Journal of Food Science and Technology (Iran)

Homepage:www.fsct.modares.ir

Scientific Research

Investigating the inhibitory effect of poulk extract (Stachys schtschegleevii) on biofilm forming *Streptococcus* in vitro

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ARTICLE INFO

Article History:

Received:2024/8/18

Accepted:2025/2/23

Keywords:

Biofilm,

Bioactive compounds,

Streptococci,

Poulk extract.

DOI: 10.22034/FSCT.22.163.138.

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ABSTRACT

Biofilms act as a natural form of life for some bacteria, which in pathogenic species are critical factors in causing infectious diseases. The aim of this research was to investigate the antimicrobial effects of *Stachys schtschegleevii* extract on biofilm forming *Streptococcus* isolated from the water supply system of industrial layer pullet farms. The results of biofilm production were determined by the tissue culture plate method and showed that both isolated *Streptococcus* species had the ability to produce biofilm. In order to investigate the antimicrobial activity of poulk extract, it was prepared at a concentration of 200 mg mL^{-1} and less (by preparing serial dilution). The bioactive compounds of the extract, which included total phenolic content, total flavonoid content, and antioxidant capacity, were obtained as 83 mg GAE mL^{-1}, 24 mg QE mL^{-1}, and 65%, respectively. Antimicrobial activity was measured by the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of the extract for *Streptococcus* spp. that was obtained 12.5 and 25 mg mL^{-1} for the one derived from water storage source (B4) and 25 and 50 mg mL^{-1}, respectively for *Streptococcus* obtained from water supply system of the poultry house (S2-6). In this research, the effect of 3 mg L^{-1} sodium hypochlorite on survival and growth of these bacteria was investigated by agar well diffusion method, and the results indicated that the *Streptococcus* isolated from water supply system of the poultry house (S2-6) was resistant to it while B4 isolate was sensitive.

[DOI: 10.22034/FSCT.22.163.138]

1. Introduction

Water plays a significant role in birds' health. Ensuring the quality of drinking water is essential to maintain their health. Also, a large part of the chicken body (55-75%) is made up of water, so the quality of the water consumed by poultry is serious for the final consumer, humans [1]. In Europe, the quality standards for drinking water for poultry farms were derived from the regulations for human drinking water [2]. Currently, there is no legal requirement to assess the microbial contamination of drinking water lines in industrial poultry houses [3]. As a result, the responsibility for maintaining the hygiene of the water lines lies primarily with the poultry farmer, usually done at intervals between rearing periods [4].

Streptococcus includes a range of bacteria that can form biofilms on mucous membranes and increase their survival. The formation of biofilms is a common feature among both commensal and pathogenic *Streptococci*, whereas, in commensal *Streptococci*, biofilms are part of their life form, while in pathogenic species, biofilms are critical factors in the development of infectious diseases [5]. Biofilms are microbial communities on surfaces enclosed within a self-produced matrix composed of one or more bacterial species. Biofilms play a prominent role in human infections, with more than 80% of microbial diseases associated with biofilm formation, and the development of antibiotic resistance in biofilms is a common problem [5].

Since water supply lines are used in the poultry industry, microorganisms have colonized their internal surfaces and, with further development, have developed into biofilms [6]. Adherent bacteria are only affected to a minimal extent by cleaning and disinfection methods [4, 7]. The inefficiency of conventional methods highlights the need for improvements in the control of biofilms [8]. Chlorine-based disinfectants at concentrations of 3 mg L^{-1} have been widely used as a common disinfectant in poultry water lines for many years, while prolonged exposure to chlorine can lead to bacterial resistance, often accompanied by antibiotic cross-resistance [9]. Therefore, innovative approaches to combat biofilm production, antibiotic resistance, and pathogenicity of bacterial species are necessary [10]. Also, concerns about the emergence of antibiotic-resistant bacteria have led to the development of non-antibiotic additives. For this purpose, various methods such as biocides, antibiotics, and ion coatings are also used [11]. Recently, there has been a growing interest in aromatic plants and their extracts as growth and health promoters [12].

