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ORIGINAL ARTICLE / ÖZGÜN MAKALE

EVALUATION OF THE POTENTIAL ANTIBACTERIAL ACTIVITY OF *BERBERIS CRATAEGINA* DC.: BY FOCUSING ON QUORUM SENSING INHIBITION OF *CHROMOBACTERIUM VIOLACEUM* ATCC 12472

BERBERIS CRATAEGINA DC'NİN ANTİBAKTERİYEL AKTİVİTE POTANSİYELİNİN DEĞERLENDİRİLMESİ: CHROMOBACTERIUM VIOLACEUM ATCC 12472*'DE ÇEVREYİ ALGILAMA SİSTEMİ İNHİBİSYONUNA ODAKLANARAK*

Fatma Tuğçe GÜRAĞAÇ DERELİ[1], Evren ARIN[2], Ebru ÖNEM[3]*

[1]Süleyman Demirel University, Faculty of Pharmacy, Department of Pharmacognosy, 32260, Isparta, Türkiye

[2]Süleyman Demirel University, Vocational School of Health Services, Department of Anesthesia, 32260, Isparta, Türkiye

[3]Süleyman Demirel University, Faculty of Pharmacy, Department of Pharmaceutical Microbiology, 32260, Isparta, Türkiye

ABSTRACT

Objective: *In the present study described here, we set out to look into the quorum sensing inhibitory activity of the methanolic seed extract of Berberis crataegina* DC. *through quorum sensing - controlled inhibition of violacein pigment production in Chromobacterium violaceum* ATCC 12472. *In addition, the antibacterial activity of the extract on various Gram-negative and Gram-positive standard strains was evaluated. Also, phenolic contents in the extract were detected by using HPLC analysis.*

Material and Method: *"The phytochemical profile of the seed extract was performed by High-Performance Liquid Chromatography technique. Antibacterial activity assays were performed on the extract using the agar well method and inhibition of the violacein pigment production was investigated spectrophotometrically.*

* **Corresponding Author / Sorumlu Yazar:** Ebru Önem
e-mail / e-posta: ebruonem@sdu.edu.tr, **Phone / Tel.:** +902462110329

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Result and Discussion: *According to antibacterial activity results Gram-negative bacteria were more resistant than Gram-positive bacteria and violacein pigment production was inhibited by 66 % percentage. Phytochemical analysis results also showed that the major component in the extract is chlorogenic acid, with a value of 1974.91 µg/ml.*
Keywords: *Antibacterial, HPLC, phenolic content, quorum sensing*

ÖZ

Amaç: *Burada açıklanan çalışmada, Berberis crataegina* DC*'nin metanolik tohum ekstresinin çevreyi-algılama sistemi üzerindeki inhibe edici aktivitesini Chromobacterium violaceum* ATCC 12472*'de viyolasin pigment üretiminin çevreyi-algılama sistemi kontrollü inhibisyonu yoluyla araştırılması amaçlandı. Ek olarak, ekstrenin çeşitli Gram negatif/Gram pozitif standart suşlar üzerindeki antibakteriyel aktivitesi değerlendirildi. Ayrıca HPLC analizi ile ekstrenin fenolik içeriği tespit edildi.*
Gereç ve Yöntem: *Bitki ekstresinin fitokimyasal içeriği yüksek performanslı sıvı kromotografisi tekniği ile belirlenirken antibakteriyel aktivite agar kuyucuk yöntemi ile belirlenmiştir. Viyolasein pigment üretimi ise spektorofotometrikyöntem ile araştırılmıştır.*
Sonuç ve Tartışma: *Yapılan çalışmalar sonucunda antibakteriyel aktivite testinde Gram-pozitif bakterilerin Gram-negatif bakterilere göre bitki ekstresine daha duyarlı olduğu görülürken, ekstrenin viyolaseyin pigment üretimi üzerinde % 66 oranında bir inhibisyona sahip olduğu belirlenmiştir. Fitokimyasal içeriğin belirlendiği analiz zonucuna göre ise klorojenik asit 1974.91 µg/ml konsantrasyon değeri ile ekstredeki major bileşen olarak tespit edilmiştir.*
Anahtar Kelimeler: *Antibakteriyel, çevreyi algılama, fenolik bileşen, HPLC*

