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RESEARCH ARTICLE

Study of *Ageratum conyzoides* L. (Asteraceae) Phytochemical Composition as Quorum-sensing Inhibitors to Combat Antibiotic-resistant Bacterial Infections

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Abstract: The rise of antibiotic-resistant bacterial infections has intensified the search for plant-derived quorum-sensing (QS) inhibitors as alternative therapeutic strategies. *Ageratum conyzoides* L. has a long history of traditional use as an antimicrobial agent against multidrug-resistant pathogens, yet the phytochemical basis of its QS-inhibitory activity remains incompletely characterized. This study aimed to investigate the phytochemical composition of *A. conyzoides* and evaluate its potential as a QS inhibitor and antioxidant. Bioassay-guided fractionation of the methanol extract was conducted using flash chromatography on a C18 column. High-performance thin-layer chromatography (HPTLC) was used to establish the phytochemical profile of active subfractions. Antioxidant activity was assessed using the DPPH radical scavenging, ferric reducing antioxidant power (FRAP), and ABTS Trolox equivalent antioxidant capacity assays. QS inhibition was evaluated by measuring violacein production in *Chromobacterium violaceum* CV026 and pyocyanin production in *Pseudomonas aeruginosa* PAO1. Fractionation identified subfraction F2-5-3 as an effective inhibitor of both violacein and pyocyanin production. HPTLC analysis revealed the presence of phenolic acids and flavonoids in this fraction, with total phenolic and flavonoid contents of 99.94 ± 0.04 mg/g gallic acid equivalent (GAE) and 16.82 ± 0.21 mg/g quercetin equivalent (QE), respectively. The fraction also demonstrated significant iron reduction capacity, ABTS scavenging activity, and a DPPH IC_{50} of 14.36 ± 3.76 µg/mL. These findings provide scientific validation for the traditional use of *A. conyzoides* and support its potential as a promising source of QS-inhibiting phytochemicals for combating antibiotic-resistant infections.

Keywords: *Ageratum conyzoides*, Quorum sensing inhibition, Antimicrobial resistance, Phytochemical analysis, HPTLC, Flavonoids, Antioxidant activity, *Pseudomonas aeruginosa*

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Introduction

Bacterial infections persist as one of the most serious global health issues driven by increasing antibiotic resistance [1-3]. The misuse and overuse of antimicrobials in healthcare, veterinary, and agricultural practices are major factors contributing to the widespread development of drug-resistant pathogens [4, 3]. The lack of effective antibiotics threatens the proper treatment of millions of patients [1, 3]. Moreover, drug-resistant also compromise animal and plant health, reduce farm productivity and threaten food security [3]. Quorum sensing (QS) is an adaptive mechanism of bacterial cell-to-cell communication regulated by population density and is related to toxin production and environmental stress resistance [5-6]. The opportunist pathogens, such us Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, and Streptococcus mutans, all exploit QS to enhance their infectious potential, improve surface adhesion, form biofilm, and evade immune responses [7, 8, 6]. Infections become more dangerous and more difficult to treat. QS inhibition is therefore an alternative approach that highlights innovative antibiotic strategies for the control of antimicrobial resistance and virulence in pathogenic bacteria [9]. In this context, medicinal plant-derived QS inhibitor molecules have been found to show significant potential for QS inhibition [6, 10]. Medicinal plants serve as valuable source of natural QS inhibitors attributed their rich diversity of bioactive secondary metabolites, such as phenolic acids, flavonoids, terpenoids, and alkaloids [11-13, 6]. These compounds have been shown to quench autoinducers synthesis, obstruct receptor binding, and suppress the activation of genes underpinning pathogenicity in various studies [14-17].

Natural phenolic acids, such as salicylic acid, caffeic acid, exert significant anti-virulence activity against P. aeruginosa and S. aureus, by preventing formation development and repressing the genes expression. [18-19, 10]. Gallic acid, derived from Campsis grandiflora, acts as an QS autoinducer inhibitor [20]. In the terpenoid group, eugenol and phytol reduce pyocyanin production and P. aeruginosa motility [21-22]. Flavonoids, such as quercetin, catechin, and naringenin, have been shown to modulate QS gene, suppress biofilm and decrease pyocyanin and elastase production [23, 16-17]. This suggests that natural QS inhibitors may provide effective approaches to mitigate bacterial virulence by interfering with QS regulated pathway [6].

