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Acacia dudgeoni Craib. ex Holl (Mimosaceae): Potential Inhibitor of Biofilm Formation and Quorum Sensing in *P. aeruginosa* PAO1

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Authors' contributions

This work was carried out in collaboration among all authors. Author VO wrote the protocol, performed the statistical analysis and wrote the first draft of the manuscript. Authors IK, AR and MC managed the analyses of the study. Author MK designed the study and managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The study aims to assess the ability of *Acacia dudgeoni* bark to antagonize quorum sensing system, expression of virulence factor and biofilm formation.
Place and Duration of Study: The study was conducted at the Laboratory of Applied Biochemistry and Chemistry (LABIOCA), University Joseph KI-ZERBO between December 2018 to April 2019.
Methodology: Methanol extract from *A. dudgeoni* stem bark, was used for the investigations. The reporters strain *Chromobacterium violaceum* CV026 and *Pseudomonas aeruginosa* PAO1 were used to measure the impact of extract on quorum sensing controlled violacein and pyocyanin production. *P. aeruginosa* PAO1 was used to measure the impact on biofilm formation.
Results: At different concentrations (50-400 µg/mL) *A. dudgeoni* methanol extract quenched the

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quorum sensing mechanism. Significant inhibition of virulence factor, pyocyanin was recorded (66% of inhibition at 400 µg/mL) without affect negatively the growth of *P. aeruginosa* PAO1. The formation of biofilm was also affected with a reduction up to 59% at the concentration of 400 µg/mL.

Conclusion: The anti-quorum sensing and anti-biofilm properties of this medicinal plant could serve as a source in the developpement of new effective anti- quorum sensing drugs.

Keywords: Acacia dudgeon; biofilm; quorum sensing; virulence factors; Pseudomonas aeruginosa PAO1.

1. INTRODUCTION

In the natural environment, microorganisms are attached to a surface, organized into structured communities, and embedded in an exopolymer matrix. This mode of development, termed biofilm, has become particularly important when it has been established to be involved in a large number of bacterial infections [1,2,3]. The infectious process is characterized by the formation of biofilm and the production of many virulence factors. These phenotypic modifications (biofilm formation and resistance) are correlated with the systems of communication between bacteria termed "Quorum Sensing" [4]. This system is considered to be a key regulatory mechanism in ecological adaptation and pathogenesis, particularly in *P. aeruginosa* [5].

In recent years, particular interest has been given to the characterization of potential new targets specific to bacteria attached to surfaces. The choice of the Quorum Sensing system as a target is validated by its position in the regulation of the expression of virulence factors and the synthesis of exopolymers [6]. In many Gram negative pathogenic bacteria, the QS system is based on the production of small diffusible molecules called acylhomoserine lactones (acyl-HSL) [7]. These acyl-HSL diffuse in the medium and activate a transcriptional regulator which caused the expression of target genes [2].

P. aruginosa possess three QS systems, lasR/lasI, rhlR/rhlI and qscR. These systems play an important role in the control of the expression of genes involved in virulence and biofilm formation [2]. Biofilm formation and QS mechanisms allows *P. aeruginosa* to be protected against immunological and antimicrobial agents [3]. In recent years, many *in vitro* assays have been developed for the research of anti-QS agents in natural products. Medicinal plants with potent anti-QS and anti-biofilm activities have been reported [7,8,9]. In our previous investigations, we demonstrated that *Acacia seyal* Del bark reduces significantly QS-controlled virulence factors production and biofilm formation [10]. These results lead to explore *Acacia* species, traditionally used in the treatment of infectious diseases for the research of anti-QS agents. This study aimed to assess its anti-QS and anti-biofilm activities.

2. METHODOLOGY

2.1 Bacterial Strains and Growth Conditions

Pseudomonas aeruginosa PAO1 and *Chromobacterium violaceum* CV026 used to assess anti-QS and anti-biofilm activities were provided from the Laboratoire de Biotechnologie Vegetale (Université Libre de Bruxelles, Belgium). Both strains were grown in Luria-Bertani (LB) broth medium at 37ºC for PAO1and 30ºC for CV026.