INTRODUCTION

The misuse, abuse and over-use of antibiotics have resulted in severe resistance problems that constitute a grave threat to the health of humans [1]. Today, there is an urgent need to discover ways to solve the problem of antibiotic resistance, and natural resources have gained importance at this point. In research in this field, the evaluation of antibacterial and antiquorum-sensing activities of medicinal herbs is becoming more popular day by day [2].

Quorum sensing (QS) is a mechanism for cell-to-cell signalling that can regulate the expression of genes responsible for the pathogenesis of microorganisms. Many medically important human pathogenic bacteria use this communication system for the conjugation, production of virulence factors, biofilm formation, motility, adherence and drug resistance [3]. QS was first described in the marine bacteria *Vibrio fischeri.* In 1970, Nealson et al. discovered that *V. fischeri* strains could communicate through secreting peptidic signalling molecules. This allowed bacteria to control bacterial behaviours in order to survive [4]. Since the QS mechanism has a key role in the pathogenesis of infectious disorders, it has been a target of interest in the research of novel anti-virulence agents [5].

In the present study described here, we aimed to investigate the QS inhibitory activity of the methanolic seed extract of *Berberis crataegina* DC. (*BC*) through QS-controlled inhibition of violacein pigment production in *Chromobacterium violaceum* ATCC 12472. In addition, the antibacterial effect of the extract on various Gram-positive and Gram-negative standard strains was evaluated.

MATERIAL AND METHOD

Plant Extraction Procedure

Seeds of *BC* were collected from Tufanbeyli/Adana on the date of August 30, 2021. The herbarium sample was authenticated by Assoc. Prof. İlker Çinbilgel and has been submitted to the Herbarium of Igdir University Biodiversity Application and Research Center with voucher number INWM00000103. To prepare the methanolic extract, 5 g of powdered dried seeds were subjected to maceration with 50 ml of 95 % methanol. The extract was filtered through Whatman No. 1 filter paper, and the filtrate was evaporated to dryness at 36 °C using a rotary evaporator (Heidolph Hei-Vap Rotary Evaporator). At the end of the process, the crude extract remaining in the flask was weighed and the amount recorded, then dissolved with dimethyl sulfoxide (DMSO) and transferred to a vial.

Phytochemical Screening

The phytochemical characterization of the seed extract was performed by High-Performance Liquid Chromatography (HPLC) technique. Conditions for HPLC analysis are presented in Table 1.

Table 1. Chromatographic conditions

Chromatographic conditions	Time (min.)	A (%)	B (%)
Detector: RF-10AXL Fluorescence Detector (Ex 295nm- Em 330 nm)	0	93	7
	20	72	28
Autosampler: SIL-20AC prominence	28	75	25
System controller: LC-20AT prominence	35	70	30
Pump: LC-20AT prominence	50	70	30
Degasser: DGU-14a	60	67	33
Column heater: CTO-10 A vp	62	58	42
Column: Gl Sciences İnertsil SIL 100A (250 mm × 4.6 mm), 5 µm	70	50	50
Column temperature: 25 °C	73	30	70
Mobile phases: A: Hexane, B: 2-propanol (98 :2)	75	20	80
Flow rate: 0.8 ml / min.	80	0	100
Injection volume: 10 µl	81	93	7

Test Organisms

In order to evaluate the antibacterial effect of the plant extract, a total of 6 strains, 2 Gram-negative strains [*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* PAO1] and 4 Gram-positive strains [*Staphylococcus aureus* ATCC 25923, *Enterecoccus faecalis* ATCC 29212, *Bacillus cereus* ATCC 11778, Methicillin-Resistant *Staphylococcus aureus* ATCC 43300] were included in the study. *C. violaceum* (ATCC 12472) strain was used to evaluate the inhibition of the violacein pigment production

Screening Seed Extract for Antibacterial Activity

Antibacterial activity assays were performed on the extract using the agar well method. This is a semi-quantitative test method for indicating the capacity of antimicrobials to inhibit microorganisms' growth [6].