Ageratum conyzoides L. (Asteraceae) is one such medicinal plant, widely used in traditional medicine to manage wounds, burns and various skin infections [24]. Many studies have reported therapeutic properties with antioxidant, antimicrobial activities [25-27]. Secondary compounds, such as phenolics acid, flavonoids, terpenoids, chromenes, coumarin, phenols and steroids, are the predominant active chemical constituents in this plant and could serve as QS inhibitors [28]. The potential of these molecules as alternatives to antibiotics and their role in the development of novel therapeutic strategies against multi-resistant bacterial infections are essential to promote the safe use of medicinal plants [28-29]. The research into these QS-inhibiting compounds requires more in-depth studies. This study builds on our previous research on A. conyzoides, which highlighted its anti-motility, antibiofilm and anti-quorum sensing potential [30-31]. This work was carried out to investigate the QS-inhibitor in A. conyzoides methanol extract.

Materials and Methods

Biological Materials

Plant Material and Extraction

The whole plant of Ageratum conyzoides was collected from a local field in Gampela (Ouagadougou, Burkina Faso). A voucher specimen was deposited at the National Herbarium of Burkina Faso (Voucher number: 8755). The plant material was air-dried, finely ground and, extracted by maceration with 80 % methanol (1:10; m/v, 24 hours). The resulting extract was concentrated using a rotary vacuum evaporator. The purification of the methanol extract was carried out before chromatographic analysis. The methanolic extract was already subjected to an initial liquid-liquid fractionation with organic solvents, previously delineated in our earlier work [30]. This resulted in the organic fractions F1, F2, F3, F4, corresponding to the hexanolic, chloroformic, ethyl acetate, and butanolic fractions, respectively. The antimicrobial activities of these organic fractions were evaluated against C. violaceum CV026 (at 24 and 48 hours) and P. aeruginosa PAO1 (at 8 and 18 hours). The most active fraction, F2, was selected for this study. This fraction was further purified by flash chromatography and analysed using the High-performance Thin Layer Chromatography (HPTLC).

Bacterial Strains and Growth Conditions

Chromobacterium violaceum CV026 is a biosensor strain unable to produce violacein autonomously without an external source of acyl-homoserine lactone (C6-HSL) [32]. This strain has been used to investigate disruption in bacterial quorum-sensing mechanisms. Pseudomonas aeruginosa PAO1 wild-type strain was used for quantitative analysis of virulence factors, including pyocyanin and motility. All strains were cultured in Luria-Bertani (LB) broth (1 % tryptone, 1 % NaCl, 0.5 % yeast extract, w/v) [33]. Stock cultures were maintained at -80 °C in culture medium supplemented with 50 % glycerol (v/v) for long-term storage.

Methods

Flash Chromatography Analysis

Flash chromatography (Puri Flash 215) was used to purify the F2 fraction. A total de 1.0 g was completely solubilised in 5.0 mL of methanol and introduced into a pre-loading C18 column. The solution was then passed through a second C18 column (30 mm, 25 g, 15 bar). Elution was conducted using a binary solvent system comprising hexane (Solvent A) and ethyl acetate (solvent B), with a gradient composition of 60 % solvent A and 40 % solvent B for 20 minutes (flow rate: 15.0). The compound exhibited differential adsorption within the separation column, allowing effective fractionation the mixture. Detection was performed using a UV-visible detector with a scanning range of 200-600 nm. The detector recorded absorbance profiles for individual compounds, facilitating both their separation and automated fraction collection into tubes. The resulting sub-fractions were grouped according to their thin-layer chromatography (TLC) profiles. Finally, the biological activities of these sub-fraction groups were evaluated using C. violaceum CV026 and P. aeruginosa PAO1 bioassay models.

High-Performance Thin-Layer Chromatography (HPTLC) Analysis

The High-performance Thin Layer Chromatography (HPTLC, CAMAG CATS version) was performed on a 200 ×100 mm HPTLC silica gel 60 F254 plate with a thickness of 0.20 mm (MERCK) [34]. The samples (5 µL, 5 mg/mL) were applied onto the HPTLC plate as a band (position Y: 8.0 mm, length: 8.0 mm; width: 0 mm) at a constant rate of 150 nl/s. the loaded plate was then developed in an automatic development chamber using a mobile phase composed of hexane: ethyl acetate (60:40, v/v). The plates were heated at 100oC for 3 minutes. After cooling, a solution of aminoethylphenylborinate (NEU) followed by polyethylene glycol 400 (PEG 400), was applied. Finally, the chromatographic bands were visualised under UV 254 nm and 366 nm with a CAMAG visualizer.

