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Anti-quorum sensing and anti-biofilm activities of *Securidaca longepedunculata* Fresen, an endangered species from Burkina Faso

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The emergence of bacterial resistance to antibiotics is a serious challenge to the global health system. The QS inhibition is one of the approaches to fight against antibiotic resistance in bacteria. *Securidaca longepedunculata* is a medicinal plant that roots are the only organ used against microbial diseases. This species is threatened with extinction due to the massive use of its roots in phytotherapy. In this study, the anti-QS and anti-biofilm activities of *S. longepedunculata* leaves methanolic extract at 100 to 400 µg/mL was assessed against the bacterial strains *Chromobacterium violaceum* CV026 and *Pseudomonas aeruginosa* PAO1. The results showed anti-QS and anti-biofilm activity of *S. longepedunculata* leaves which reduced violacein production in *C. violaceum* CV026 by 12 to 59%. The virulence factor pyocyanin in *P. aeruginosa* PAO1 was inhibited from 13 to 46%. Biofilm formation was significantly inhibited (41%) at 400 µg/mL.

Key words: *Securidaca longepedunculata*, anti-quorum, anti-biofilm.

INTRODUCTION

National Institutes of Health (NIH) estimated that more than 80% of human microbial infections are associated with biofilms which is present in about 65% of chronic infections (Jamal et al., 2018). Biofilms are aggregations of microorganisms which live in an extracellular matrix. This matrix is composed by extracellular polymeric substances (EPS), including polysaccharides, nucleic acids, proteins and lipids, at a liquid interface (Li et al., 2020). In biofilms, the bad penetration of antibiotic, reduced nutrition and growth, adaptive stress responses, and persister cells formation would constitute a multi-layered defense system (Stewart, 2002). Cells growing in the biofilm are resistant to both antibiotic therapy and the host immune defense system. This situation is the cause of recurrent and recalcitrant infections (Li et al., 2020). Bacteria attached to a surface and growing in a biofilm are protected against the action of antibiotics, biocides and other control methods (Abebe, 2020). Persistent cells are a subpopulation of bacteria which can transiently survive under the lethal effect of antibiotic treatment and thus contribute to the strong resistance of biofilms (Dincer et al., 2020).

Bacteria control the expression profile of genes according to the size of the microbial population through

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the mechanism of quorum sensing (QS), which allows to form different forms of biofilm (Abebe, 2020). QS acts on the development of the biofilm and controls the production of virulence factors (enzymes, toxins) necessary for resistance to phagocytosis (Li et al., 2020). One of the strategies proposed for overcoming resistance in biofilm is the use of QS inhibitors (Li et al., 2020).

Pseudomonas aeruginosa is an opportunistic bacterium responsible for chronic infections which occurred in hospitals especially in patients suffering from cystic fibrosis. The biofilm formation and the production of several virulence factors characterize the infection process. At the same time, the sessile growth mode leads to a reduction of bacterial sensitivity to both host defenses and antimicrobial agents. Quorum sensing, which is the inter-bacterial communication system, is correlated with their biofilm production and resistance (Ciofu and Tolker-nielsen, 2019).

Medicinal plants offer a variety of phytochemicals with a new potential control of microbial diseases, due to the spectrum of secondary metabolites present in the extracts, which include phenolic, quinones, flavonoids, alkaloids and terpenoids (Asfour, 2018).

As a result, the commonly used plants in ethnomedicine constitute an alternative to search bioactives compounds against virulence factors or their production.

Securidaca longepedunculata is a highly medicinal plant whose roots and stem bark are the only organs used against bacterial disease and for the treatment of chronic wounds in traditional medicine (Mongalo et al., 2015). This plant is threatened because of the massive use of its roots (Compaoré et al., 2018).

This study aimed to assess anti-QS and anti-biofilm activities of *S. longepedunculata* leave extract.

MATERIALS AND METHODS

Bacterial strains and growth conditions

The Plant Biotechnology Laboratory of Université Libre de Bruxelles (Belgium) has kindly offered *P. aeruginosa* PAO1 and *Chromobacterium violaceum* CV026 strains for the biological activities assessment. Luria-Bertani (LB) broth was used as culture medium (37°C for PAO1 and 30°C for CV026).