The bacteria strains used in the study were inoculated into Luria-Bertani Broth (LBB) medium and waited for 24 hours at 37°C in the incubator. The bacterial cultures were adjusted to 0.5 McFarland (10^8/ml) turbidity standard and spread on Muller Hinton Agar plates. Six mm diameter wells were opened on the media and 100 µl of the plant extract was loaded into the wells. Gentamicin (40 µg/ml) was used as positive control. After overnight incubation at 35°C, by evaluating the zone sizes, antibacterial activity was discovered. The test was performed in triplicate.

The Determination of the Inhibition of the Violacein Pigment Production

Violacein is a violet colour secondary metabolite produced by diverse microorganisms to protect from environmental stress factors [7]. The production of this indole derivative compound is mediated by QS and is essential for biofilm formation [8]. As a reference strain, *C. violaceum* ATCC 12472 has the ability to produce this pigment, and thus, it is widely preferred for QS inhibition studies [9].

In this research, the inhibitory effect of the seed extract on the QS-regulated violacein production in *C. violaceum* ATCC 12472 was investigated spectrophotometrically [10]. A hundred µl (60 mg/ml concentration) of the seed extract was added to 5 ml of LBB containing *C. violaceum* ATCC 12472 and incubated overnight in a shaker incubator at 30ºC. At the end of the incubation, the bacterial culture was centrifuged at 14500 rpm for 10 minutes. After discarding the supernatant, 5 ml of DMSO was added and vortexed for 30 seconds. Centrifugation was performed at 14500 rpm for 10 minutes and the absorbance of the supernatant was read at 585 nm. LBB containing *C. violaceum* was used as a positive control. The percentage of violacein inhibition was quantified as follows**:**

Percentage of violacein inhibition = (Control OD585 nm – Sample OD585 nm/Control OD585 nm) × 100

OD: Optical Density

RESULT AND DISCUSSION

BC, also traditionally known as "Karamuk" in Turkey, is a medicinal shrub belonging to the Berberidaceae family [11]. It naturally grows in Europe and Asia. All parts of this plant are used in traditional medicine systems worldwide [12]. While there has been a plethora of report published that has described *BC* have antioxidant, anti-inflammatory, analgesic and febrifuge activities, little is known about its antimicrobial and anti-quorum sensing properties [13- 15]. In a study conducted by Kaya et al., chitosan-based edible films prepared from *BC* seed oil and fruit extract were found to have high antioxidant, anti-quorum sensing and antimicrobial activity [16].

We have investigated the antibacterial effect of the extract on various Gram-positive and Gram-negative standard strains.

Data on antibacterial screening are depicted in Table 2. Based on the results, the extract possesses a similar activity on Gram-positive bacteria, however, it is not active against Gram-negative bacteria at the studied concentration (60 mg/ml). Moreover, the extract showed antibacterial activity on Gram-positive bacteria, similar to the antibiotic gentamicin, which was chosen as the reference. The findings of this study support the possibility that *BC* could be used in the treatment of infections caused by selected Gram-positive bacteria.

Table 2. Results of the antibacterial agar well diffusion test (mm)

Sample	***E. faecalis* ATCC29212**	***S. aureus* ATCC 25923**	***MRSA* ATCC 43300**	***B. cereus* ATCC 11778**	***P.aeruginosa* PAO1**	***E.coli* ATCC 25922**
Methanolic seed extract of *BC*	17.0±0.0 *ns*	16.0±1.0 a**	17.7±1.2 a*	19.0±0.0 *ns*	*ne*	*ne*
CN (40µg/ml)	18.0±0.0	14.3±0.6 b	14,3±0.6 b	19.3±0.6	15.7±0.6	13.3±0.6

*ns insignificant; ne none-effective; *The averages of zones within columns with the same letter are not importantly different by LSD's at p<0.05. **The averages of zones within columns with the same letter are not importantly different by LSD's at p<0.01.CN gentamicin.*

In the present study, we have researched for the first time the QS inhibitory activity of the methanolic seed extract of *BC* through QS-controlled inhibition of violacein pigment production in *C. violaceum* ATCC 12472. As evident from Figure 1, the extract produced a 66% inhibition in the violacein production at 60 mg/ml concentration, and this result was found to be statistically significant.

