil of this plant has antibacterial effects [13]. Due to these reasons, in this study, biofilm formation by *S. flexneri* isolates was investigated and the anti-biofilm properties of *P.harmala* and *C.sativus* on *S. flexenarii* clinical isolates were evaluated.

2. METHODS

2.1. Bacterial Collection and Identification

A total of thirty *S. flexneri* clinical isolates were prepared at the Microbiology Research Center, Ilam University of Medical Sciences. Ilam, Iran. Then, *S. flexneri* were obtained from Ahvaz, Iran, by a a standard method [14,15].

2.2. Cell Culture

The *P. harmala* and *C. sativus* ethanolic extracts were applied to determine their cytotoxic effect on a vero cell line. Then, the MTT assay was performed by the MTT assay kit (Sigma, United States).

2.3. Toxicity Assay

The cells were inoculated in 96-well microplates and cellular density was determined. Then, the cells encountered different concentrations of the *P. harmala* and *C. sativus* extracts. The MTT assay was performed and the absorbance of the transformed dye was measured at a 600nm wavelength.

2.4. Biofilm Formation Assay

Initially, 0.5 McFarland solution of *S. flexneri* was prepared. Then, we inoculated 200 uL of broth media (LB broth) with a 0.5 McFarland solution of *S. flexneri* in 96 microplates for the evaluation of biofilm formation. Henceforth, the culture incubated for 24 hours at 35°C, so, the experiment was performed in triplicate. LB broth without *S. flexneri* was a negative control.

2.5. Semi-quantification of Biofilm Biomass

In this study, we used the methodology defined by Mowat *et al*. [16].

2.6. Analysis of Biofilm Formation

The ability of biofilm formation in all *S. flexneri* isolates was measured by absorbance in the crystal violet stain. In addition, the capacity of all of the strains to form a biofilm was compared with biofilm-forming *S. flexneri* controls. Furthermore, we measured biofilm formation for each sample by analyzing the absorbance of the crystal violet. In this process, each isolate can create a biofilm mass in 24 hours which is eventually compared with the control. Finally, the isolates were divided into three categories based on biofilm formation. These groups included biofilms with 75% of the biomass of the positive control, moderately adherent biofilms with 25-75% biomass or weak biofilms with 25% of the biomass of the positive control.

2.7. Determination of Anti-biofilm Properties of *P. Harmala* and *C. Sativus*

The bacterial suspension was inoculated in 96 microplates. Different concentrations of *P. harmala* (1-35µg/ml) and *C. sativus* (1-100µg/ml) were applied. Then, the biofilm formation assay was performed.

3. RESULTS

3.1. Biofilm Formation by *S. flexneri*

Initially, the bacteria were confirmed by phenotypic methods. Furthermore, we discovered biofilm formations as a significant factor in *S. flexneri* clinical isolates; while the largest number of clinical isolates with a strong biofilm structure (n=11). In some *S. flexneri* clinical isolates, a moderate biofilm formation was also significant (n=10). Nevertheless, *S. flexneri* isolate was also observed with a weak biofilm formation (n=8). In addition, strains with no biofilm were very low and negligible (n=1). These results are summarized in Fig (**1**).

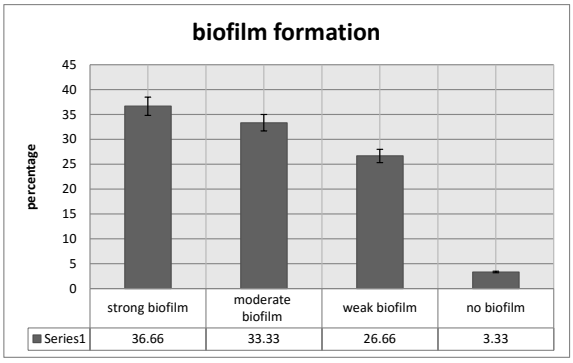

Fig. (1). The biofilm formation in *S. flexneri* clinical isolates.

3.2. *P. harmala* as An Anti-biofilm Formation in *S. flexneri*

The IC_{50} of *P. harmala* was 35 µg/ml. In this study, eleven isolates (36.66 percentages of samples) were observed to be able to produce a strong biofilm. Different concentrations of *P. harmala* was tested for all of them. *P. harmala* in a concentration of 30µg/ml could eradicate the biofilm formation (Fig. **2**).

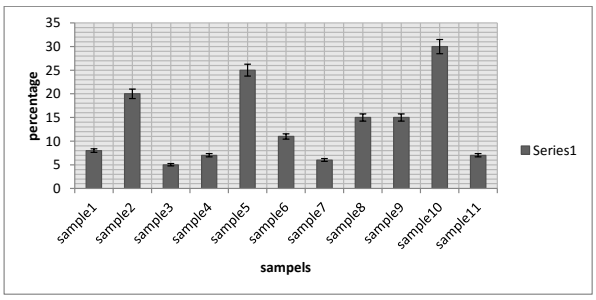

Fig. (2). Anti-biofilm Properties of *P. harmala* for eleven isolates. The highest concentrations were reported as final concentrations.

3.3. *C. sativus* as An Anti-biofilm Formation in *S. flexneri*

The IC_{50} of *C. sativus* was 100 µg/ml. Different concentrations of *C. sativus* were tested for eleven isolates (36.66 percentages of samples) with a strong biofilm formation. *C. sativus* in a concentration of 60µg/ml easily eradicated the biofilm of *S. flexenary* (Fig. **3**).

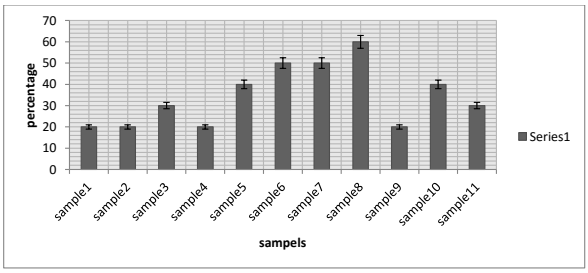

Fig. (3). Anti-biofilm Properties of *C.sativus* for eleven isolates. The highest concentrations were reported as final concentrations.

4. DISCUSSION

The biofilm formation causes many serious problems in the development of effective therapies for the treatment of infectious diseases [17]. However, the inherent ability of biofilm production in some bacteria has created many challenges in medical science [18]. Also, biofilm formation can cause widespread complications in the treatment of diseases and in the maintainence of human health [19].

In addition, biofilm formation is a community of microorganisms, which results in many infections and diseases causing problems at biological and environmental level [20].

CONCLUSION

In fact, one of the main mechanisms of bacterial survival in different environments is the ability to produce biofilms [21]. Moreover, bacteria that have the capacity to create biofilms can escape the host immune system, and therefore, cause chronic infections [22]. Meanwhile, *S. flexneri* employs several strategies to escape the immune system; one of the most important strategies is the ability to produce a biofilm [23]. In some studies, the effective factor in biofilm formation by *S. flexneri* was investigated [24]. Also, in several studies, *S. flexneri* infection was investigated but there remained a huge and significant gap in our knowledge for how to make *S. flexneri* capable of surviving in stress conditions [25]. Our data demonstrated that biofilm formation is a significant factor in *S. flexneri* clinical isolates. Furthermore, our results declare that medicinal plants can be used as a suitable candidate for the treatment of biofilm formation caused by *S. flexneri*. However, it seems that *in vivo* studies and more extensive studies in the field are necessary.

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

The current research approved by Ethical committee of Ilam University of Medical Sciences, Iran.

HUMAN AND ANIMAL RIGHTS

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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