Spectral Analysis

A chemometric analysis of A. conyzoides powder and its fractions was conducted using near-infrared (NIR) spectroscopic (10,000 to 4,000 cm-1) analysis [35]. A precisely measured sample of 2.0 ± 0.1 g of A. conyzoides powder (in a Petri dish) and 1.0 mL of each fraction (dissolved in methanol) were transferred into a vial and positioned within the optical path of a spectrophotometer (Spectrum NFT-NIR, Perkin Elmer, USA). For each sample, three replicate measurements were acquired, with spectra recorded in transmittance mode. The raw spectra data underwent pre-processing steps, including baseline correction, derivation, and normalisation.

Quantification of Total Phenolic and Flavonoid Content

The total phenolic content in all fractions was determined spectrophotometrically at 600 nm using the Folin-Ciocalteu method, with gallic acid as the reference standard (R2 = 0.9952), [36]. Results were expressed as milligrams of gallic acid equivalents per gram (mg/g GAE). The total flavonoid content was assessed using the aluminium chloride complexation assay at 415 nm with quercetin as standard (R2 = 0.9995) [37]. The results were reported as milligrams of quercetin equivalents per gram (mg/g QE).

Antioxidant Activities

The antioxidant activities of the fractions (DPPH, ABTS and FRAP) were evaluated according to the method described by [38-40]. The absorbance for DPPH (2,2-diphényl-1-picrylhydrazyl) was measured at 515 nm, and the scavenging activity was quantified as the IC50 value. The ABTS radical cation decolourization assay was performed by generation ABTS•+ (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radicals with absorbance mesured at 734 nm. The results were expressed as milligrams of Trolox equivalent per gram of extracts (mg TE/g). The FRAP (Ferric Reducing Antioxidant Power) assay

assessed the reduction of ferric ions (Fe3+) to ferrous ions (Fe2+) in the presence of an antioxidant. The reaction was monitored by measuring the absorbance at 593 nm. Results were expressed as millimoles of ascorbic acid equivalent per gram of extract (mmol AAE/g), reflecting the electron-donating capacity of the sample. Quercetin, gallic acid and ascorbic acid were used as reference standards throughout the assays.

Violacein Inhibition Assay

Quorum Sensing (QS)-mediated violacein production was carried out using C. violaceum CV026 [41-42]. Violacein biosynthesis was induced by adding N-hexanoyl-L-homoserine lactone (C6-HSL, final concentration 10 µM). CV026 inoculum (100 µL, 107 CFU/mL) was cultured in 1.880 mL of LB medium supplemented with C6-HSL and 20 µL of the sample, the incubated for 24 and 48 hours. Salicylic acid was used as the positive control, and DMSO 1 % as the negative control. After incubation, 1 mL of each culture was centrifuged (7000 rpm, 10 min), the supernatant was carefully removed, and the pellet was resuspended in 1 mL of 100 % DMSO to ensure complete solubilization of the violacein, followed by another centrifugation under identical conditions. The absorbance was measured at 585 nm. Violacein production was quantified based on the absorbance ratio (A585nm/A600nm). The results were expressed as a percentage of inhibition as follows:

Inhibition (%) = [(Control ratio (DSMO 1%) - Samples ratio / Control ratio (DMSO 1%)] × 100]

Pyocyanin Inhibition Assay

The inhibition of pyocyanin production in P. aeruginosa PAO1 was evaluated at 8 hours and 18 hours following the method of [17]. The fractions (150 µL) were mixed with 750 µL of PAO1 inoculum (107 UFC/mL) in LB medium. At each time point, pyocyanin was extracted from 500 µL of bacterial suspension using 400 µL of chloroform, followed by recovery in 300 µL of hydrochloric acid solution (0.2 N), resulting in a pink aqueous phase. Absorbance was measured a 380 nm. Salicylic acid was used as a positive control, DMSO 1% as a negative control, and the pyocyanin amount was estimated based on the absorbance ratio (A380nm/A600nm). The results were expressed as a percentage of inhibition as follows:

Inhibition (%) = [(Control ratio (DSMO 1%) - Samples ratio / Control ratio (DMSO 1%)] × 100]

Statistical Analysis

Graphs were generated using GraphPad Prism 8.0.2 (GraphPad Software Inc., San Diego, CA, USA). And statistical analysis was performed with XLStat and GraphPad. One-way Anova followed by a Tukey post-test was applied with $p \leq 0.05$ considered significant. Principal component analysis (PCA) was conducted using SIMCA P+ 16 software to discriminate powder of A. conyzoides, totum and fractions. All experiments were conducted in triplicate, and the data were presented as mean ± standard deviation.

