Biological Journal of Microorganism
4th Year, Vol. 4, No. 16, Winter 2016
Received: December 29, 2014/ **Accepted:** February 18, 2015. **Page:** 109- 120

Antibacterial activity of Espand (*Peganum harmala*) alcoholic extracts against six pathogenic bacteria in planktonic and biofilm forms

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Abstract

Introduction: Microbial biofilms have attracted interest in recent years because they have become the most important cause of nosocomial infections. This study was aimed to examine the antibacterial activities of *Peganum harmala* extracts on the development of microbial biofilms and planktonic form of six pathogenic bacteria which include *Staphylococcus aureus*, *Bacillus cereus*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae*.

Materials and methods: Antimicrobial activities of the crude extracts against the planktonic form of bacteria were evaluated by using disc diffusion method, minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) values were determined by a macrobroth dilution technique. Anti- biofilm effects of the extracts were assessed by microtiter plate method.

Results: According to the results, *P. harmala* extracts could inhibit test bacteria in planktonic form. To inhibit biofilm formation, biofilm metabolic activity and eradication of established biofilms, efficiency of the extracts depended on concentration. The highest inhibitory effects of *P. harmala* extracts were observed on biofilm formation of *S. aureus* (90.28%) also, the greatest demolish were observed on *S. pneumonia* biofilm (77.76%). These extracts cause dramatically decrease the metabolic activity of bacteria in biofilm structures, in this case the decrement of *B. cereus* were highest (69.98%) compared to other tested bacteria.

Discussion and conclusion: Therefore, it can be suggested that *P.harmala* extracts applied as antimicrobial agents against testing bacteria particularly in biofilm forms.

Key words: Antimicrobial effect, Biofilm, *Peganum harmala*, Pathogenic bacteria

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Introduction

Biofilms are complex structures consisting of surface- attached bacteria surrounded by a self- produced extracellular polymer matrix and are currently recognized as the predominant bacterial life- style (1 & 2). Typically, anywhere there is a flow of water, organisms and a solid surface, a biofilm could be formed. It is well established that bacteria contained within biofilms exhibit increased resistance to antimicrobial treatments compared to individual cells grown in suspension (3). Several mechanisms can account for the increased antibiotic resistance in biofilms, including the physical barrier formed by exopolymeric substances (4), a proportion of dormant bacteria that are inert toward antibiotics (5), and resistance genes that are uniquely expressed in biofilms (6). The ability of biofilm- embedded bacteria to resist clearance by antimicrobial agents points to the importance of a continuous search for novel agents. These agents should be effective against bacteria orwork in synergy with the currently available antimicrobial agents. Novel strategies are therefore required to deal with these biofilm- mediated infections. Currently; a renewed interest in natural substances has focused attention on plants rich in bioactive compounds well known for their antimicrobial properties. Plants produce an enormous array of secondary metabolites and it is commonly accepted that a significant part of this chemical diversity serves to protect plants against microbial pathogens (7).

Peganum is a small genus belonging to the family Zygophylaceae and mainly distributed in the Mediterranean region, also found in central Asia, North Africa and has also been distributed in America and Australia (8 and 9). From ancient times, *Peganum harmala* was claimed to be an important medicinal plant and it was used in global folk medicine (10). This plant has been considered for the treatment of a variety of human ailments, such as lumbago, asthma, colic, jaundice and as a stimulant emmenagogue (11). The smoke of its seeds is traditionally used as a disinfectant. Its seed extracts are known to contain *β*- carbolinalkaloids, anthroquinons and a small quantity of flavonoid glycosides (10 & 12). There are reports that alkaloids in *P. harmala* seed are mainly responsible for different pharmacological activities including antibacterial, antifungal, antiprotozoaland antiviral effects (13 & 14), in addition to vasorelaxant (15), anti-spasmodicandanti- histaminic effect (16), spontaneous effect (17), anti-hemosporidian effect (18), anticancer and anti- nociceptive effect (19), antitumor effect (20) or antineoplastic effects (21).