Plant material collection and extraction

The leaves of *S. longepedunculata* were harvested in Badara locality (Region of "Haut Bassin"). Voucher specimen (CI : 16713) was identified and deposited in the herbarium of "Université Joseph KI ZERBO", Burkina Faso. The powder from dried samples was extracted with methanol for one day. An evaporator was used to concentrate the extracts under vacuum before drying them.

Assessment of inhibition of violacein production in *C. violaceum* CV026

The method of Choo et al. (2006) was used to assess the ability of *S. longepedunculata* extract to affect negatively the QS system through its effect on violacein production in *C. violaceum* CV026. In the presence of exogenous N-hexanoyl-L-homoserine lactone (C6-HSL; Sigma-Aldrich), *C. violaceum* CV026 which is a mutant strain is capable of producing violacein. Briefly, to the mixture of *S. longepedunculata* extract (in DMSO) and C6-HSL, a diluted culture of *C. violaceum* CV026 (18 h at 30°C) was added. The final concentrations were 100 to 400 µg/mL for the extracts and 10 µM for C6-HSL. After tubes incubation at 30°C (24 h, 175 rpm), bacterial growth was assessed by measuring the bacterial turbidity (OD600 nm). The bacterial culture (1 mL) was first centrifuged (7000 rpm, 10 min) and then the violacein was dissolved in 1 mL of DMSO. Quantification of violacein production was performed by measuring its absorbance at 585 nm.

Inhibition of pyocyanin production in *P. aeruginosa* PAO1

The QS controls the pyocyanin production. The inhibitory capacity of *S. longepedunculata* leaves extract on this production was assessed according to the method of Ouedraogo and Kiendrebeogo (2016). Briefly, the extract was used to make a series (dilution by half) of concentrations (in DMSO) which were each added overnight to a culture of *P. aeruginosa* PAO1. The final concentrations of the extracts were 100 to 400 µg/mL. Bacterial growth was assessed by measuring the bacterial turbidity (OD_{600nm}) after 18 h incubation (37°C, 175 rpm) of tubes. To assess the production of pyocyanin (A_{380}), the supernatant was used.

Biofilm formation and quantification

The ability of *S. longepedunculata* extract to inhibit biofilm formation was assessed according to the method of Vandeputte et al. (2010). A volume of 200 µL of *P. aeruginosa* PAO1 culture was supplemented with leaves extract solution for final concentrations ranging from 100 to 400 µg/mL in round-bottomed wells. The supernatant was removed after 24 h incubation at 37°C and the biofilm was fixed with methanol after washing with distilled water. A crystal violet solution (0.1% in water) was added to the wells followed by 30 min incubation at room temperature. The crystal violet stained was dissolved with 200 µL of acetic acid (33% in water) in order to read solution absorbance at 590 nm.

Statistical analysis

One way analysis of variance (ANOVA) followed by Tukey test of Graph Pad Prism software was used to determined statistical significance; p value ≤ 0.05 was considered significant (n=3).

RESULTS

Anti-QS activity of *S. longepedunculata* leaves

To search anti-QS compounds, the strain of *C. violaceum* CV026 is indicated. The leaves extract at different concentrations (100-400 µg/mL) was used to assess the inhibitory capacity of *S. longepedunculata* on violacein production after 24 h of growth. Figure 1A shows that compared to the control (DMSO at 1%), the production of violacein is reduced by 12 to 59% by *S. longepedunculata* leaves extract in concentration-dependent manner. The growth of *C. violaceum* CV026 is not affected by this