2.2 Plant Material Collection and Extraction

The barks of *A. dudgeoni* were collected in Loumbila region (12°29' 19.5 N; 001° 23'30.5 W), Burkina Faso. The identification of sample was confirmed in the laboratory of vegetal ecology (university Joseph KI-ZERBO, Burkina Faso)). The barks were dried, powdered and soaked during 24 h in methanol. The extract was concentrated in a vacuum evaporator (Büchi Labortechnik AG, Postfach, Flawil, Switzerland) and dried.

2.3 Inhibition of Violacein Production in *C. violaceum* CV026

The ability of *A. dudgeoni* bark extract to quench the QS system was assessed by its capacity to inhibit violacein production in *C. violaceum* CV026 according to Choo et al. [11]. *C. violaceum* CV026 is a mutant able to produce violacein in the presence of exogenous N-

hexanoyl-L-homoserine lactone (C6-HSL; Sigma-Aldrich Chemie GmbH, Darmstadt, Germany). Briefly, *C. violaceum* CV026 culture (18h at 37ºC) was diluted and added to *A. dudgeoni* bark extract dissolved in DMSO (50-400 µg/mL final concentration) in presence of C6-HSL (10 µM final concentration). Tubes were incubated during 24 h at 30ºC, 175 rpm. Bacterial turbidity (OD_{600nm}) was measured to assess bacterial growth. One mL of bacterial culture was used for violacein quantification. Tubes were centrifuged at 7000 rpm for 10 min and violacein was then dissolved in DMSO (1 mL). The production of violacein was quantified by measuring the absorbance at 575 nm.

2.4 Inhibition of Pyocyanin Production in *P. aeruginosa* PAO1

The capacity of *A. dudgeoni* bark extract to inhibit QS-controlled pyocyanin production was assessed according to the procedures described by Ouedraogo and Kiendrebeogo [12]. Briefly, different concentrations of bark extract dissolved in DMSO (50-400 µg/mL final concentration) were added to overnight culture of *P. aeruginosa* PAO1. Tubes were incubated for 18 h at 37ºC, 175 rpm. Bacterial turbidity (OD_{600nm}) was measured to assess bacterial growth. Supernatant was used for pyocyanin determination (A_{380}).

2.5 Biofilm Formation and Quantification

Anti-biofilm activity of *A. dudgeoni* bark extract was assessed according to the method described by Vandeputte et al. [7]. In each well of 96-well round-bottom was introduced 200 µL of *P. aeruginosa* PAO1 culture supplemented with different concentrations of bark extract (50-400 µg/mL). Plate was incubated for 24 h at 37ºC. After removing of the supernatant of each well, the biofilms were washed with distilled water, fixed with methanol and dried. Crystal violet dissolved in water (0.1%) was added to each well and plates were incubated for 30 min at room temperature. Finally, 200 µL of acetic acid (33% in water) were used to dissolve the crystal violet stained and the absorbance of the solution was read at 590 nm.

2.6 Total Polyphenol and Flavonoid Contents Determination

Total polyphenol of *A. dudgeoni* bark extract was determined according to the Folin–Ciocalteu method described by Lamien-Meda et al. [13]. Plant extract dissolved in methanol was mixed with Folin-Ciocalteu Reagent (0.2 N) and 5 min later supplemented with sodium bicarbonate (75 g/L). After incubation (1 h, room temperature), absorbance was measured at 760 nm. Gallic acid was used to generate a standard calibration curve and total polyphenol content was expressed as mg gallic acid equivalent for 100 mg of plant extract (mg GAE/g).

Total flavonoid was determined according to the procedures described by Lamien-Medaet al. [13]. Plant extract dissolved in methanol was mixed with aluminium trichloride (2% in methanol). Absorbance was subsequently read at 415 nm after incubation (10 min, room temperature). Quercetin was used to plot a standard calibration curve and total flavonoid content was expressed as mg of Quercetin equivalent to 100 mg of plant extract (mg QE/g).

2.7 Statistical Analysis

One way analysis of variance (ANOVA) followed by Tukey test of GraphPad Prism software was used to determined statistical significance, *p* value <.05 was considered significant (n=3).