In some study, antibacterial activity of *P. harmala* was confirmed. According to these researches, *P. harmala* extracts can inhibit planktonic form of many bacteria such as *Streptococcus pyogenes*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Escherichia coli* (22), *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Klebsiella pneumoniae* (23). The object of this study was to examine the antibacterial activities of alcoholic extracts

of *P. harmala* against the biofilm of six pathogenic bacteria and their free- living forms. These bacteria included *S. aureus*, *Bacillus cereus*, *Streptococcus pneumoniae*, *P. aeruginosa*, *E. coli* and *K. pneumoniae*. However, a study with focusing on anti- biofilm activity of *P. harmala* has not yet been documented.

Materials and methods

Plant Collections, Identifications and extraction: Fresh plant P. harmala was collected in spring season locally in April, 2012 from Kerman, Iran. The taxonomical identification of the plant was confirmed by Dr. Mirtajadini at the department of biological sciences, Shahid Bahonar university of Kerman, Kerman, Iran.

Plants were extracted as described by El-Mahmood (24) with some modifications. Freshly collected seed and stem of *P. harmala* were rinsed with running water, shade- dried and then powdered using an electrical blender (Bosch Limited, Germany). For successive extraction, ten gram (10 g) of plant powder was macerated in 100 ml of ethanol 80% and methanol 96% (Pars chemic co., Kerman, Iran). Different extracts were prepared using the maceration process for 30 hat 38°C under constant shaking and filtered with What man No. 1 filter paper. The extracts were evaporated to dryness under reduced pressure using a rotary evaporator and then were incubated at 37°C to complete evaporation solvent. One hundred milligram (100 mg) of each methanolic and ethanolic extracts was carefully taken in a standard measuring flask and the appropriate volume of DMSO 1% were added to dissolve the extracts. Then each extract was made up to 1 ml by adding sterile NB medium and the solution was then filter sterilized with 0.22 µm mixed cellulose ester membranes (Millipore^{TM}; MA, USA). The extracts obtained were kept in sterile dark bottle at 4°C for furtheruse.

Test microorganisms and media: Microorganisms used in this study included six bacteria species, three Gram- positive (S.aureus, B.cereus and S. pneumoniae) and three Gram- negative (P. aeruginosa, E. coli and K.pneumoniae). The tested microbial species were clinical isolates provided by the Faculty of Medicine, Department of Microbiology, Kerman Medical University, Kerman, Iran.

The test microorganisms were maintained in NB/glycerol (20%) at - 80°C. Nutrient agar (NA, Merck, Germany) containing Luria- Bertani (LB, Merck, Germany) was used to activate *S.pneumoniae* while nutrient agar was used for other bacteria. The Mueller Hinton Agar (MHA, Merck, Germany) medium was used for disc diffusion assay and Nutrient broth (NB, Merck, Germany) was used for the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) determination. The Mueller Hinton agar was also used for the determination of the MBC on the species. The tryptic soy broth (TSB, Merck, Germany) medium was used for anti-biofilm assay. For *S. pneumoniae*in all assessments, medium was enriched by increasing LB.

Disc Diffusion Assay: The antimicrobial susceptibility of the tested bacteria to P. harmala alcoholic extracts was performed as recommended by the Bauer- Kirby disc diffusion method (25). Five hundred microliters (500µl) of 18 h culture of tested bacteria adjusted to 10^8CFU/ml was poured and uniformly spread on MHA.

In the following, the sterile 6 mm blank paper disks (Padtan Teb Inc., Tehran, Iran), saturated with filter sterilized plant extract at the prepared concentration (100 mg/ ml) for about 2 h and allowed to dry at 37°C for 5 h. Ciprofloxacin (2 mg/ ml) was used as a positive control; 96% of methanol and 80% of ethanol were used as a reagent control. Each of the discs was placed on the inoculated plates and they were incubated at 37°C for 18h. The diameters of the inhibition zones were measured in millimeters (26).

MIC and MBC assays: MICs and MBCs were determined by a Macrobroth dilution method as recommended by the Clinical and Laboratory Standards Institute (27) using NB as the test medium. Overnight cultures of bacteria were diluted to yield a final concentration of 5×10^5 CFU/ml. Different concentrations of extract were prepared from serial two- fold dilutions method by dissolving extract stock concentration (100 mg/ ml) in sterile culture medium (NB). 1 ml of each standard bacterial suspension and different concentrations of extract (0.05 - 100 mg/ ml) were added to tubes containing 1 ml NB. These tubes were incubated at 37°C for 18 h. The first tube in the above series without sign of visible growth was considered as the MIC. Controls included NB plus extracts, NB plus Ciprofloxacin (2 mg/ ml) plus cells, cells plus NB plus sterile distilled water and NB plus solvents.