The genus *Stachys* is known for its ability to produce a wide range of natural compounds consisting of phenolic acids, flavonoids, phenylethanoid and phenylpropanoid glycosides, saponins, iridoids, diterpenoids, and steroids, which exhibit various biological properties [13, 14]. Plant extracts are composed of numerous biochemical compounds having antioxidant, antimicrobial, anti-inflammatory, anticoccidial, and anthelmintic properties [15]. The plant belongs to a highly diverse family of plants including about 300 species in that family [16]. There are approximately 34 distinct species of it in Iran, 13 of which are endemic [17]. Poulk (*Stachys schtschegleevii*) is one of the 34 *Stachys* species that has been used in traditional medicine to treat inflammatory respiratory diseases such as asthma, sinusitis, and colds and is also known for its antibacterial properties and effectiveness in treating rheumatism and urinary tract infections. In addition, the methanol extract derived from the aerial parts of *S.schtschegleevii* has analgesic and anti-inflammatory properties [18].

One of the methods for determining the antimicrobial activity of various compounds is the dilution method in broth culture medium to investigate their minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), in which two-fold dilutions

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of the antimicrobial agent are added to a liquid culture medium in a 96-well microtiter plate, and then a standardized microbial suspension with a 0.5 McFarland turbidity is inoculated into each well and incubated under controlled conditions, and microbial growth is assessed using a spectrophotometer or microplate reader [19].

In a study by Chitsaz et al. (2006), the essential oil, hydro-extract (boiled), and methanol extract of poulk were comparatively investigated on four bacterial species containing *Streptococcus pyogenes*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, and the results indicated that the methanol extract had a significant inhibitory effect on *Streptococcus pyogenes* and *Staphylococcus aureus*. In this study, the MIC and MBC for *Streptococcus pyogenes* were equal, and their value was 12.5 mg mL^{-1} [20].

The present study was conducted to investigate the antimicrobial activity of poulk extract on biofilm-producing *Streptococcus* bacteria isolated from the water supply lines of industrial layer pullet farms.

2.Material and Methods

1- Preparation of hydroethanolic extract of *Stachys schtschegleevii*

To prepare the extract of *Stachys schtschegleevii*, the plant leaves and flowers were collected from the Arasbaran region and turned into powder by hand grinding at 25°C within 3 days in dry shade. Extraction from the powder of *Stachys schtschegleevii* was done by maceration method (cold method) with 70% hydroethanolic solvent (70:30) in laboratory temperature (25°C) for 4 days with stirring [21]. The extract was separated from the pulp using a filter cloth, then ethanol was separated from the extract by rotating vacuum evaporator at a temperature of 55°C. The obtained extract was filled in dark glasses and stored in the refrigerator until the laboratory analyses were performed [21].

2- Determination of bioactive compounds

2-1- Total Phenolic Contents (TPC**)**

The amount, of 50 µL extract was analyzed by the Singleton et al. method [22] at 765 nm by spectrophotometer (JENWAY 6405 UV/V) to determine total phenolic contents. The TPC was quantified and expressed as gallic acid equivalent (µg GAE mL^{-1} extract).

2-2- Total Flavonoid Contents

The colorimetric method using aluminum chloride with absorbance at 510 nm by spectrophotometer (JENWAY 6405 UV/V) was utilized to estimate the total flavonoid contents in the extract (50 µL) as quercetin equivalent (µg QE mL^{-1} extract) [23].

2-3- Antioxidant capacity

The antioxidant capacity was determined by the DPPH (2,2-Diphenyl-1-picrylhydrazyl) inhibitory activity and absorbance value at 517 nm using spectrophotometer (JENWAY 6405 UV/V) [24] and the inhibitory activity of the extracts (150 µL extract) was expressed as DPPH (2,2-Diphenyl-1-picrylhydrazyl) inhibition percent using the formula:

$\% \text{ inhibition} = (A_C - A_S / A_C) \times 100$

Where A_C represents the absorbance of the control (DPPH) solution and A_S is the absorbance of the sample.

3- Isolation and identification of *Streptococci* spp.

Bacterial strains were isolated from water samples obtained from industrial layer pullet farms and then examined and identified by the microbiology laboratory. The strains were grown and maintained in Brain-heart infusion agar medium (BHI Agar, Merck 1.13825.0500) [25]. Each isolate was identified morphologically in a pure culture based on its staining reaction, shape, size, and arrangement. Gram-positive, medium-sized, non-sporulated cocci underwent further biochemical examination. Oxidase (Merck Cat # 13300), catalase (Merck Cat # 11351) tests, and cultivation in Oxidative-Fermentative medium (Merck Cat # 10282) were done. In the oxidase

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test, a pure bacterial colony was spread using an inoculating loop on an area where a few small drops of the prepared solution from the kit had been placed. After a few minutes, the color of the solution was examined. In the catalase test, a drop of normal saline was placed on the slide and the pure bacterial colony was dissolved in the normal saline, and then a drop of hydrogen peroxide was added to the normal saline and the result was examined. Finally, the bacterial strains that could produce biofilm were examined in this study [26].