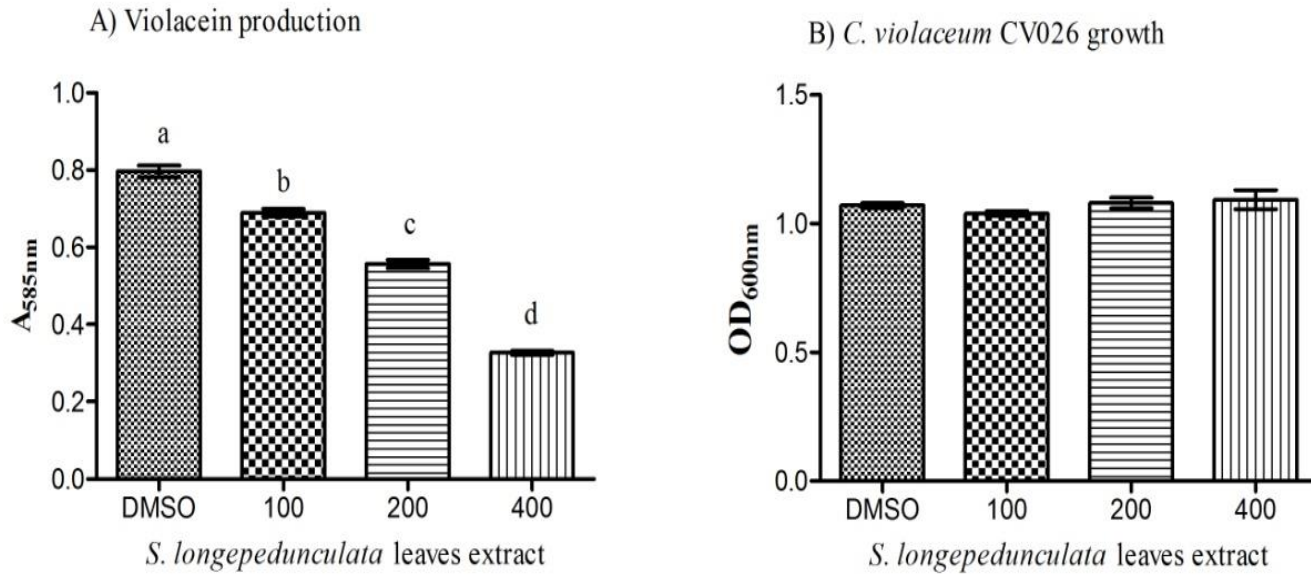

Figure 1. Concentration-dependent manner inhibitory activity of *S. longepedunculata* leaves extract on violacein production in *C. violaceum* CV026. A) Extraction and quantification (A_{585}) of violacein was performed as described in materials and methods section. B) Growth of CV026 assessed at 600 nm. The negative control used was DMSO at 1%. The difference in superscript letters assigned to histograms indicates that values are significantly different ($p < 0.05$).

reduction (Figure 1B). These results confirm that *S. longepedunculata* leaves extract contains anti-QS compounds.

S. longepedunculata leaves extract affects QS-controlled extracellular virulence factor production

The leaves extract reduced significantly the production of violacein, thus showing an inhibitory effect of *S. longepedunculata* on QS system. The QS system in *P. aeruginosa* controls the production of pyocyanin which is the virulence factor. The redox cycle of host cells is altered by a blue-green phenazine pigment which is the pyocyanin produced in culture medium (Liu and Nizet, 2009). The assessment of *S. longepedunculata* ability to inhibit pyocyanin production showed significant reduction effects ranging from 13 to 46% in dose-dependent manner (100 to 400 µg/mL) of leaves extract (Figure 2A). The growth of *P. aeruginosa* PAO1 was not affected by the extracts (Figure 2B).

S. longepedunculata leaves extract affect biofilm formation

The formation of *P. aeruginosa* biofilm PAO1 is related to QS system (Jimenez et al., 2012). Based on anti-QS activity observed, the ability of *S. longepedunculata* leaves extract to inhibit biofilm formation of *P. aerugionosa* PAO1 was evaluated.

Figure 3 shows that biofilm formation is significantly inhibited by *S. longepedunculata* leaves extract at different concentrations (100-400 µg/mL). An inhibition of 41% was recorded at the concentration of 400 µg/mL.

DISCUSSION

Bacterial biofilms are difficult to control and show high resistance to antibiotics (Koo et al., 2017). The destruction of a fully formed biofilm necessarily involves the use of molecules capable of penetrating its structure or destructuring it (Paluch et al., 2020).

