Avicenna J Clin Microb Infec. 2018 February; 5(1):e61772. doi: 10.5812/ajcmi.61772.

Published online 2018 February 17. **Research Article**

Antibiofilm Activity of *Dracocephalum polychaetum* Extract on Methicillin-Resistant *Staphylococcus aureus*

Mohammad Mehdi Yaghoobi,[1,*] Mouj Khaleghi,[2] Hajar Rezanejad,[1] and Paria Parsia[2]

[1]Department of Biotechnology, Institute of Science and High Technology and Environmental Sciences, Graduate University of Advanced Technology, Kerman, IR Iran
[2]Department of Biology, Faculty of Science, Shahid Bahonar University of Kerman, Kerman, IR Iran

[*]*Corresponding author*: Mohammad Mehdi Yaghoobi, Department of Biotechnology, Institute of Science and High Technology and Environmental Sciences, Graduate University of Advanced Technology, End of Haft-bagh Highway, P.O. Box: 7631818356, Kerman, IR Iran. Tel: +98-3431623209, Fax: +98-3433776617, E-mail: m.yaghoobi@kgut.ac.ir

Received 2017 September 15; **Revised** 2018 January 08; **Accepted** 2018 January 14.

Abstract

Background: Antibiotic resistance among biofilm-producing pathogenic bacteria is a major health concern today. Plants, as a rich source of medicinal compounds, are interestingly explored for discovering new antibiotics.
Objectives: In this study, the effect of *Dracocephalum polychaetum* Bornm extract on the growth, biofilm formation, and expression of biofilm-related genes in methicillin-resistant *Staphylococcus aureus* (MRSA) was explored.
Methods: Antimicrobial activity of *D. polychaetum* aerial part extract in MRSA samples was evaluated by agar well diffusion method. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extract were determined according to the CLSI manual. Inhibition of biofilm formation was analyzed by microtitre plate method. Expression of *icaA*, *icaD*, *bap*, *sar*, and *agr* genes was studied by Reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) technique.
Results: The antimicrobial effect of the extract against 20 MRSA strains was appropriate. The MIC and MBC of the extract were in the range of 0.781 - 25 mg mL^{-1} and 1.56 - 50 mg mL^{-1}, respectively. Biofilm formation was inhibited in all of the isolated MRSA strains in sub-MIC concentrations. PCR results demonstrated that half of the samples had both *icaA* and *icaD* genes, about 1/3 had *icaA* gene, and 1/6 had merely *icaD* gene. RT-qPCR data showed that the expression of *sar*, *bap*, *icaD* and *icaA* genes was significantly reduced by the extract.
Conclusions: Collectively, the results demonstrated that *D. polychaetum* not only had anti-staphylococcus effects against MRSA but also suppressed biofilm formation both at phenotype and gene expression levels. Its effects are comparable to the effect of other *Dracocephalum sp.* Further in vivo studies can reveal the potential application of this plant against MRSA strains.

Keywords: Microbial Drug Resistance, Biofilm, Medicinal Plant, Gene Expression, *Staphylococcus aureus*

1. Background

Staphylococcus aureus is a major source of nosocomial infections and mortality. Overcoming methicillin-resistant *S. aureus* (MRSA) infections is an obstacle due to the resistance to beta-lactam antibiotics (1). The virulence of *S. aureus* strains is due to their adherence and invasion, which is associated with biofilm formation. Biofilm is a barrier to diffusion of some antibiotics (2).