MBC was determined by culturing one standard loop of the tubes with no apparent growth on MHA plates and subsequent incubation at 37°C for 24 h. The least concentration that inhibited colony formation on agar was considered as MBC for the extract.

Inhibitory assay for biofilm formation: Biofilm formation was measured as described by O'Toole and Kolter (28) with some modifications. Three concentrations of extract were prepared from serial two-fold dilutions method by dissolving extract stock concentration (100 mg/ ml) in sterile culture medium (TSB). 100 µl of each concentration (12.5- 50 mg/ ml) were added into the wells of sterile 96- well polystyrene microtiter plate. The standard bacterial culture for biofilm assessments was prepared from the cells, were cultured at 37°C overnight and re- suspended at an optical density at 600 nm (OD600) of 0.2 in 1ml of TSB. Then 100µl of these suspensions were added to each well and incubated at 37°C for 24 h. Controls included TSB plus extracts (extract control), TSB plus Ciprofloxacin (2 mg/ ml) plus cells (positive control), cells plus TSB plus sterile distilled water (Negative control) and TSB (media control).

The formation of biofilm was quantified by measuring the absorbance of CV-stained biofilm at 630 nm and by total viable counts (29). After 24 h of exposure, media were discarded and each well was

washed three times with phosphate buffer saline (PBS) to remove non- adherent cells. Adherent microorganisms were fixed by adding 150 µl of methanol 96% for 15 min. The contents of the wells were then aspirated and 200 µl of crystal violet 1% (Gram color- staining set for microscopy – Merck, Germany) were added to wells to stain for 20 min. Excess stain was rinsed off with running tap water and the plates were air- dried at room temperature. To measure the absorbance of adherent cells, the CV was re- solubilized with 160 µl of acetic acid glacial 33% (Merck, Germany) and the absorbance was measured with a microtiter plate reader (ELX- 800, Biotec, India) at 630 nm. The percentages of biofilm inhibition in the presence of different concentrations of extracts were calculated employing the ratio between the values of OD_{630nm} with and without the extracts.

Eradication of established biofilms: Initially, 100µl of the standard bacterial culture (OD 600=0.2) was transferred into sterile 96- well polystyrene microtitre plates and incubated at 37°C for 24 h. After removing the supernatant, the plates were then washed with sterile distilled PBS. Thereafter, three different concentrations (12.5- 50 mg/ ml) of herbal extracts were added to the established biofilm followed by incubation for 24 h at 37 ºC (30).The control wells were the same as those described in inhibition of biofilm formation assays. Further Eradication of a preformed biofilm was quantified by crystal violet staining and reading the absorbance at 630 nm. The percentages of reduction biofilm structures in the presence of different concentrations of extracts were calculated employing the formula as described earlier.

Effect of extracts on biofilm metabolic activity: The effect of the P. harmala on the metabolic activity of pre- formed biofilm was measured as reported by Ramage and Lopez- Ribot (31). At first established biofilms were washed twice with PBS, then extracts (12.5- 50 mg/ ml) were added to each well and plates were incubated for 24 h at 37◦C. Afterwards, 50 µl of a Triphenyl Tetrazolium Chloride (TTC, Merck, Germany) solution was added and it was further incubated in the dark at 37◦C for 3 h. The colorimetric change was measured at 490 nm using a microtiter plate reader (ELX- 800, Biotec, India). The control wells were the same as those described in inhibition of biofilm formation assays. The percentages of reduction of biofilm metabolic activity in the presence of different concentrations of extracts were calculated employing the formula as described earlier.