This study showed that *S. longepedunculata* leaves methanolic extract have anti-QS activity, inhibit virulence factors and biofilm formation. High concentrations of *S. longepedunculata* extract did not show bactericidal activity against *C. violaceum* and *P. aerugionosa*. This absence of bactericidal activity shows that the leaves of *S. longepedunculata* act only through their inhibitory effects on QS and the formation of the biofilm. An inhibition of biofilm formation would make bacteria accessible and sensitive to the immune system and to antibacterial. This result would suggest a possible combination of *S. longepedunculata* leaves and some antimicrobial compounds in external treatment. Previous work has reported that leaves of *S. longepedunculata* are rich in polyphenol, flavonoids, and alkaloids (Karama et al., 2018). The biological activities of *S. longepedunculata* observed in this work are obviously due to the nature of bioactive compounds contained in its leaves. Vasavi et al.

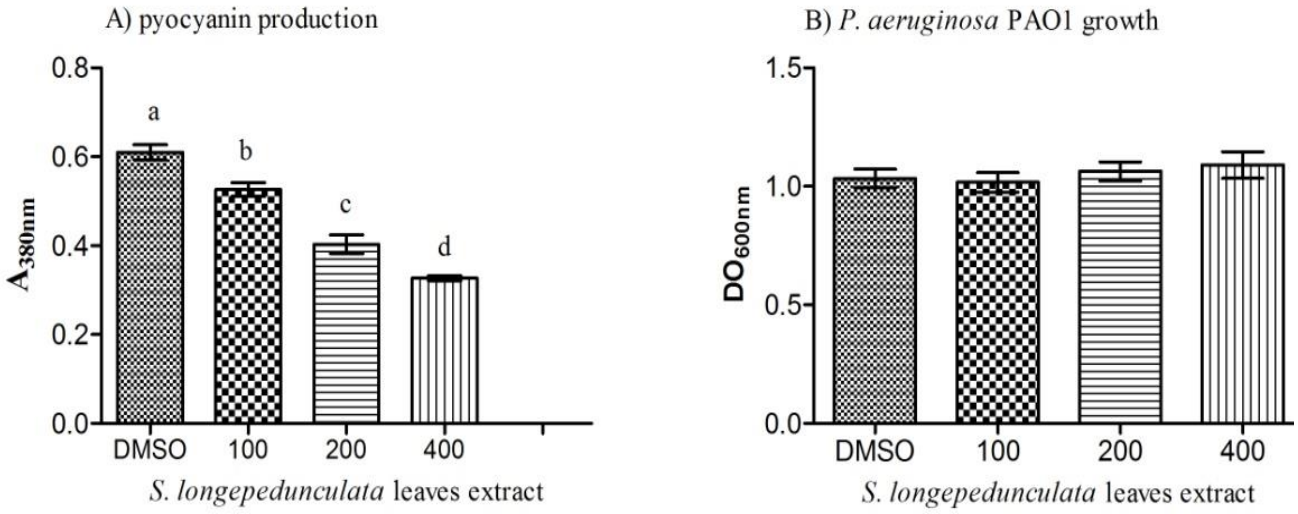

Figure 2. Effect of *S. longepedunculata* extract on *P. aeruginosa* PAO1 pyocyanin production. A) Pyocyanin production; B) Growth. Histogram with different letters is significantly different ($p < 0.05$).