3. RESULTS AND DISCUSSION

3.1 Anti-QS Activity

The anti-QS activity of *A. dudgeoni* bark was evaluated using *C. violaceum* CV026. This strain is able to produce violacein controlled by the QS system and is an excellent strain for the research of anti-QS compounds. Different concentrations (50-400 µg/mL) were used to assess the ability of *A. dudgeoni* bark extract to inhibit the production of violacein after 24 h of growth. As shown in Fig. 1A *A. dudgeoni* bark extract reduced violacein production by 25% to 69% compare to the control in concentration-dependent manner. *C. violaceum* CV026 growth was not negatively affected (Fig. 1B) confirming the presence of anti-QS compounds in *A. dudgeoni* bark extract.

3.2 Quantitative Analysis of Pyocyanin Production

A. dudgeoni bark extract quenched the QS system by reducing significantly the production of violacein. So, we evaluated the ability of this extract to inhibit the production of pyocyanin, a

virulence factor controlled by the QS system in *P. aeruginosa* PAO1. Pyocyanin produced in the culture medium is a blue-green phenazine pigment which alter the redox cycle on host cells [14]. It also involved in the apoptosis of neutrophils by repressing the immune response [15,16]. At the concentrations of 50 to 400 µg/mL *A. dudgeoni* bark extract showed a pronounced inhibitory effect on pyocyanin production with a reduction ranging from 33% to 66% (Fig. 2A) in dose-dependent manner. The extract did not affect the growth of *P. aeruginosa* PAO1 at these concentrations (Fig. 2B).

3.3 *A. dudgeoni* Bark Extract Affects Biofilm Formation

QS system is known to be implicated in the formation of biofilm of *P. aeruginosa* PAO1 [2]. The anti-QS activity of *A. dudgeoni* bark extract leads to evaluate its ability to inhibit the formation of biofilm in *P. aerugionosa* PAO1. Analysis of Fig. 3 showed that *A. dudgeoni* bark extract reduced significantly the formation of biofilm at different concentrations (50-400 µg/mL). At the concentration of 400 µg/mL the inhibitory effect recorded was 59%.

3.4 Total Polyphenol and Flavonoid Contents

The amount of total polyphenol was 13.12 ± 1.12 mgGAE/g in addition to a high content of total flavonoid (5.31 ± 0.11 mgQE/g) suggesting that the methanol bark extract from *A. dudgeoni* is rich in phenolic compounds.

Our investigation demonstrated that *A. dudgeoni* bark is a potent source of anti-QS compounds. Natural products which downregulated the QS system are able to reduce the pathogenicity of bacteria. Thus, *A. dudgeoni* with the capacity to inhibit the production of the extracellular virulence factor, pyocyanin, controlled by QS as well as biofilm formation is an excellent medicinal plant for the research of compounds in the

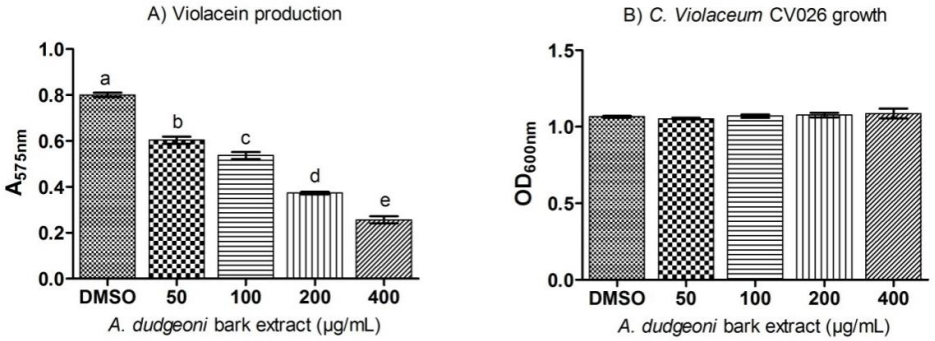

Fig. 1. Dose-dependent inhibitory effect of *A. dudgeoni* bark extract on violacein production in *C. violaceum* CV026. A) Violacein production. B) Growth of CV026. Dimethyl sulfoxide DMSO (1%) was used as negative control

Histogramm with different letters in superscript are significantly different (p < .05)

