Original Research Article

Anti-virulence Activity of Three Medicinal Plants: *Cassia occidentalis* L., *Crossopteryx febrifuga* (Afzel ex G. Don) Benth. and *Zanthoxylum zanthoxyloides* (Lam) Zep. and Timl

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ABSTRACT

This study aimed to assess the ability of *Cassia occidentalis*, *Crossopteryx febrifuga* and *Zanthoxylum zanthoxyloides* traditionally used for the treatment of infectious diseases, to reduce the production of virulence factors. Methanol extracts from *C. occidentalis* (leaves and stem), *C. febrifuga* (leaves and stem) and *Z. zanthoxyloides* (Stem bark) were used for the investigations. The reporter strain *Pseudomonas aeruginosa* PAO1 was used to measure the impact of extracts on elastase and pyocyanin production. Antioxidant activity was measured through 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays. All extracts at the concentration of 100 µg/mL inhibited significantly the production of pyocyanin without affect negatively the growth of *P. aeruginosa* PAO1 with a reduction of 39%, 52% and 28% respectively for *C. occidentalis*, *C. febrifuga* and *Z. zanthoxyloides*. *C febrifuga* showed the highest inhibition level on the production of elastase with a rate of 48%. The results demonstrated varying level of reduction of pyocyanin and elastase production in the reporter strain. Moreover, the antioxidant polyphenols evidenced are capable to reduce the oxidative stress induced by pyocyanin. The antioxidant and anti-virulence properties of these medicinal plants could justify their traditional use in the treatment of infectious diseases.

Keywords: Cassia occidentalis, Crossopteryx febrifuga, Zanthoxylum zanthoxyloides, virulence factors, Pseudomonas aeruginosa PAO1

1. INTRODUCTION

Pathogenic bacteria are dynamic organism able to evolve quickly and adapt to strong selective pressure leading to the emergence of strains resistance to antibiotics [1]. This situation is so delicate that the WHO warned that we will enter the post-antibiotic area if the current trends are still observed, and previously treatable infectious diseases will cause deaths [2]. Hence, the discovery of new antibacterial drugs with novel targets is urgently needed [3]. In recent years, targeting bacterial virulence instead of their viability provided a new approach for the control of infectious diseases. the production of virulence factors is controlled by a cell to cell communication system termed quorum sensing (QS) [4]. This system in Gram negative bacteria is based on the production of small diffusible molecules, acylhomoserine lactones (acyl-HSL) [5]. These HSLs cause the activation of a transcriptional regulator which will then trigger the expression of virulence genes [6]. The

disruption of this system is the main anti-virulence strategy for the treatment of recalcitrant bacterial infection [7].

One of the model organisms use to assess anti-virulence property is *P. aeruginosa*, an opportunistic pathogen responsible of nosocomial infections. It pathogenicity is due to its arsenal of virulence factors. Associate to its inherent resistance to several classes of antibiotics *P. aeruginosa* causes chronic infections particularly in immunocompromised patients [4]. The QS system in *P. aeruginosa* control the production of virulence factors such as exoproteases (elastase, alkaline protease), phenazines (pyocyanin) that promote the generation of reactive oxygen species [8].

Recently, many systems related to the study of medicinal plants as anti-virulence sources had increased. Their capacity to combat bacterial infections without promoting resistance have been demonstrated [3,4,9]. In our recent investigations, we showed that *Anogeisuss leiocarpus* (DC) Guill. and Perr. traditionally used to treat infectious diseases affect negatively QS-controlled virulence factors production and gene expression [10]. These results permit to explore Burkina faso flora for the research of anti-virulence compounds from medicinal plants. Ethnobotanical searches indicated that *C. occidentalis*, *C. febrifuga* and *Z. zanthoxyloides* are used in the treatment of infectious diseases such as typhoid fever, respiratory infections, infected wounds, dental diseases [11,12,13]. This study aimed to assess their anti-virulence and antioxidant potentialities.

2. METHODOLOGY

2.1 Bacterial strains and growth conditions

Pseudomonas aeruginosa PAO1 used to assess anti-virulence property was provided from the laboratory of plant biotechnology (free university of Brussels, Belgium) and grown in Luria-Bertani (LB) broth medium at 37°C.