Statistical analysis: Statistical analysis was carried out using SPSS Version 18.0 for Windows. Analysis of variance (ANOVA) was used to evaluate any significant differences between the control and treated groups observed in the presence of different concentrations of the extracts. *P value*< 0.01 denoted a statistically significant difference (SPSS, version 18, USA). All tests were performed in triplicate.

$$\% \text{ inhibition} = \frac{(\text{OD negative control} - \text{OD media control}) - (\text{OD test} - \text{OD extract control})}{(\text{OD negative control} - \text{OD media control})} \times 100$$

Results

Inhibitory efficiency of P. harmala extract against planktonic form of pathogenic bacteria: The zone of inhibition (ZOI) for methanolic and ethanolic extracts of *P. harmala*were shown in table (1), also the MIC and MBC values of these extracts were illustrated in table (2). In disc diffusion analysis, the extracts of *P. harmala* have the best inhibitory effect on *S. aureus, B. cereus* and *E. coli.* In this test ethanolic extracts were better growth inhibitor. Although these extracts didn't have any inhibitory effect on other tested clinical bacterial pathogens.

The results of *P. harmala* extracts in broth medium, show that these extracts have sufficient inhibitory effect (below the concentration used in disc 0.19- 3.12 mg/ ml) on a tested bacteria. Then it was concluded that the inhibitory effect of these plant extracts in broth medium is more than solid medium.

Based on the disc diffusion results, the inhibitory effect of ethanolic extracts on planktonic forms is more than methanolic extracts, however for *B. cereus* and *P. aeruginosa* there wasn't any significant difference between inhibitory effects of methanolic or ethanolic extract (Table 2). The best inhibitory effect (ZOI) of *P. harmala* was shown against *B. cereus.*

The inhibitory effects of P. harmala extracts against biofilm structures: The inhibitory efficiency of each concentration of *P. harmala* extracts on preventing biofilm formation, demolished of biofilm structures and inhibition of metabolic activity of biofilm were shown in fig.s (1), (2) and (3). According to the F value of the ANOVA analysis on tested data, it was confirmed that inhibitory efficiency of *P. harmala* extracts were significant at 0.01 level (*P value*<0.01).

Table 1- Antibacterial activity of *P.harmala* alcoholic extracts against test microorganisms by using disc diffusion method (Zone of inhibition in mm).

Bacteria	Methanolic extract	Ethanolic extract	Ciprofloxacin	Solvent control
Staphylococcus aureus	12.3±1.1	15±0.8	23±0.6	-
Bacillus cereus	12±1.4	21.7±1.2	21±0.4	-
Streptococcus pneumonia	-	-	9.4±0.1	-
Pseudomonas aeruginosa	-	-	24.6±0.3	-
Escherichia coli	10.4±1.2	12.6±0.4	22.6±0.7	-
Klebsiella pneumoniae	-	-	12±0.9	-

Table 2- *P. harmala* minimal inhibitory concentrations (MICs) and minimal bactericidal concentrations (MBCs) of test bacteria.

Bacteria	MIC methanolic extract (mg/ ml)	MIC ethanolic extract (mg/ ml)	MBC methanolic extract (mg/ ml)	MBC ethanolic extract (mg/ ml)
Staphylococcus aureus	0.39	0.19	0.78	0.39
Bacillus cereus	1.56	1.56	3.12	3.12
Streptococcus pneumonia	3.12	0.78	12.5	3.12
Pseudomonas aeruginosa	0.78	0.78	1.56	1.56
Escherichia coli	1.56	0.39	6.25	0.78
Klebsiella pneumoniae	3.12	0.39	12.5	0.78