Polysaccharide intercellular adhesin (PIA) encoded by *icaA*DBC operon and biofilm-associated protein (Bap) are major components of *S. aureus* PIA-dependent biofilm. The collaboration between *ica* operon genes leads to the expression of capsule polysaccharide phenotype. The expression of *ica* locus is tightly regulated and affected by genetic and environmental conditions. *SarA* is a positive regulator of the operon while icaR suppress it. Surface protein bap promotes cell adhesion and enables biofilm formation even in the absence of PIA. Bap is crucial for the attachment and biofilm accumulation. The accessory gene regulator (*agr*) system is involved in the switch from the synthesis of cell surface proteins during the exponential growth phase to the synthesis of toxins and degradative proteins in the stationary phase. The expression of *agr* can repress the capacity of *S. aureus* to form biofilm (3). The regulation and interaction between different factors involved in *S. aureus* biofilm is intricate and not fully identified. Recently, molecular techniques, such as PCR, are employed for verifying the presence and analyzing the expression of biofilm-related genes in *S. aureus* (4, 5).

Medicinal plants are widely being utilized for centuries in Iran. Nowadays, plant extracts are used to kill pathogens including *S. aureus* and their anti-bacterial effects on *S. aureus* have been demonstrated (6). *Dracocephalum polychaetum* Bornm (family Lamiaceae) is a native and exclusive species to Kerman province of Iran. This plant is used

in traditional medicine in Kerman as a food additive and for stomachache.

2. Objectives

There is no published study on the antimicrobial effects of *D. polychaetum*. Thus, we explored the antimicrobial and anti-biofilm effects of MIC and MBC of *D. polychaetum* extract on the growth and biofilm formation of MRSA.

3. Methods

3.1. Sampling and Extraction

The aerial parts of *D. polychaetum* were collected in spring from Laleh zaar area at N 29.49 E 56.81 (Kerman, Iran) and were confirmed by a herbarium specialist (voucher No 1059). The samples were dried in dark and 100 g were milled and extracted by maceration method. The dried extracts were stored at -20°C freezer.

3.2. Collection and Identification of Methicillin-Resistant S. aureus

S. aureus samples were collected from 30 abscesses and wounds from patients hospitalized in Shafa hospital of Kerman, Iran. The samples were cultured on Blood agar medium (Merck) at 37°C for 24 hours. Gram staining followed by biochemical tests for catalase, coagulase, oxidase, and mannitol fermentation was done for identification of *S. aureus*. Oxacillin disk-diffusion (one μg) assay was used for identification of resistant strains. The diameter of the corona of growth prevention was determined according to the clinical and laboratory standards institute (CLSI) standard (7). MIC of oxacillin was determined according to the Felten method. The samples were frozen and stored at -80°C freezer.

3.3. Evaluating Antimicrobial Property

3.3.1. Agar Well Diffusion Assay

The *S. aureus* strains were inoculated by spreading over Muller-Hinton agar plate surface, which contained a hole with a diameter of 6 mm. Then, 50 μL extract solutions (50 mg mL^{-1}) were introduced into the wells and plates were incubated at 37°C for 24 hours. Subsequently, the diameters of inhibition growth zones (mm) were measured. *S. aureus* ATCC 6538 (PTCC, IROST, Tehran, IRAN) was used as standard strain. Sulfamethoxazole/Trimethoprim as a positive control antibiotic and DMSO as a negative control were applied.

3.3.2. Assessing MIC and MBC of the Plant Extract by Macrodilution Method

Ten dilutions of the extract (0.05 - 50 mg mL^{-1}) were prepared by serial dilution, added to the microbial suspension containing 107 CFU/mL in Muller-Hinton broth medium, and incubated at 37°C for 24 hours. Then, the minimum concentration of the extract that inhibited bacterial growth was selected for the next steps. This concentration was added to the bacterial medium and incubated at 37°C for 24 hours. If no growth was observed, it was considered as MBC, and if any growth was observed, it was considered as MIC.