HPLC chromatogram of the methanolic seed extract of *BC* shows the presence of gallic, protocatechuic, *p*-hydroxybenzoic, chlorogenic, caffeic, benzoic, cinnamic, *o*-coumaric acid and, rutin, quercetin. Chlorogenic acid had the highest concentration (1974.91 µg/ml) followed by benzoic acid with a concentration of 548.43 µg/ml, then *p*-hydroxybenzoic acid with a concentration of 278.55 µg/ml, with the presence of caffeic acid, rutin, protocatechuic acid, quercetin, cinnamic acid, *o*-coumaric acid and gallic acid with concentrations of 244.06, 189.55, 94.17, 32.47, 15.42, 7.75 and 7.42 µg/ml,

respectively (Table 3). The chemical structures of determined compounds in the extract are shown in Figure 2.

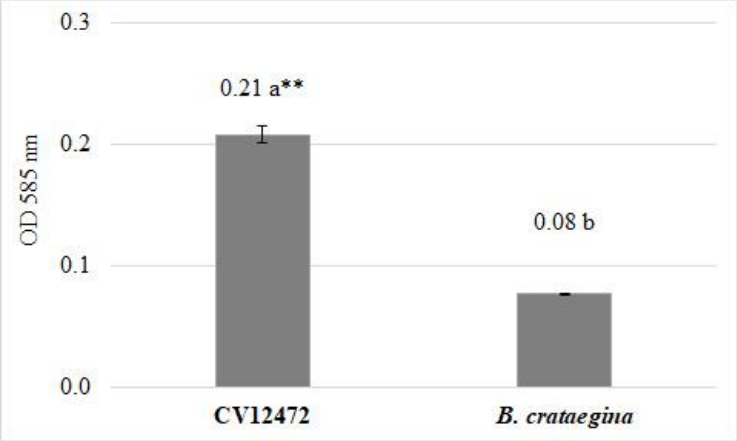

Figure 1. Results of the violacein quantification assay

Table 3. Concentrations of the main phenolic compounds identified in the methanolic extract of *BC* seeds

Phytochemicals	**Concentrations (µg/ml)**
Chlorogenic acid	1974.91
Benzoic acid	548.43
***p*-hydroxybenzoic acid**	278.55
Caffeic acid	244.06
Rutin	189.55
Protocatechuic acid	94.17
Quercetin	32.47
Cinnamic acid	15.42
***o*-coumaric acid**	7.75
Gallic acid	7.42

In the literature, there is a large number of reports related to the antibacterial and anti-virulence properties of phenolic phytocompounds. It is well documented that many phenolic secondary metabolites' main target has been the inhibition of the QS system [17-20]. In a study undertaken to research the *in vitro* anti-QS potential of chlorogenic, caffeic, and gallic acids against *S. aureus* it was found that these compounds inhibit the biofilm formation and bacterial adhesion in a dose-dependent manner. It has been determined that phenolic acids interfere with the stability of the cell membrane and the metabolic activity of the cells of *S. aureus* [21]. Similarly, in molecular docking study conducted by Mostafa et al., *p*-hydroxy benzoyl protocatechuic acid glucose, *p*-hydroxy benzoyl galloyl glucose, epicatechin and caffeoylmalic acid were reported being the main active compounds for the anti-QS activity of the methanolic extracts prepared from *Salix tetrasperma* stem bark and flowers. In this study, it was determined that these compounds help for decreasing the spread of bacterial cells by inhibiting protease and hemolysin activity [22]. According to the results of an experiment by Fratianni et al., the presence of rutin in the ethanolic extract prepared from the aerial parts of *Hypericum connatum* is held responsible for anti-QS activity against *C. violaceum* [23]. In a publication examining the effects of dietary phytochemicals (such as flavonoids) as QS inhibitory agents, the inhibition mechanisms of these compounds have been determined as interfering with acylated homeserine lactone (AHL) activity, modulating the synthesis of AHLs and inhibiting the aggregation of pathogenic bacteria known to be modulated by QS [24].