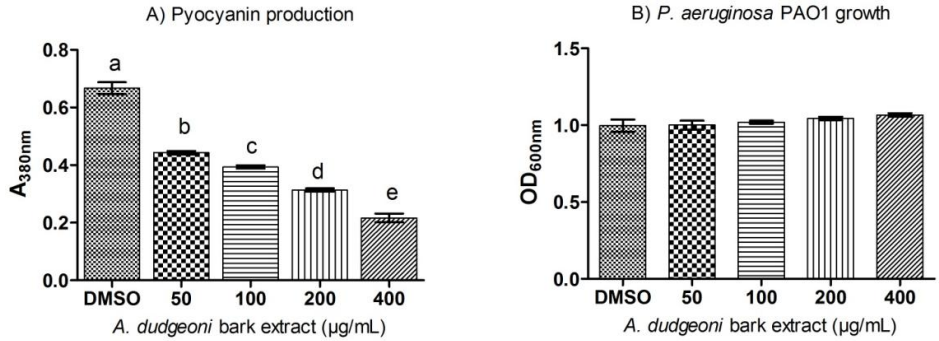

Fig. 2. Effect of *A. dudgeoni* extract on *P. aeruginosa* PAO1 virulence factor production. A) Pyocyanin production; B) *P. aeruginosa* PAO1 growth

Histogramm with different letters in superscript are significantly different (P<.05)

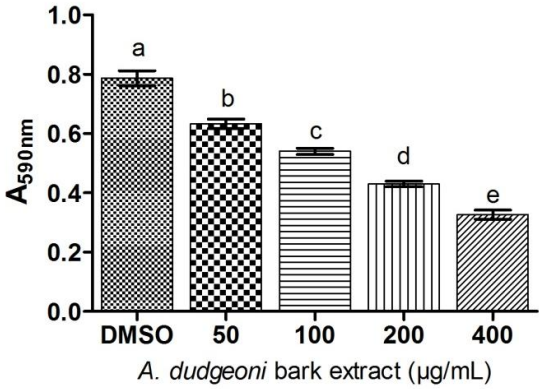

Fig. 3. Effect of *A. dudgeoni* extract on *P. aeruginosa* PAO1 biofilm formation
Histogramm with different letters in superscript are significantly different (P<.05)

treatment of diseases caused by bacteria resistant to antibiotics. Other investigations demonstrated the anti-QS activity of *Acacia* species. The *in vitro* investigation of [17] demonstrated the capacity of green pod of *Acacia nilotica* to inhibit the production of violacein by up to 100% in a concentration-dependent-manner. Biofilm formation in *P. aeruginosa* PAO1 is positively regulated by QS. Several medicinal plants such *Conocarpus erectus*, *Chamaesyce hypericifolia*, *Callistemon viminalis*, *Bucida buceras* with anti-QS activity demonstrated inhibition of biofilm formation [8].

Previous studies demonstrated the contribution of phenolic compounds to the anti-QS and anti-biofilm activity of medicinal plants on pathogenic bacteria. Catechin, isolated from *Combretum albiflorum* reduces at sub-inhibitory concentration (4 mM) significantly the production of pyocyanin and affects negatively the formation of biofilm [7]. The polyphenol curcumin (used at 1.5 and 3 µg/mL) from *Curcurma longa*, attenuates *P. aeruginosa* PAO1 biofilm formation [18]. Also, [3] demonstrated that oleanolic aldehyde coumarate (at 200 µM) from Bark of *Dalbergia trichocarpa* inhibits pyocyanin and elastase production (respectively 64% and 19% of inhibition) and biofilm formation (44% of inhibition).

Thus, *A. dudgeoni* could be use in further investigations for the isolation and identification of compounds which interfere with the mechanism of QS.

4. CONCLUSION

Our in vitro investigation showed that *A. dudgeoni* bark extract is a potential source of anti-QS phytomolecules. It exhibited anti-virulence and anti-biofilm potentialities against *P. aeruginosa* without affecting its growth. These biological properties contribute to the valorization of medicinal plants used in the treatment of diseases caused by pathogenic bacteria resistant to available antibiotics. In future investigations, we will evaluate the ability of the anti-QS molecules from *A. dudgeoni* to interfere either with the mechanisms of perception or production of homoserine lactones (lasI/lasR, rhlI/rhlR QS systems).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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