3.4. Assessing Biofilm Inhibition by the Extract by Microtitre Plate Method

Bacterial samples were cultured in Tryptic soy broth (TSB) medium until turbidity growth equal to 0.5 McFarland standard. In each well, a 1:1 ratio of the microbial suspension and extract (0.05 - 50 mg mL^{-1}) was added and incubated at 37°C for 24 hours without shaking. Subsequently, the wells were evacuated, the biofilm structures were fixed by ethanol, stained with 2% crystal violet, and the absorbance of the wells was measured at 492 nm using Biotek ELISA reader. The percentage of biofilm inhibition was calculated by the following formula:

$$\text{Inhibition percent} = \left[\frac{(C-B)-(T-B)}{(C-B)}\right] \times 100 \quad (1)$$

In this formula, C is the mean absorbance of control wells, B is the mean absorbance of blank wells, and T is the mean absorbance of treated wells. In the negative control well, just medium was added and in the positive control well, medium and bacteria (without extract) were added.

3.5. DNA Extraction and PCR

To verify the presence of *icaA* and *icaD* genes, DNA was extracted from 2 mL of 24 hours cultured *S. aureus* strains by lysozyme digestion method. PCR was done on 1 μL template DNA, with 200 μM dNTPs, 400 nM forward and reverse primers of *icaA* and *icaD* genes (Table 1) and 1 U Taq DNA Polymerase in 25 μL volume reaction.

The amplification cycles were carried out in a thermal cycler machine (Mastercycler; Eppendorf). The reaction condition was 94°C for 4 minutes as initial denaturation, followed by 35 cycles of 94°C for 60 seconds, annealing for 60 seconds, and 72°C for 60 seconds. A final extension step at 72°C was enforced for 10 minutes. The PCR products were visualized by EtBr on 1% agarose gel electrophoresis.

Table 1. The Sequence of Primers and PCR Product Length

Genes	Primer Sequence PCR 5' to 3'	Annealing Temperature (°C)	Product Size (bp)
icaA	CCTAACTAACGAAAGGTAG	49	1315
	AAGATATAGCGATAAGTGC		
icaD	AAACGTAAGAGAGGTGG	49	381
	GGCAATATGATCAAGATAC		
bap	CCCTATATCGAAGGTGTAGAATTG	62	971
	GCTGTTGAAGTTAATACTGTACCTGC		
sar	CGGTACCGTTGATTTGGGTAGTATGC	55	867
	TTGCCATGGTTAAAACCTCCC		
agr	GTAGAGCCGTATTGATTCC	60	463
	GTATTTCATCTCTTTAAGG		
16 SrRNA	AGAGTTTGATCCTGGCTCAG	60	1400
	GACGGGCGGTGTGTACAA		

3.6. RT-qPCR

3.6.1. RNA Extraction Following Treatment with D. Polychaetum Extract

10^6 CFU/mL of *S. aureus* were inoculated on TSB medium containing sub-MIC concentration (0.4 mg mL^{-1}) of the extract and incubated at 37°C for 12 hours. Subsequently, the medium was centrifuged at 10,000 × g for 20 minutes. RNA was extracted from the pellet by NucleoSpin®RNAII (MACHEREY-NAGEL; Germany) according to the manufacturer's company. The same bacterial sample without treatment was served as control.

3.6.2. cDNA synthesis

cDNA was synthesized by Viva 2-steps RT-PCR Kit (Vivantis, Malaysia) on five μg from total RNA with one μL random hexamer, according to the manufacturer's company. Two negative controls, without template and without M-MuLV RT, were also employed in every cDNA synthesis run.

3.6.3. qPCR

Real-time RT-qPCR was accomplished using 10 μL 2X qPCR GreenMaster (Jena Bioscience GmbH, Germany), 2 μL cDNA, and 150 nM of each primer (Table 1) in a 20-μL final volume reaction on Rotor-Gene 3000 (Corbett research, Australia). Temperature conditions were as follows: 95°C for 30 seconds, 45 cycles of 95°C for 15 seconds, and 66°C for 40 seconds. Melting analysis was induced as 72 - 99°C ramp with one-degree increments every 5 seconds. The data were gathered on FAM/SYBR channel, exported to an Excel worksheet, and analyzed with LinRegPCR (v 2015) program (8). The initial concentration of every sample was normalized against 16 S reference gene concentration to obtain the expression value. Untreated *S. aureus* cDNA template was selected as calibrator and each experiment was biologically and technically repeated at least 3 times.