Results and Discussion

Results

First Flash Chromatography Analysis of Fraction F_2

The first flash chromatography of the chloroform fraction (F2) produced 59 sub-fractions (Fig. 1). TLC analysis enabled them to be grouped into 07 subs-fraction groups: F2-1, F2-2 (1.8-1.10: 8 mg), F2-3 (1.11-1.19: 250 mg), F2-4 (1.20-1.25:54 mg), F2-5 (1.26 - 1.30:150 mg), F2-6 (1.31 - 1.2.2:146 mg), F2-7 (2.3 - 2.15:6 mg) (Figure 1). The overall yield was 5.10 %. The F2-5 fraction was the most abundant in quantitative terms.

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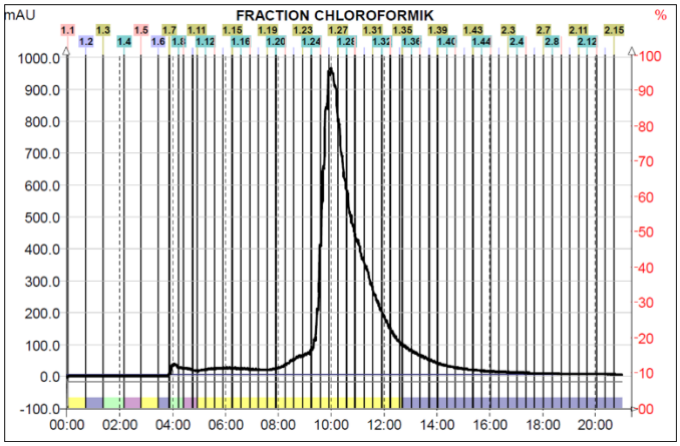

Fig. 1: Chromatogram of the F2 fraction

Eluent system: Hexane: Ethyl acetate. A total of 59 sub-fractions (1.1 to 2.15) were collected.

Anti-QS activity of F2 Subfractions on C. Violaceum CV026 and P. Aeruginosa PAO1

The groups of the seven fractions were evaluated for their capacity to inhibit C6-HSL induced synthesis of violacein in C. violacein CV026 and to suppress pyocyanin production in P. aeruginosa PAO1. (Fig. 2). As shown in Figure 2, only F2-5 exhibited a significant inhibitory effect on both bacterial pigment secretion ($p < 0.05$). Indeed, F2-5 has reduced violacein production by over 60 % (65 % at 24 hours and 63 % at 48 hours) and suppressed pyocyanin production by more than 40 % (42.4 % at 8 hours and 46.3 % at 18 hours). By comparison, the parent fraction F2 showed lower inhibition (40.1 % at 8 hours and 34.7 % at 18 hours), similar to the salicylic control (43.9 % at 8 hours and 49.0 % at 18 hours).

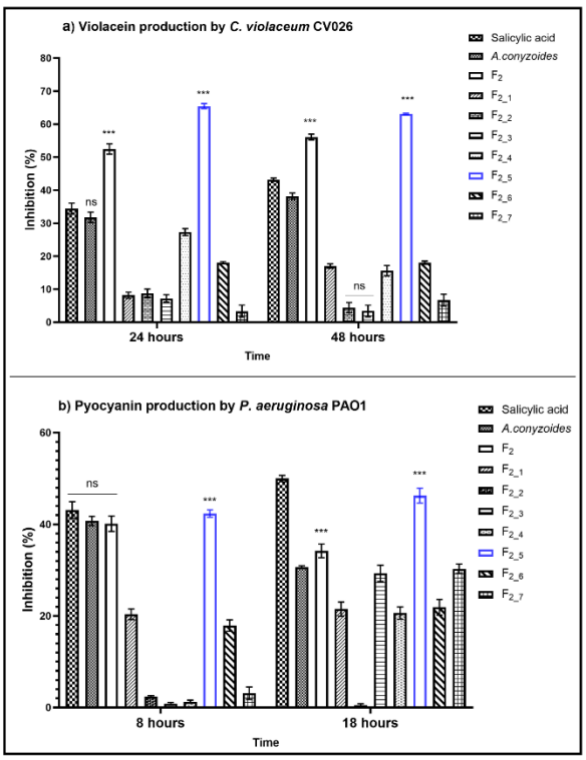

Fig. 2: Effects of Subfractions from the first flash chromatography on C. violaceum CV026 (a) and P. aeruginosa PAO1 (b). Statistically significant differences at P<0.001 (***), P<0.01 (**), P<0.05 (*), ns: not significant