4- Biofilm production by Tissue Culture Plate (TCP) method

In the TCP assay, colonies obtained from Agar medium were inoculated to Tryptic Soy Broth (TSB, Merck 1.05459.0500) medium in a tube and incubated for 24 h at 37°C, then the microbial suspension was prepared at a specific wavelength and transferred to 96-well microplates. In this way, each well received 20 µL of microbial suspension and 180 µL of TSB culture medium and incubated at 37°C for 24 h. Biofilm production was measured in triplicate using 180 µL of TSB. Therefore, 180 µL of culture medium and microbial suspension were poured into all the wells. Finally, 5 to 6 wells were designated as negative controls, and 180 µL of TSB medium was added to each of these wells [27].

Following a 24-hour incubation period, the 96-well microplates were emptied and rinsed with distilled water to determine the level of biofilm production in each well. Subsequently, 200 µL of 99% methanol was poured into each well for fixation at room temperature for 15 minutes. After discarding the contents of the wells, the microplate was left to dry at room temperature for 1 hour. The wells were stained by 200 µL of 1% crystal violet (Neutron pharmachemical Co. C.I. 42555) and left for 15 minutes at room temperature, followed by three washes with distilled water [28]. In the final stage, 200 µL of 30% glacial acetic acid (Merck CAS # 100063) was introduced into each well, followed by incubation at room temperature for 30 minutes. Subsequently, the optical density of the wells was assessed at 570 nm utilizing an ELISA reader (Hiperion Microplate reader, MPR4+) [29]. Compared to the control well, the average optical density (AOD) of the three wells was determined. The results were recorded as a) no biofilm formation when the average optical density was less than or equal to the control well, b) weak biofilm formation when the average optical density was less than twice that of the control well, c) biofilm development when the average optical density was more than twice that of the control well, and d) strong biofilm formation when the average optical density was more than four times that of the control well.

5- Determining effects of poulk extract on *Streptococci* spp. *in vitro*:

5-1- Determining Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC*) in vitro*

100 µL of Mueller Hinton Broth (Merck 1.10293.0500) and serial dilutions of antimicrobial agent (poulk extract) were prepared and poured into all microplate wells, except the first well, to accomplish the MIC test. The first well contained only 100 µL of poulk extract, and the second well contained 100 µL of culture medium and 100 µL of poulk extract. Dilution continued from the second well to the 9th well. After completing the dilution step, 100 µL was removed from the 9th well to have 100 µL of solution in all wells containing serial dilutions of poulk extract. The poulk extract was not added into the 10th well and was used as a bacterial control, which was positive due to the absence of poulk extract. The 11th well was considered to be extract-free and also without bacteria and was used as a culture medium control. The test result in the last well, which was a control for poulk extract and did not contain bacteria, was negative (Table 3).

In the next step, biofilm-producing *streptococcus* were cultured for 24 h. A turbidity of 0.5 McFarland standards (1.5×10^8 CFU mL^{-1}) was

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prepared and added to the wells. 20 µL of resazurin reagent (SIGMA-ALDRICH Lot # MKCL9251) was also added to the wells to be observed in blue color [30]. The 0.5 McFarland turbidity of bacteria and resazurin reagent were prepared in a broth culture medium. In the first well, which did not contain culture medium, a 10^{-2} dilution of 0.5 McFarland turbidity, and in the last two wells, pure resazurin was poured. Then, the wells were incubated for 24 h at 37 °C, and the color changes of the wells were evaluated. Bacterial growth and pH changes caused the reagent color to change from blue to red (Figure 1).

To approve the MBC test results, the stab culture method on a plate containing an agar culture medium was used [30]. The specified well in the MBC test, along with one well before and one well after it, were analyzed. To achieve greater certainty and observe the inhibition of growth and also the destruction of bacteria, more wells were taken and stab cultured on a microplate (Figure 2).