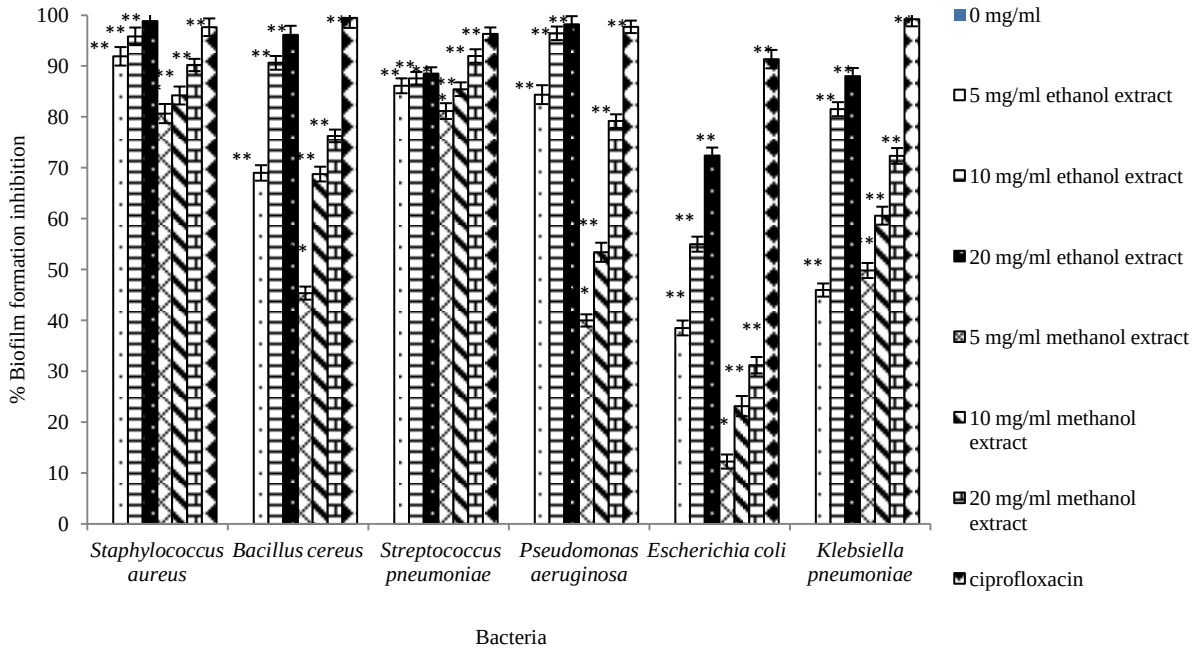

Fig. 1- Percent reduction of biofilm formation for test bacteria treated with different concentrations of *P.harmala*for 24 h. * Differences between control (no inhibition) and treatment with extracts (* p < 0.05, ** p <0.01).

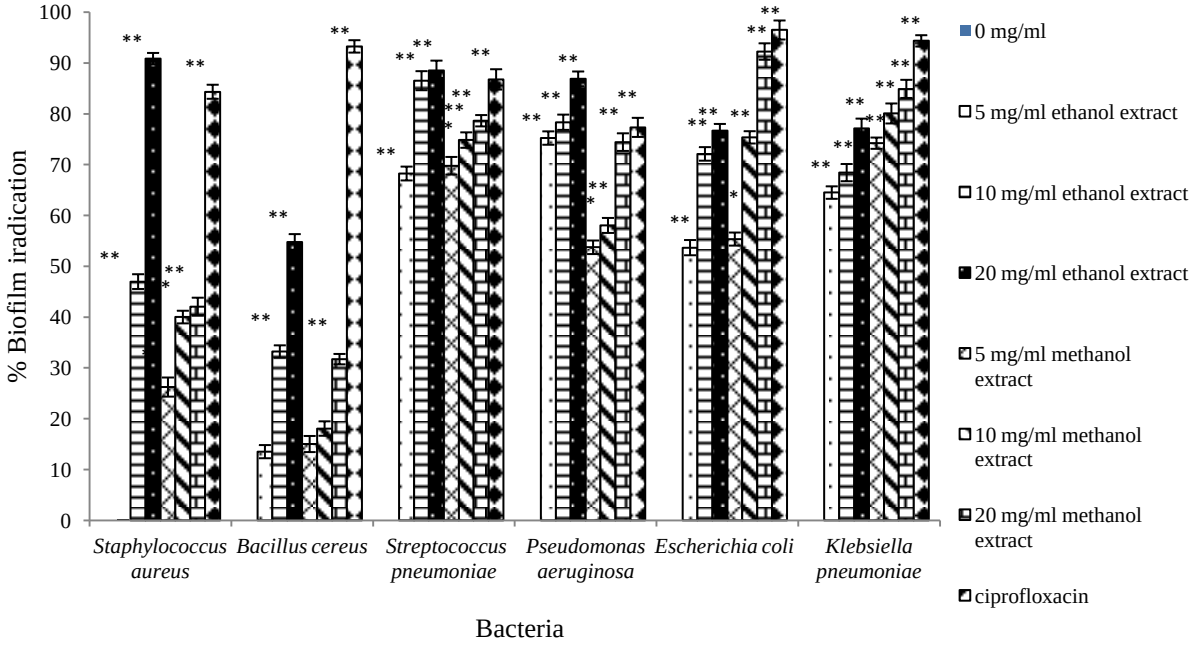

Fig. 2- Percent disruption of biofilm formation for test bacteria treated with different concentrations of *P.harmala* for 24 h. * Differences between control (no inhibition) and treatment with extracts (* p < 0.05, ** p <0.01).