2.2 PLANT MATERIAL COLLECTION AND EXTRACTION

C. febrifuga (leaves and stem), *C. occidentalis* (leaves and stem) and *Z. zanthoxyloides* (stem bark) were collected in Gampela region, Burkina Faso. The identification of sample was confirmed in the laboratory of vegetal ecology (university Ouaga 1 Pr JosephKI-ZERBO, Burkina Faso) and the voucher specimens have been deposited (01ID 15929, 02ID 15930 and 03ID15940). Dry plant powder (50 g) was soaked in methanol (500 mL) for 24 h. After filtrated, extract was concentrated in a vacuum evaporator (Büchi Labortechnik AG, Postfach, Flawil, Switzerland) and dried.

2.3 Inhibition of pyocyanin and elastase production in *P. aeruginosa* PAO1

The ability of plant extracts to inhibit the production of pyocyanin was assessed according to previously described procedures [10]. Overnight culture of *P. aeruginosa* PAO1 was diluted and supplemented with plant extract dissolved in DMSO. After 18 h of incubation at 37 °C, 175 rpm, tubes were sampled to assess bacterial growth through turbidity (OD_{600nm}). Supernatant was used for pyocyanin determination (A_{380nm}). Pyocyanin was extracted successively with chloroform and 0.2 M HCl.

Elastase production contained in the supernatant was assessed according to [14]. Briefly, 750 µL cell free supernatant was added to 250 µL elastin congo red solution (5 mg/mL in 0.1 M Tris-HCl pH 8; 1 mM $CaCl_2$) and the mixture was incubated at 37 °C for 16 h at 200 rpm.

The mixture was centrifuged at 3000 g for 10 min and absorbance was read at 495 nm to estimate elastase activity.

2.4 Total polyphenol and flavonoid contents determination

Total polyphenol in plant extracts was determined according to the Folin–Ciocalteu method described by [15]. 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/ 100mg).

Total flavonoid was determined according to the procedures described by [15]. 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/100 mg).

2.4 Antioxidant assays

antioxidant activity was measured through 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays described by [15]. Briefly, DPPH solution (200 µL, 0.02 mg/mL in methanol) was supplemented with 100 µL of plant extract dissolved in methanol. The mixture was then incubated for 15 min in darkness at room temperature and absorbance measured at 517 nm. Results were expressed as sample concentration scavenging 50% of DPPH radicals (IC50). Quercetin was used as positive controls.

2.5 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.

3. RESULTS AND DISCUSSION

3.1 Antioxidant activity, total polyphenol and flavovoid content

Total polyphenol and total flavonoid of plant extracts were quantified as well as their antioxidant capacity through radicals DPPH scavenging activity (Table 1). As shown, *C. febrifuga* extract exhibited the highest total polyphenol (23.91 ± 0.84 mg GAE/100 mg) while *C. occidentalis* extract contains the highest total flavonoid (3.87 ± 0.02 mg EQ/100 mg of QE/100 mg of extract). An interesting radical scavenging activity was pointed out. *C. febrifuga* extract exhibited the best antioxidant activity compared to quercetin.

Table 1. Polyphenol contents and antioxidant activity of methanol extract

Plants/References compound	Total phenolic content mg GAE/100 mg extract	Total flavonoids content mg QE/100 mg extract	DPPH IC50: µg/ml

C. occidentalis	5.93 ± 0.83[c]	3.87 ± 0.02[a]	162.73 ± 3.75[a]
C. febrifuga	23.91 ± 0.84[a]	2.95 ± 0.16[b]	5.7 ± 0.26[c]
Z. zanthoxyloides	7.85 ± 0.32[b]	1.12 ± 0.01[c]	154.8 ± 3.95[a]
Quercetin			11.2 ± 1.16[b]

**Mean ± Standard error of means of three experiments; Values with different letter in superscript are significantly different (p<.05).*

3.1 Anti-virulence activity of plant extracts

The plant extracts (100 µg/mL final concentration) were incubated in *P. aeruginosa* PAO1 culture to evaluate their ability to inhibit the production of pyocyanin and elastase, two QS-controlled virulence factors. These virulence factors play an important role in the degradation of host tissues during infection. Pyocyanin is a blue-green pigment secreted by *P. aeruginosa* in the culture medium and capable to increase the stress oxidative on host cells by altering the redox cycle [16]. It also able to induce apoptosis of neutrophils leading to a repression of the immune response [17,18]. The zinc metalloprotease namely elastase (lasB) is involved in the degradation of immunological agents and elastin that is a major component of the respiratory epithelium [19]. The analysis of Fig. 1A showed that all extracts at 100 µg/mL significantly affect the production of pyocyanin after 18 h compared to control (DMSO) with a reduction of 39%, 52% and 28% respectively for *C. occidentalis*, *C. febrifuga* and *Z. zanthoxyloides*. The effect of plant extracts on elastase production was also tested. As shown in Fig. 1B, after 18 h, all extracts significantly affect negatively the production of elastase. *Z. zanthoxyloides* was the least active giving a reduction of 22%. *C febrifuga* and *C occidentalis* reduce respectively 48% and 45% the production of elastase. The plant extracts had no effect on bacterial growth (Fig. 2), none of the extracts did not show any bactericidal or bacteriostatic activity against *P. aeruginosa* suggesting that the inhibitory effect observed must be an interference with the QS system.