3.7. Statistical Analysis

The obtained data were analyzed by one-way ANOVA and Duncan's test (SPSS software V. 16). The P value less than 0.05 was considered statistically significant. All tests were repeated at least three times.

4. Results

4.1. Identification of Methicillin-Resistant S. aureus

To identify the isolated clinical samples, agar disk-diffusion assay was applied. According to the results of the MIC on 20 isolates, the diameter of inhibition growth zones due to oxacillin was < 13 mm. The MIC of oxacillin on these samples was also > 2 mg mL^{-1} (data not shown). Accordingly, these strains were considered as MRSA and employed for the next experiments.

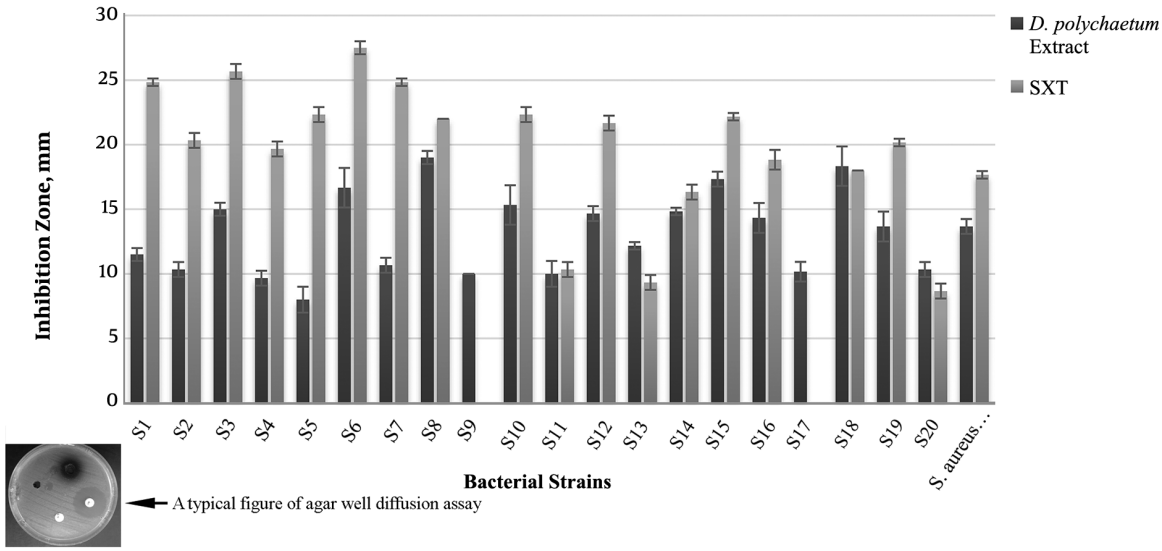
4.2. Antimicrobial Activity of D. polychaetum Extract

As the results of agar well diffusion assay on 20 bacterial samples showed, the plant extract had adequate anti-staphylococcus property (Figure 1). The diameter of inhibition growth zones was ≤ 10mm in 40% of the samples, 11 - 15 mm in 35% of the samples, and more than 15 mm in 25% of the samples. The most and least antimicrobial activity was observed in S8 (19 ± 0.5 mm) and S5 (8 ± 1 mm) isolates, respectively.

4.3. Determination of MIC and MBC of D. polychaetum

The survey of antimicrobial activity of *D. polychaetum* (in the range of 0.05 - 50 mg mL^{-1}) demonstrated that the extract had both inhibitory and bactericidal effects (Table 2). The inhibitory and lethal effects in different strains were in the range of 0.781 - 25 mg mL^{-1} and 1.56 - 50 mg mL^{-1}, respectively.