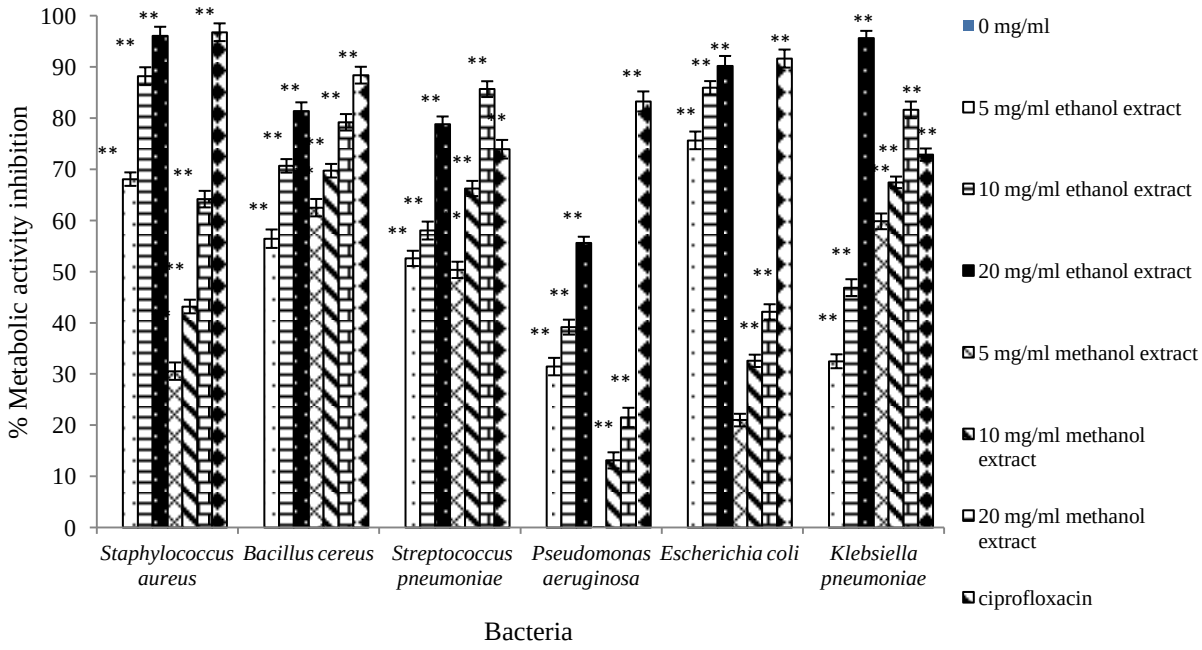

Fig 3- Percent reduction of biofilm metabolic activity for test bacteria treated with different concentrations of *P.harmala*for 24 h. * Differences between control (no inhibition) and treatment with extracts (* *P value*< 0.05, ** *P value* <0.01).

Based on the examination performed on biofilm structures, it was concluded that the type of bacteria and concentration of extracts were significant at 0.01 percent level (*P value*<0.01), but the type of solvent used in extraction process was only efficient on inhibition of metabolic activity of biofilm (*P value*= 0.017). Indeed, the inhibition of biofilm formation and demolished of biofilm structures in the treatment with extracts of *P. harmala* is independent from the type of solvent (*P value*= 0.09). The concentration of each extract has a direct relation with inhibitory effect that the inhibitory effect increases with increasing concentration.