In such a way that 2 µL of the culture medium containing bacteria and poulk extract was transferred into the MRS agar culture medium (Merck 1.10660.0500) plate for stab culture. After cooling at room temperature, the plates were incubated (37 °C and 24 h). After the aforementioned period, the bacterial growth or lack of growth was examined to determine the antimicrobial effect [30].

5-2- Determining effects of NaClO on *Streptococci* spp. *in vitro*

In this study, the effect of 3 mg L^{-1} sodium hypochlorite (neutron chemistry) on bacterial survival and growth through the agar well diffusion method was investigated [31]. A turbidity of 0.5 McFarland standards of *Streptococci* spp. culture was prepared and spread uniformly with a sterile cotton swab on a 5 mm thick Mueller Hinton agar (Merck 1.05437.0500) plate with a 6 mm diameter well in the center. Afterward, 30 µL of sodium hypochlorite solution (3 mg L^{-1}) was poured into the well and placed in an incubator at 37 °C for 24 h. Then, the inhibition of microbial growth around the well was examined. The diameter of the inhibition zone was measured and recorded, along with the results of some diagnostic tests (shown in Table 4) [31].

Statistical Analysis

The data of this study were analyzed in a completely randomized design to investigate the effect of different concentrations of poulk extract on biofilm-producing *Streptococcus* using SPSS software (version 23, USA). A comparison of means was performed with Duncan's test ($p<0.05$). All treatments were performed in triplicate (n=3).

3.Results and Discussion

1- Determination of bioactive compounds in the extract

The total phenolic content, total flavonoid content, and antioxidant capacity of poulk extract are summarized in Table 1.

Table 1- Total phenolic content, Total Flavonoid and Antioxidant capacity of poulk extract

Scientific name	Total phenolics (mg GAE mL^{-1})	Flavonoids (mg QE mL^{-1})	Antioxidant capacity (%)
Stachys schtschegleevii	83 ± 2.6	24 ± 3.5	65 ± 2.5

2- Biofilm production by Tissue Culture Plate (TCP) method

To investigate biofilm production using the tissue culture plate (TCP) method, the optical density of the wells was evaluated at 570 nm by an ELISA reader [29]. The average optical density of the

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three wells of water storage source (B4) *Streptococci* spp. was 0.217, which was weak in biofilm production, considering its difference from the control well. Also, the average optical density of the three wells of the hall water supply system source (S2-6) *Streptococci* spp. was 0.458, and considering its difference from the control well, this isolate had a strong biofilm production capacity (Table 2).

Table 2-Biofilm production by Tissue Culture Plate (TCP) method.

	Obtained biofilm production	
Sample	B4 (Storage)	S2-6 (Hall)
ELISA Titer*	0.217	0.458
Difference with Titer	0.131	0.462
Interpretation	weak	Strong

*ELISA titers (reported as mean) are obtained at 570 nm wavelength (n= 3).

3- Effects of poulk extract on *Streptococci* spp. *in vitro*:

3-1- Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of the extract

Table 3- The results of MIC and MBC tests of Poulk extract on *Streptococci* spp.

		Poulk extract concentration (mg mL^{-1})											
		1	2	3	4	5	6	7	8	9	10	11	12
Sample location	Test type	200	100	50	25	12.5	6.25	3.125	1.56	0.78	Bacterial control	Culture medium control	Extract control 2
Storage (B4)	MIC	-	-	-	-	-	+	+	+	+	+	-	-
	MBC	-	-	-	-	+	+	+	+	+			
Hall (S2-6)	MIC	-	-	-	-	+	+	+	+	+	+	-	-
	MBC	-	-	-	+	+	+	+	+	+			

As can be seen in Table 3, the Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of the poulk extract for the *Streptococci* spp. isolated from the water storage source (B4) were observed at concentrations of 12.5 and 25 mg mL^{-1}, respectively, and for the *Streptococci* spp. obtained from the hall water supply system (S2-6) at concentrations of 25 and 50 mg mL^{-1}, respectively, which are also shown in Figure 1. Figure 2 also shows the results of examining the ability to inhibit pathogens by the agar diffusion method with an inoculating needle on an agar plate culture medium. The well specified in the MBC test, along with several wells before and after it, was cultured on a microplate as a spot to obtain more certainty and observe the inhibition of growth and destruction of bacteria, which in this experiment also confirmed the results of the MBC test observed in Table 3.