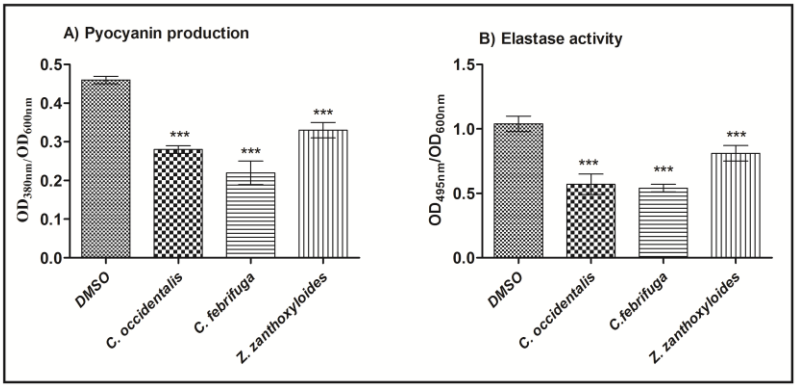

Fig. 1. Effect of plant extracts on *P. aeruginosa* PAO1 virulence factors production. A) Pyocyanin production; B) elastase activity.

**** Significantly different compared with Dimethyl sulfoxide (DMSO) used as control (P<.05).*

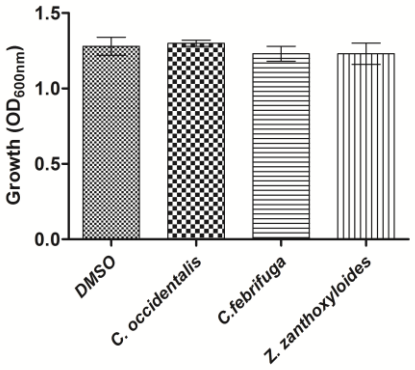

Fig. 2. Effect of plant extracts on *P. aeruginosa* PAO1 growth

Due to its capacity to produce proteases and toxins *P. aeruginosa* degrades host tissues [8]. Natural products with anti-virulence property contribute to reduce the pathogenicity of pathogens resistant to antibiotics. Our investigations demonstrated that methanol extract from *C. occidentalis*, *C. febrifuga* and *Z. zanthoxyloides* exhibits an interesting antioxidant capacity along with anti-virulence activity. Pyocyanin is involved in the pathogenicity of *P. aeruginosa* by reducing molecular oxygen into reactive oxygen species leading to a persistent oxidative stress [8]. Thus, the secretion of pyocyanin in persons infected by *P. aeruginosa* lead to chronic inflammation. Polyphenols and flavonoids of plant extracts which are responsible for their antioxidant activity could therefore contribute to the reduction of the oxidative stress caused by pyocyanin and thus reduce inflammatory intensity with a subsequent benefit for healing process. Also, polyphenols of these extracts could be responsible for the anti-virulence properties. Apigenin whith anti-virulence activity [20] have been isolated from *C. occidentalis* [21]. Other anti-virulence polyphenols such as caffeic acid, rutin, quercetin, kaempferol and coumarin in *Z. zanthoxyloïdes* have been reported [22,23]. The in vitro investigations of [24] showed that caffeic acid decrease pyocyanin production in *P. aeruginosa*. [20] demonstrated that quercetin, kaempherol and coumarin possess anti-virulence properties against, *Staphylococcus aureus* and *Escherichia coli*. The presence of polyphenol in *C. febrifuga* have been also reported [25]. Antioxidant and anti-virulence activities could contribute to protect host tissue against pathogens and to ameliorate the response of host immune.

4. CONCLUSION

This study demonstrated the antioxidant and anti-virulence activities of *C. occidentalis*, *C. febrifuga* and *Z. zanthoxyloides*. Phenolic compounds of these medicinal plants might be responsible for the anti-virulence property demonstrated. These biological properties contribute to the valorization of these plants in the management of diseases caused by bacterial multiresistance to antibiotics. In future investigations, the ability of the anti-QS molecules from these plants to interfere either with the expression of genes controlled by the QS (lasI/lasR, rhlI/rhlR) systems will be evaluated.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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