Figure 1. Antimicrobial Activity of *D. polychaetum* Extracts Against *S. aureus* Isolates as Calculated by Well Diffusion Method



The data are presented as the mean of inhibition zone diameter ± SD. The inhibitory effect of the extract on the growth of all isolates (except S11, S13, S18, and S20 isolates) was significantly stronger than the effect of the positive control drug (SXT) ($P < 0.05$). The S9 and S17 isolates were completely resistant to SXT, so their value is zero.

4.4. Determination of Anti-Biofilm Effect of D. polychaetum

According to our findings, biofilm formation was inhibited in all of the isolated MRSA strains in sub-MIC concentrations (Table 2). To evaluate biofilm inhibition, strains with the diameter of inhibition growth zone ≥ 15 mm were selected. Biofilm formation was strongly inhibited in all strains at 1.56 and 3.125 mg mL^{-1} concentrations. The most sensitive isolates were S3, S8, and S18 (Figure 2).

4.5. Evaluation of the Presence of icaA and icaD Genes

The presence of *icaA* and *icaD* genes in those strains whose diameter of inhibition growth zone was ≥ 15 mm was explored. The results revealed that 50% of the samples had both *icaA* and *icaD* genes (e.g. S8, S10 and S18), about 33% had *icaA* gene, and about 17% had *icaD* gene only. According to the results, the S8 strain was selected for analyzing the expression of genes following treatment with the extract.

4.6. Exploring the Expression of Genes Involved in Biofilm Formation

The expression of the five genes *agr*, *sar*, *bap*, *icaD*, and *icaA* in the S8 strain was quantified by RT-qPCR. As the data in Figure 3 show, the *D. polychaetum* extract significantly reduced the expression of *icaD*, *icaA*, *bap*, and *sar* in comparison with the control group ($P < 0.05$). However, the expression of *agr* did not show any significant variation.

5. Discussion

Our results demonstrated that the *D. polychaetum* extract has anti-MRSA property (Figure 1). Evaluating the MIC and MBC of the extract revealed its inhibitory and lethal effects on MRSA (Table 2). Meanwhile, surveying anti-biofilm property of the extract showed that it inhibited biofilm in MRSA at sub-MIC concentrations (0.1 - 3.125 mg mL^{-1}). If the MIC of a plant extract is in the range of 8 - 64 mg/L, it's activity is considered moderate to weak and if it is between 64 and > 256 mg/L, its activity is very weak (9). Therefore, the observed antibacterial activity of *D. polychaetum* is considered strong and significant in comparison with other species of *Dracocephalum* genus.

Lee et al. reported that essential oil of *D. foetidum* had an antimicrobial activity against most studied pathogenic bacteria (MIC = 26 - 2592 $\mu g\ mL^{-1}$) (10). Likewise, Kamali et al. also showed that different extracts of *D. kotschyi* had moderate antimicrobial effects (MIC = 0.781 - 12.5 mg mL^{-1}) (11). Asghari et al. reported that *D. kotschyi* extract had an adequate antimycobacterial activity (12). Moreover, in another study, the antimicrobial activity of essential oils of *D. polychaetum* and *D. surmandinum* was reported (13).

The main component of the essential oil of *D. polychaetum* is monoterpenes (70% perillaldehyde and 17% limonene). In addition, there are two flavonoids apigenin and luteolin in the methanolic extract of aerial part of *D. polychaetum* (14). Both monoterpenes and flavonoids have

Table 2. MIC, MBC, and Anti-Biofilm Activity of the *D. polychaetum* Extract Against MRSA Strains