1	2	3	4	5	6	7	8	9	10	11	12

A) MIC test, after incubation (Storage: B4)

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B) MIC test, after incubation (Hall: S2-6)

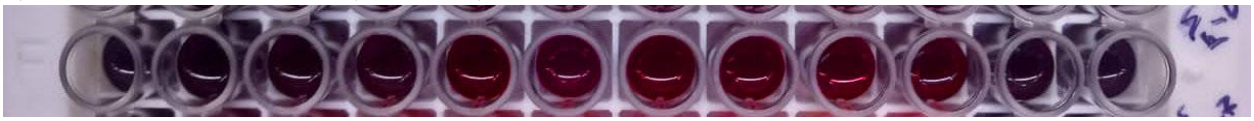

Figure 1- MIC tests by Resazurin reagent: A) After incubation (Storage) and B) After incubation (Hall).

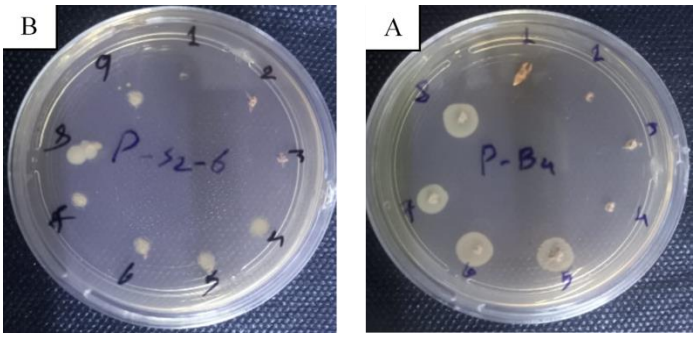

Figure 2- To ensure more certainty the inhibition of growth and destruction of bacteria, they were taken from wells and cultured on a microplate as a spot. A) Microplate (Storage: B4) – MBC – B) Microplate (Hall: S2-6) – MBC

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4- Determining effects of NaClO (3 mg L^{-1}) on *Streptococci* spp. *in vitro*

According to the results of biochemical tests shown in Table 4, as well as the study of the effect of sodium hypochlorite (3 mg L^{-1}) (Table 4 and Figure 3) on the obtained isolates, the two isolates obtained from the layer pullet farm were two species of *Streptococcus*, and their difference in the size of the zone of inhibition against sodium hypochlorite indicates their difference in terms of bacterial resistance to one of the common disinfectants in the poultry industry.

Table 4- Morphological characteristics, enzymatic activity of isolated bacteria and the effect of 3 mg L^{-1} NaClO on *Streptococci* spp.

	NaClO (3 mg L^{-1})						
Sample location	Catalase	Oxidase	OF*	Morphology	Genus	Cl Inhibitory zone (mm)	Biofilm**
Storage (B4)	-	-	F	Cocci	*Streptococcus*	8	+
Hall (S2-6)	-	-	F	Cocci	*Streptococcus*	0	+

*Oxidative-Fermentative Test, ** Both had the ability to form biofilm.

In Table 4, in the oxidase test, given that there was no change in the color of the solution, the tested bacteria lacked the cytochrome oxidase enzyme, and the oxidase test result was negative. In the catalase test, if the test was positive, many bubbles should have been observed, but given that no bubbles were observed, the catalase test result was also negative.

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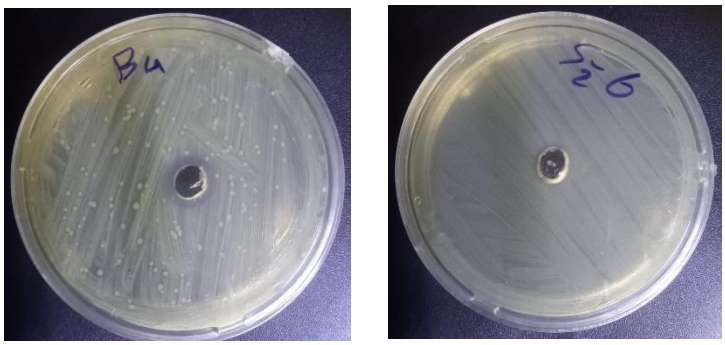

Figure 3- Agar well diffusion tests using NaClO solution: A) *Streptococci* spp. derived from storage (B4), B) *Streptococci* spp. derived from Hall (S2-6) water supply system.