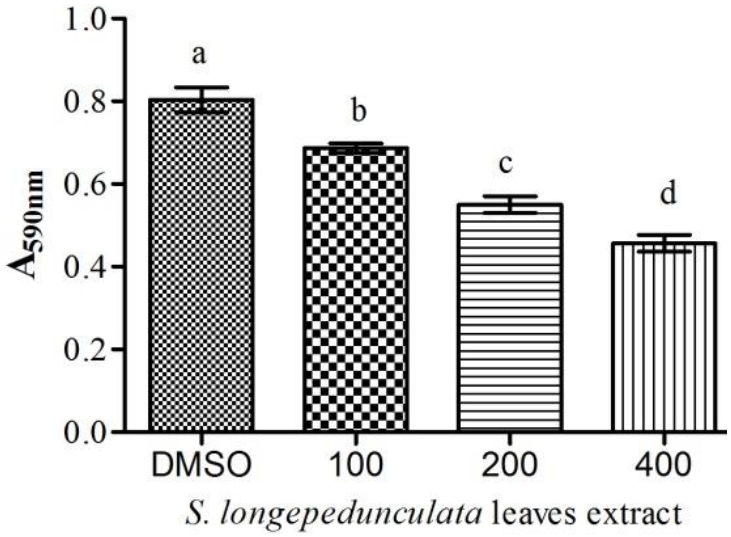

Figure 3. Activity of *S. longepedunculata* extract on *P. aeruginosa* PAO1 biofilm formation. Histograms with different superscript letters are significantly different ($p < 0.05$).

(2016) reported that plant flavonoids have the ability to interfere with inter-microbial communication and have anti-biofilm activity. Karama et al. (2020) reported the presence of compounds such as quercetin, chrysin, rutin, isorhamnetin, luteolin, gallic acid, ellagic acid, ferulic acid and tannic acid in leaves of *S. longepedunculata*. The anti-QS activity against *C. violaceum* of various flavonoids such as quercetin and luteolin have been reported (Bali et al., 2019). Rekha et al. (2016) reported that quercetin showed good inhibition of pyocyanin production.

The anti-biofilm activity of rutin against multidrug-resistant *P. aeruginosa* has been reported (Deepika et al., 2018). Wang et al. (2017) showed that rutin significantly inhibited the biofilm formation of *Streptococcus suis* without impairing its growth *in vitro*.

Likewise, the anti-QS and anti-biofilm effect of luteolin and chrysin have been reported against Gram-positive and Gram-negative bacteria (Cho et al., 2015; Shen et al., 2014).

Another study showed quercetin activity against virulence factors production and biofilm formation of *P. aeruginosa* PAO1 (Ouyang et al., 2016). Significant inhibitions were recorded with quercetin against

pyocyanin, protease and elastase production and biofilm formation (Quecan et al., 2019). Other work had reported that quercetin inhibited the production of violacein in *C. violaceum* 12472, at 50 and 100 µg/mL, respectively (Vasavi et al., 2014).

In addition, Vikram et al. (2010) have shown that quercetin removes *Escherichia coli* O157: H7 and *Vibrio harveyi* biofilm formation. Tannic acid has reduced the QS regulated violacein production up to 47.7% (Sivasankar et al., 2019). According to a past study (Karama et al., 2018), the leaves of *S. longepedunculata* showed an alkaloid content of 245 µg/g of methanolic extract. Studies reported that alkaloids have shown the ability to reverse biofilm resistance (Othman et al., 2019; Su et al., 2020).

The anti-QS and biofilm formation inhibitory activity of *S. longepedunculata* leaves extract would be due to different bioactive compounds it contains. The results observed with S*. longepedunculata* extracts show that this plant could be used against microbial infections of *P. aeruginosa*. However, subsequent studies could accurately identify anti-QS and biofilm inhibitor molecules through bioguided screening. As *S. longepedunculata* is threatened with extinction, the leaves could be used instead of the roots in phytotherapy and thus contribute to a sustainable use of this plant in the management of microbial disease. The biological activity observed with the extract of *S. longepedunculata* would be due to the properties of anti-quorum sensing and anti-biofilm compounds in the phytochemistry of its leaves.

Conclusion

This study showed that methanolic extracts from *S. longepedunculata* leaves have the ability to inhibit significantly the production of QS-controlled factors including violacein and pyocyanin from *C. violaceum* and *P. aeruginosa*, respectively and the formation of biofilm. The extract of *S. longepedunculata* did not affect negatively the growth of bacteria used.

S. longepedunculata leaves would act only by inhibiting QS and biofilm formation. The anti-QS activity of leaves shows that they could be used in the treatment of *P. aeruginosa* infections. This work results show that *S. longepedunculata* leaves would contain anti-QS and anti-biofilm compounds that could be identified by further studies.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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