Bacteria	*D. polychaetum* Extract (mg/mL)		
	MIC	MBC	Anti-Biofilm
S1	12.5	25	3.125
S2	12.5	25	3.125
S3	1.56	3.125	0.39
S4	25	50	3.125
S5	12.5	25	3.125
S6	3.125	6.25	1.56
S7	12.5	25	3.125
S8	0.781	1.56	0.1
S9	12.5	25	1.56
S10	3.125	6.25	0.78
S11	12.5	50	3.125
S12	3.125	6.25	0.2
S13	3.125	6.25	0.39
S14	1.56	3.125	0.39
S15	3.125	6.25	0.78
S16	3.125	6.25	0.2
S17	6.25	12.5	0.2
S18	0.781	1.56	0.2
S19	12.5	25	3.125
S20	12.5	25	3.125
***S. aureus* ATCC 6538**	3.125	6.25	1.56

antimicrobial activities against some pathogenic bacteria including *S. aureus* (15, 16). Hence, we can attribute the observed antimicrobial properties of *D. polychaetum* to ingredients such as these monoterpenes or flavonoids. However, verifying this conclusion needs further chemical and biological analyses.

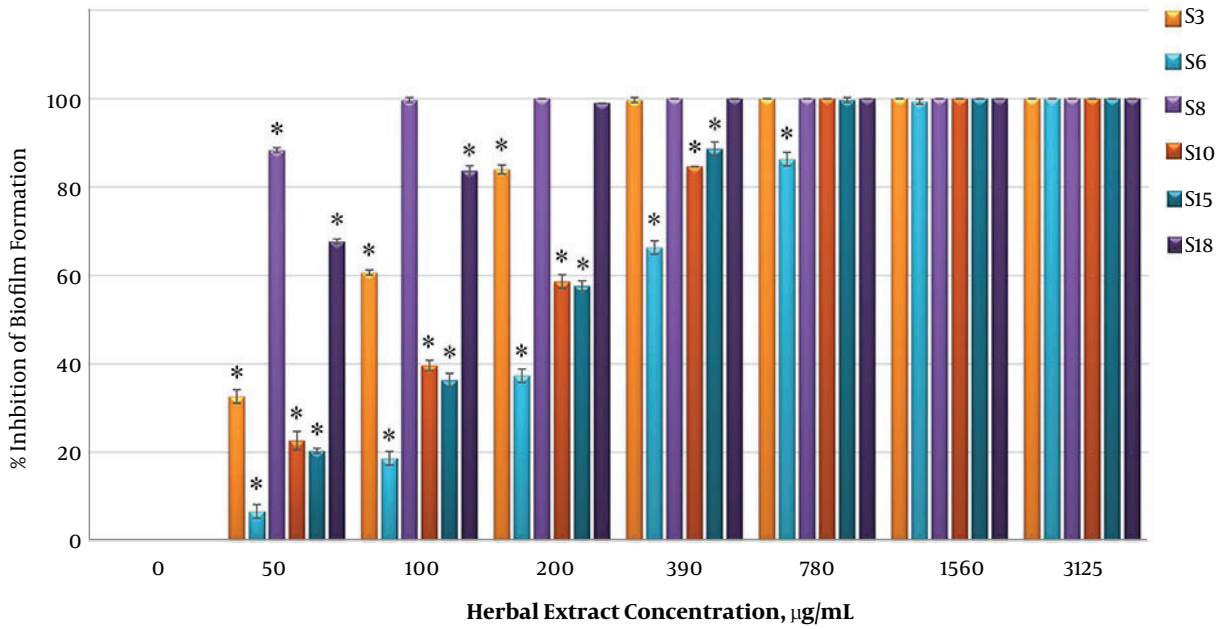
The results of gene expression analysis demonstrated that *D. polychaetum* extract reduced the expression of all study genes significantly, except *agr* (Figure 3). The *icaA* and *icaD* genes have important roles in biofilm formation and exopolysaccharide synthesis. Therefore, the drop-off in their expression indicates a reduction in biofilm formation. The observed antibiofilm property of the extract supports a decrease in the expression of *icaA* and *icaD* genes. In a recently published article, *icaA* and *icaD* were downregulated following treatment of *S. aureus* with gallic acid. The polysaccharide slime formation also reduced (17).