The results of the experiments showed that the hydroethanolic extract of *Stachys schtschegleevii* had a strong inhibitory effect *in vitro* on *Streptococci* spp. isolated from the water supply system of industrial layer pullet farms that were capable of producing biofilms. There are various reports around the world about the antibacterial effects of *Stachys schtschegleevii*, especially in traditional medicine in the Iranian community for the treatment of some infections [32, 33], so in this study, the antibacterial effects of *Stachys schtschegleevii* extract were studied *in vitro*.

In this study biofilm-forming bacteria in industrial layer pullet farms were investigated, and two *Streptococci* spp. were isolated from different sources. It was determined that these species have different abilities to produce biofilms, and even these species showed different sensitivity to treatment and common disinfectants. Biofilms of microorganisms form a network surrounded by an external matrix of cellular polymeric materials (EPS). These microorganisms can adhere to a surface or move freely in a liquid medium. The structure of extracellular polymeric substances is complex and includes polysaccharides, proteins, extracellular DNA, and metabolic byproducts [34].

The effectiveness of disinfectants in controlling biofilm-forming bacteria is highly dependent on the concentration and duration of contact. According to Fraise (2008), a successful disinfectant can destroy more than 99% of the biofilm upon exposure to the biofilm-forming bacteria [35]. Common biofilm-forming bacteria in poultry and food industries include *Salmonella* spp., *Staphylococcus* spp., *Listeria monocytogenes*, *Escherichia coli*, *Klebsiella pneumonae*, *Campylobacter jejuni*, *Streptococcus agalactiae* [11].

It has been reported that the active extracts were bacteriostatic rather than bactericidal [36]. This finding was similar to the bacteriostatic activity of *S. glutinosa* against *Vibrio cholerae* reported recently [37]. The antibacterial activity of *S. schtschegleevii* is mainly due to the moderate polarity or polar compounds, such as phenolics present in the polar MeOH extract. Antimicrobial activity of other species of the genus *Stachys* has also been reported [38, 39, 40]. The present findings support at least partially the traditional uses of this plant, especially for bacterial infections and inflammation treatment. Several studies on the antibacterial and antifungal activity of *Stachys* species also confirmed the results of the present study.

In one research, the minimum inhibitory concentration (MIC) of extracts was determined by the resazurin microtiter assay [41]. The methanolic extract was the most potent (MIC range 1.56–6.25 mg mL^{-1}) among the extracts. Some polyphenols such as ellagic and tannic acid show comparable biofilm inhibition mechanisms.

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However, compared to furanone they require higher concentrations to produce equivalent effects [42]. Considering the toxic effects of methanolic extract in previous studies, hydroethanolic extract was chosen as a safe option to make the product usable in various poultry and food industries. The hydroethanolic extract used in this study showed minimum inhibitory concentrations in the range of 12.5–25 mg mL^{-1} for *Streptococci* spp., which was similar to that reported by Sarker et al. (2007), and this range was due to the different *Streptococci* spp. isolated and used in this study [41]. Furthermore, hydroethanolic extract is safer than methanolic extract and can be used for human, livestock, and poultry products.

The results presented in Figures 1 and 2, together with the data in Table 3, showed that the poulk extract showed a minimum bactericidal concentration of 25 mg mL^{-1} against the *Streptococci* spp. isolated from the water storage and a minimum bactericidal concentration of 50 mg mL^{-1} against the *Streptococci* spp. isolated from the water supply system of Hall No. 2.

The purpose of comparing the antibacterial properties of the poulk extract with sodium hypochlorite (3 mg L^{-1}) was to determine whether such extracts could be used as an alternative to conventional disinfectants in the poultry industry. Given that the amounts of active ingredients, which is a necessary condition for comparing the inhibitory strength of the extract with sodium hypochlorite, were not the same, the diameter of the inhibitory zone for the extract was not measured and the diameter of the inhibitory zone was only investigated to survey the resistance of the *Streptococci* spp. to sodium hypochlorite and also the differences between the isolated species. The study of the effect of sodium hypochlorite showed that the strain isolated from the hall is stronger than the strain isolated from the water storage, which does not show a halo of non-growth when exposed to hypochlorite, and when exposed to the extract, the strain obtained from the hall showed more resistance. Accordingly, it can be said that in addition to its various proven properties, the poulk extract has better performance against bacterial species such as *Streptococcus* compared to this conventional disinfection method. On the other hand, it is not harmful to the consumer and can also be used orally.