The maximum inhibitory effects of *P. harmala* extracts on biofilm formation were observed on *S. aureus* (90.28%) and *P. aeruginosa* (86.80%) although these extracts had low efficiency to inhibit biofilm formation of *E. coli* (38.75%). For destruction of the biofilm structures, biofilm of *S.pneumoniae* were the most sensitive (77.76%) and biofilm of *B. cereus* (27.73%) were the most resistant structures among all tested clinical bacterial pathogens.

Metabolic activity of bacteria in biofilms treated with *P. harmala* extract had a remarkable decrease, the greatest reduction was observed in *B. cereus* (69.98%) and the lowest reduction was observed in *P. aeruginosa* (26.81%).

Discussion and conclusion

Despite increasing advances in medical science and the development of treatment techniques, infectious diseases are still considered as major cause of worldwide

deaths (32). Pathogenic microorganisms have different ways to deal with antimicrobial agents such as antibiotics and indiscriminate uses of these compounds have led to the development of drug resistance. Drug- resistant bacteria, is easily crossed antibiotic treatment and create many clinical problems. Even drug-sensitive bacteria that are able to form biofilms, when placed in this structure will respond differently to antibiotics (3 & 33).

Resistance of microbial biofilms to antimicrobial agents led to difficult removal of these microbial structures and cause major problems in controlling of pathogenic microorganisms and treatment of infectious disease. Biofilm producing bacteria cause more than 60% of nosocomial infections. In the recent years some researchers conducted in order to find new antimicrobial compounds against bacterial biofilms, in this case medicinal plants have a special interest (7).

The advantages use of medicinal plant for bacterial infection treatment instead of chemical antimicrobial agents includes: Many of the medicinal plants due to a combination of herbal ingredients with other elements, make balancing the biological toxicity and they have fewer side effects. Also easy access, reasonable price, and no bacterial resistant to herbs are other benefits of using medicinal plants (34).

In this research, antimicrobial effect of *P. harmala*, against selected bacterial pathogen was evaluated. These results show that the antibacterial effect of *P. harmala* extracts increased after concentrating on extracts by maceration method.

Disc diffusion analyses show that *P. harmala* extracts had high ability to inhibit the growth of *B. cereus* and *S. aureus.* However these extracts had low inhibition efficiency on *E. coli* and didn't have any inhibitory effect on other tested clinical bacterial pathogens. Although *P. harmala* extracts were inhibited, the growth of all tested bacteria in broth media was with low concentration that used in solid media. According to these results it can be concluded that antimicrobial compounds in the *P. harmala* extracts similar to other plant extracts have low diffusion in solid media than broth media, then for favorable impact on the solid media the concentrations were much higher than broth media needed.

The MIC values for *P. harmala* extracts on tested bacteria, were in 0.19 to 3.12 concentration range that these values confirmed the inhibitory ability of these plant extracts, also higher values of MBC than MIC indicated in the bacteriostatic properties of *P. harmala* extracts. The inhibitory effect of ethanolic extracts in MIC experiment was more than methanolic extract except in the case of *B. cereus* and *P. aeruginosa* that the inhibitory effects of two extracts were the same. The best inhibitory effect in MIC experiment was shown for ethanolic extract of *P. harmala* against *S. aureus* and low inhibition was found in methanolic extract against *S. pneumoniae* and *K. pneumoniae.*

Dealing with biofilm structures, the *P. harmala* extracts were efficient. The inhibitory effect of extracts has a direct

correlation to concentration and except on inhibition of metabolic activity of biofilm, efficacy of each extract was independent from type of solvent. The ability of *P. harmala* ethanolic extract in inhibition of biofilm formation was more than demolish of biofilm or preventing of metabolic activity of microbial cell in the biofilm structure. It should be noted that *P. harmala* extracts destroyed properly biofilm structures of *E. coli* bacterium and inhibited more than 50 percent of releasing cell, however the biofilm formation of this bacterium in treatment of these extracts inhibited very low. Also in *K. pneumoniae* any significant difference between various tests that performed on biofilm structures was not observed. Considering that the active components of *P. harmala* and the inhibitory mechanisms on biofilm structures was not investigated in this research it can be suggested by characterization of these compounds that it will be possible to interpret the difference inhibitory effect of these extracts between various tested clinical bacteria.