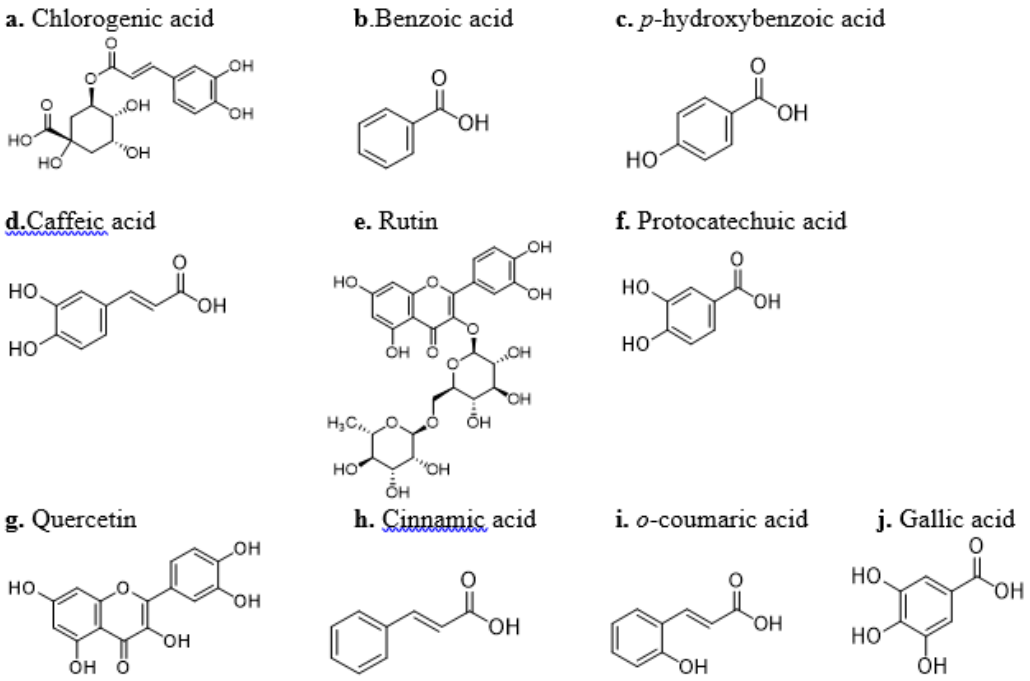

Figure 2. Chemical structures of determined phenolic compounds in the methanolic extract of *BC* seeds

Given the growing global public health problem arising from the emergence of bacterial multiple resistances to antibiotics, new drug development studies that target virulence offer hope for overcoming this problem. Therefore, we think that the current research can be an important step in the development of new agents and *Berberis crataegina* might be a promising natural weapon for fighting pathogens.

AUTHOR CONTRIBUTIONS

Concept: F.T.G.D., E.Ö.; Design: F.T.G.D., E.Ö.; Control: F.T.G.D., E.Ö.; Sources: E.Ö., E.A.; Materials: F.T.G.D., E.Ö., E.A.; Data Collection and/or Processing: F.T.G.D., E.Ö., E.A.; Analysis and/or Interpretation: F.T.G.D., E.Ö., E.A.; Literature Review: F.T.G.D., E.Ö.; Manuscript Writing: F.T.G.D., E.Ö.; Critical Review: F.T.G.D., E.Ö.; Other: -

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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