4.CONCLUSION

Replacement of chemical and somehow detrimental compounds by organic and herbal products improved the restriction of the growth and development of biofilm-generating bacteria even more effectively than the conventional disinfection systems used in industrial farms against bacteria without the possibility of harming the life of the consumer with chemical disinfectants or leaving any residue. The findings of our research showed that the ethanolic extract of the poulk herbal plant has a strong antibacterial effect on biofilm-forming *Streptococci* spp. isolated from the water supply system of the layer pullet farms, and its activity against them was bactericidal. We hope that future research can clarify the role and contribution of each of these substances in antibacterial effects along with providing the details of the effective substances in poulk herbal plant extract.

Acknowledgments

The present research was financially supported by University of Tabriz (No. 3/S, 1403.02.19).

Declaration of interest

The authors declare that there is no conflict of interest.

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[DOI: 10.22034/FSCT.22.163.138]

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[DOI: 10.22034/FSCT.22.163.138]

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مقاله علمی_پژوهشی

بررسی اثر مهاری عصاره پولک (*Stachys schtschegleevii*) بر استرپتوکوکوس تولیدکننده بیوفیلم در شرایط آزمایشگاهی

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اطلاعات مقاله

تاریخ های مقاله :

تاریخ دریافت: ۱۴۰۳/۵/۲۸

تاریخ پذیرش: ۱۴۰۳/۱۲/۵

کلمات کلیدی:

بیوفیلم،

ترکیبات زیست‌فعال،

استرپتوکوکوس،

عصاره پولک.

DOI:10.22034/FSCT.22.163.138.

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چکیده

بیوفیلم‌ها به‌عنوان شکل طبیعی زندگی برای برخی از باکتری‌ها عمل می‌کنند، که در گونه‌های بیماری‌زا، عوامل حیاتی در ایجاد بیماری‌های عفونی می‌باشند. هدف از این تحقیق بررسی اثرات ضدمیکروبی عصاره *Stachys schtschegleevii* بر روی باکتری *استرپتوکوکوس* تولیدکننده بیوفیلم جداشده از سیستم آبرسانی مزارع صنعتی پرورش پولت تخمگذار بود. نتایج تولید بیوفیلم با روش صفحه کشت بافت (TCP) تعیین شد و نشان داد که هر دو گونه *استرپتوکوکوس* جداسازی شده توانایی تولید بیوفیلم را داشتند. به منظور بررسی فعالیت ضدمیکروبی، عصاره پولک در غلظت $mg\ mL^{-1}$ ۲۰۰ و کمتر (با تهیه رقت سریالی) تهیه شد. ترکیبات زیست‌فعال عصاره که شامل محتوای فنولی کل، محتوای فلاونوئید کل و ظرفیت آنتی‌اکسیدانی بودند، به ترتیب $mg\ GAE\ mL^{-1}$ ۸۳، $mg\ QE\ mL^{-1}$ ۲۴ و ۶۵٪ بدست آمدند. فعالیت ضدمیکروبی با بررسی حداقل غلظت مهاری (MIC) و حداقل غلظت باکتری‌کشی (MBC) عصاره اندازه‌گیری شد و با توجه به نتایج بدست آمده حداقل غلظت مهاری و حداقل غلظت باکتری‌کشی عصاره پولک برای *استرپتوکوکوس* مشتق شده از منبع ذخیره آب (B4) به ترتیب در غلظت ۱۲/۵و $mg\ mL^{-1}$ ۲۵ و برای *استرپتوکوکوس* حاصل از سیستم آبرسانی سالن طیور (S2-6) به ترتیب در غلظت ۲۵ و $mg\ mL^{-1}$ ۵۰ مشاهده گردید. در این تحقیق تأثیر $mg\ L^{-1}$ ۳ هیپوکلریت سدیم بر بقاء و رشد باکتری‌های تحت مطالعه به روش انتشار در چاهک آگار بررسی شد که نتایج حاکی از آن بود که استرپتوکوکوس جدا شده از سیستم آبرسانی سالن طیور (S2-6) در مقابل آن مقاوم بود در حالی که جدایه B4 نسبت به آن حساس بود.

[DOI: 10.22034/FSCT.22.163.138]