Some researchers confirmed antimicrobial properties of *P. harmala* against different microorganisms. These studies have been carried out mostly on planktonic forms, but any researches on anti- biofilm properties of this plant have not done yet. For example Minan and Al-Izzy (35) confirmed the inhibitory effects of aqueous and ethanolic extracts of *P. harmala* against *Candida albicans*. Their research indicated that the antimicrobial property of this plant had direct relation with concentration and ethanolic extract is more effective than aqueous extract. Mazandarani et al. (23) showed the inhibitory effect of different organs of *P. harmala* against *S. aureus*, *Staphylococcus epidermidis*, *E. coli*, *K. pneumonia* and *Micrococcus luteus*, also they concluded that these plant extracts didn't have any inhibitory effect on *P. aeruginosa* and *Salmonella typhi.* Their results show that the extracts from seeds and roots of *P. harmala* have more inhibitory effect than other organs. Our results in this study are in agreement with what the researcher mentioned; however some slight differences were observed.For example our study is the first report on anti- biofilm properties of *P. harmala* against pathogenic bacteria.

According to the results of this research and other studies that performed on different parts of *P. harmala* the antimicrobial potential of this plant was confirmed and the extractions of this plant are suitable choices against pathogenic microorganisms. Hence, further investigation is recommended in order to identify and purify active components of these extracts and also to identify the mechanism of action of the seed extracts on biofilm structures in order to achieve a good source of antimicrobial agent with pathogenic microorganisms.

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بررسی اثر ضدمیکروبی عصاره‌های الکلی گیاه اسپند (*Peganum harmala*) بر فرم منفرد و بیوفیلمی ٦ باکتری پاتوژن

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چکیده

مقدمه: در سال‌های اخیر توجه به بیوفیلم‌های میکروبی به علت نقش ویژه این ساختارها در بروز عفونت‌های بیمارستانی، چشمگیر بوده است. این پژوهش به منظور بررسی اثر ضدمیکروبی عصاره‌های گیاه اسپند بر فرم منفرد و ساختارهای بیوفیلمی ۶ باکتری پاتوژن انجام شد. باکتری‌های مورد بررسی شامل *استافیلوکوکوس اورئوس*، *باسیلوس سرئوس*، *استرپتوکوکوس پنومونیه*، *سودوموناس آئروژینوزا*، *اشرشیا کلی* و *کلبسیلا پنومونیه* بودند.

مواد و روش‌ها: قابلیت مهاری این عصاره‌ها بر فرم منفرد با روش انتشار دیسک تعیین و مقادیر MIC و MBC به روش ماکروبراث دایلوشن به دست آمد. اثر ضدبیوفیلمی عصاره‌ها نیز با روش میکروتیترپلیت بررسی شد.

نتایج: براساس نتایج به دست آمده قابلیت عصاره‌های گیاه اسپند در مهار فرم منفرد باکتری‌های مورد بررسی، تایید شد. این عصاره‌ها در مهار پدیده تشکیل بیوفیلم، تخریب ساختارهای بیوفیلمی و مهار فعالیت متابولیک در این ساختارها نیز موثر بوده و غلظت هر عصاره رابطه مستقیمی با اثربخشی آن داشت. در تیمار *استافیلوکوکوس اورئوس* بیش‌ترین مهار پدیده تشکیل بیوفیلم (۹۰/۲۸ درصد)، در تیمار *استرپتوکوکوس پنومونیه* بیش‌ترین تخریب ساختارهای بیوفیلمی (۷۷/۷۶ درصد) و در تیمار *باسیلوس سرئوس* بیش‌ترین مهار فعالیت متابولیک (۶۹/۹۸ درصد) مشاهده شد.

بحث و نتیجه‌گیری: بر اساس نتایج این پژوهش می‌توان عصاره‌های گیاه اسپند را به عنوان یک عامل ضدمیکروبی مؤثر در مقابله با باکتری‌های مورد مطالعه به ویژه در شکل بیوفیلمی پیشنهاد کرد.

واژه‌های کلیدی: اثر ضدمیکروبی، بیوفیلم، *Peganum harmala*، باکتری پاتوژن

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تاریخ دریافت: ۱۳۹۳/۱۰/۰۸- تاریخ پذیرش: ۱۳۹۳/۱۱/۲۹