Sar is an essential protein for biofilm development and its expression is reduced under extract treatment (2). A decrease in the *sar* expression leads to a decrease in *ica* operon expression and subsequently biofilm production. Our finding is consistent with the role of *sar* in biofilm formation. Bap whose expression was reduced by *D. polychaetum* extract has a role in PIA independent biofilm formation. In a study, the *bap* gene was not detected in approximately 70% of human and bovine *S. aureus* isolates despite the production of biofilm (18). The reduction of *bap* expression by the extract highlights the strong role of *D. polychaetum* in suppressing *S. aureus* infection and biofilm formation through diverse mechanisms. As the *D. polychaetum* extract had no effect on the expression of *agr*, it seems that its effect on biofilm can be via other pathways.

5.1. Conclusion

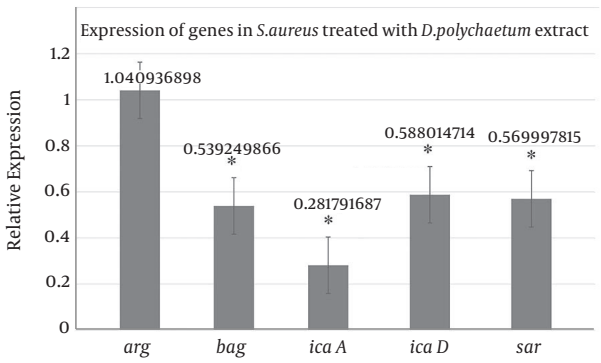
Collectively, our data indicated that *D. polychaetum* extract not only exerted antibacterial property against MRAS but also inhibited biofilm formation and decreased biofilm-related genes. Its effective components and its activity in vivo need to be revealed.

Figure 2. Biofilm Inhibition by *D. polychaetum* Extract on Strains S3, S6, S8, S10, S15, and S18 Samples in the Range of 0.05 - 3.125 mg/mL



The data show that biofilm formation was inhibited in a dose-dependent manner; so, biofilm formation was completely inhibited at concentrations > 780 μg/mL in all strains ($P < 0.05$). The S8 and S6 were the most sensitive and most resistant strains respectively. In the negative control group, no extract was applied and biofilm formation was not inhibited.

Figure 3. The Effect of *D. polychaetum* Extract on the Expression of *icaD*, *icaA*, *agr*, *bap* and *sar* Genes in the S8 Strain as Quantified by RT-qPCR



As the data show, the expression of *icaD*, *icaA*, *bap* and *sar* genes dropped off significantly ($P < 0.05$). However, the expression of *agr* has no significant changes.

Acknowledgments

This work was supported financially by the grant No. 95.1705 from Institute of Science and High Technology and Environmental Sciences graduate university of advanced technology. There is no conflict of interest between the authors.

References

1. Purrello SM, Garau J, Giamarellos E, Mazzei T, Pea F, Soriano A, et al. Methicillin-resistant Staphylococcus aureus infections: A review of the currently available treatment options. *J Glob Antimicrob Resist*. 2016;**7**:178–86. doi: 10.1016/j.jgar.2016.07.010. [PubMed: 27889013].
2. Laverty G, Gorman SP, Gilmore BF. Biomolecular mechanisms of staphylococcal biofilm formation. *Future Microbiol*. 2013;**8**(4):509–24. doi: 10.2217/fmb.13.7. [PubMed: 23534362].
3. Le KY, Dastgheyb S, Ho TV, Otto M. Molecular determinants of staphylococcal biofilm dispersal and structuring. *Front Cell Infect Microbiol*. 2014;**4**:167. doi: 10.3389/fcimb.2014.00167. [PubMed: 25505739].
4. Atshan SS, Shamsudin MN, Karunanidhi A, van Belkum A, Lung LT, Sekawi Z, et al. Quantitative PCR analysis of genes expressed during biofilm development of methicillin resistant Staphylococcus aureus (MRSA). *Infect Genet Evol*. 2013;**18**:106–12. doi: 10.1016/j.meegid.2013.05.002. [PubMed: 23669446].
5. Nourbakhsh F, Namvar AE. Detection of genes involved in biofilm formation in Staphylococcus aureus isolates. *GMS Hyg Infect Control*. 2016;**11**:Doc07. doi: 10.3205/dgkh000267. [PubMed: 27303652].
6. Lee JH, Park JH, Cho HS, Joo SW, Cho MH, Lee J. Anti-biofilm activities of quercetin and tannic acid against Staphylococcus aureus. *Biofouling*. 2013;**29**(5):491–9. doi: 10.1080/08927014.2013.788692. [PubMed: 23668380].
7. CLSI . *Performance Standards for Antimicrobial Susceptibility Testing;Twenty-Third Informational Supplement*. Wayne, PA: Clinical and Laboratory Standards Institute; 2013.
8. Ruijter JM, Ramakers C, Hoogaars WM, Karlen Y, Bakker O, van den Hoff MJ, et al. Amplification efficiency: linking baseline and bias in the analysis of quantitative PCR data. *Nucleic Acids Res*. 2009;**37**(6). e45. doi: 10.1093/nar/gkp045. [PubMed: 19237396].

9. Taylor PW. Alternative natural sources for a new generation of antibacterial agents. *Int J Antimicrob Agents*. 2013;**42**(3):195–201. doi: 10.1016/j.ijantimicag.2013.05.004. [PubMed: 23796893].
10. Lee SB, Cha KH, Kim SN, Altantsetseg S, Shatar S, Sarangerel O, et al. The antimicrobial activity of essential oil from Dracocephalum foetidum against pathogenic microorganisms. *J Microbiol*. 2007;**45**(1):53–7. [PubMed: 17342056].
11. Kamali M, Khosroyar S, Mohammadi A. Antibacterial activity of various extracts from Dracocephalum kotschyi against food pathogenic microorganisms. *Int J Pharm Tech Res*. 2015;**8**(2):158–63.
12. Asghari G, Nasr Esfahani B, Paydar P. Evaluating the effect of Dracocephalum kotschyi methanol extract on Mycobacterium tuberculosis. *Res J Pharmacogn*. 2015;**2**(3):31–6.
13. Sonboli A, Gholipour A, Yousefzadi M. Antibacterial activity of the essential oil and main components of two Dracocephalum species from Iran. *Nat Prod Res*. 2012;**26**(22):2121–5. doi: 10.1080/14786419.2011.625501. [PubMed: 22007732].
14. Mehrabani M, Roholahi S, Foruomadi A. Phytochemical studies of Dracocephalum polychaetum Bornm. *J Med Plant*. 2005;**4**(16):36–42.
15. Usman Amin M, Khurram M, Khan TA, Faidah HS, Ullah Shah Z, Ur Rahman S, et al. Effects of Luteolin and Quercetin in Combination with Some Conventional Antibiotics against Methicillin-Resistant Staphylococcus aureus. *Int J Mol Sci*. 2016;**17**(11). doi: 10.3390/ijms17111947. [PubMed: 27879665].
16. El-Haci IA, Bekhechi C, Atik-Bekkara F, Mazari W, Gherib M, Bighelli A, et al. Antimicrobial activity of Ammodaucus leucotrichus fruit oil from Algerian Sahara. *Nat Prod Commun*. 2014;**9**(5):711–2. [PubMed: 25026729].
17. Liu M, Wu X, Li J, Liu L, Zhang R, Shao D. The specific anti-biofilm effect of gallic acid on Staphylococcus aureus by regulating the expression of the ica operon. *Food Control*. 2017;**73**:613–8.
18. Khoramian B, Jabalameli F, Niasari-Naslaji A, Taherikalani M, Emaneini M. Comparison of virulence factors and biofilm formation among Staphylococcus aureus strains isolated from human and bovine infections. *Microb Pathog*. 2015;**88**:73–7. doi: 10.1016/j.micpath.2015.08.007. [PubMed: 26279194].