Second Flash Chromatography Analysis of Fraction F2

The F2-5 fraction was separated with flash chromatography for 55 minutes using chloroform as the sole eluent; The F2-5 chromatogram is shown in Fig. 3a, where a prominent high-intensity peak was observed between 5 and 10 minutes of elution. A total of 40 sub-fractions were collected and analysed by TLC. The TLC analysis has allowed the sub-fractions to be grouped into 09 clusters: F2-5-1 (91 mg), F2-5-2 (254 mg), F2-5-3 (140 mg), F2-5-4 (102 mg), F2-5-5 (78 mg), F2-5-6 (101 mg), F2-5-7 (7.4 mg), F2-5-8 (78 mg) et F2-5-9 (107 mg). F2-5-2 and F2-5-3 had the highest masses. A diagram illustrating both fractionation steps is given in Fig. 3b.

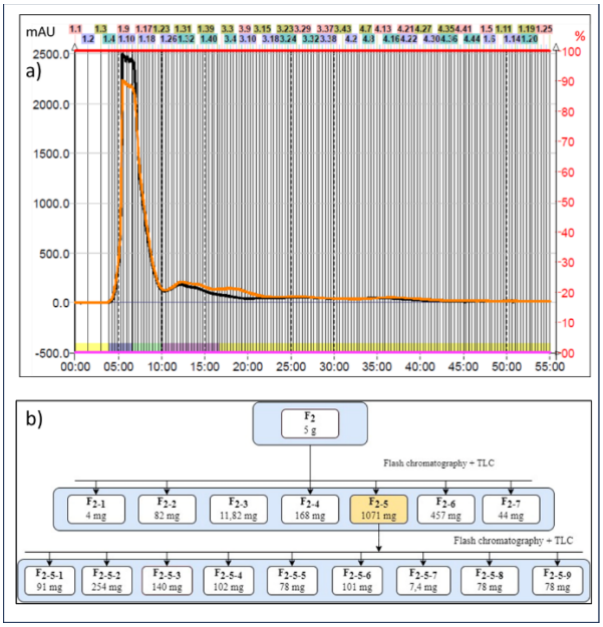

Fig. 3: (a) Chromatogram of the $F_{2\text{-}5}$ fraction; (b) bioassay-guided fractionation steps of fraction F_2

Eluent system: Hexane: Ethyl acetate. A total of 40 sub-fractions were collected, spanning retention points. Bioassay-guided fractionation steps of fraction F_2.

Effect of the Subfractions on C. Violaceum CV026 and P. Aeruginosa PAO1 Growth

Fig. 4 shows the growth of C. violaceum and P. aeruginosa with all subfractions (100 µg/mL) compared to the crude extract (A. conyzoides) and salicylic acid control at 8 hours and 18 hours. For both C violaceum (Figure 4a) and P. aeruginosa (Figure 4b), at both 8 hours and 18 hours, and for most fractions and subfractions, no significant negative impact was observed on the two bacterial strains' growth. Thus, the addition of the fractions at 100 µg/mL, did not result in any bacteriostatic or bactericidal activity.

Anti-QS Activity of F2-5 Subfractions on C. Violaceum CV026 and P. Aeruginosa PAO1

The effect of the subfractions (F2-5-1 to F2-5-9) on both bacterial strains are illustrated in Fig. 5 which shows that the subfractions F2-5-2, F2-5-3, and F2-5-4 significantly reduced violacein production compared to F2-5. The reduction was achieved without affecting bacterial growth, thus supporting the anti-QS activity of these sub-fractions. F2-5-2 and F2-5-3 exhibited statistically similar activity after 48 hours, leading to a 65 % reduction in violacein production (Fig. 5a). This finding suggests the involvement of structurally related or distinct bioactive compounds able to modulate C6-HSL signalling in C. violaceum CV026. However, for P. aeruginosa PAO1, F2-5-3 was more effective than F2-5-2 in inhibiting pyocyanin production at both 8 and 18 hours (Figure 5b). The reduction rates for F2-5-3 were 63.9 % and 62.8 %, compared to 57.8 % and 56.3 % for F2-5-2 at the same time points. F2-5-3 could contain more potent QS-inhibitory compounds than F2-5-2. In addition, after 18 hours, F2-5-3, was more active than the crude extract (A. conyzoides) and the salicylic acid control (Fig. 5).

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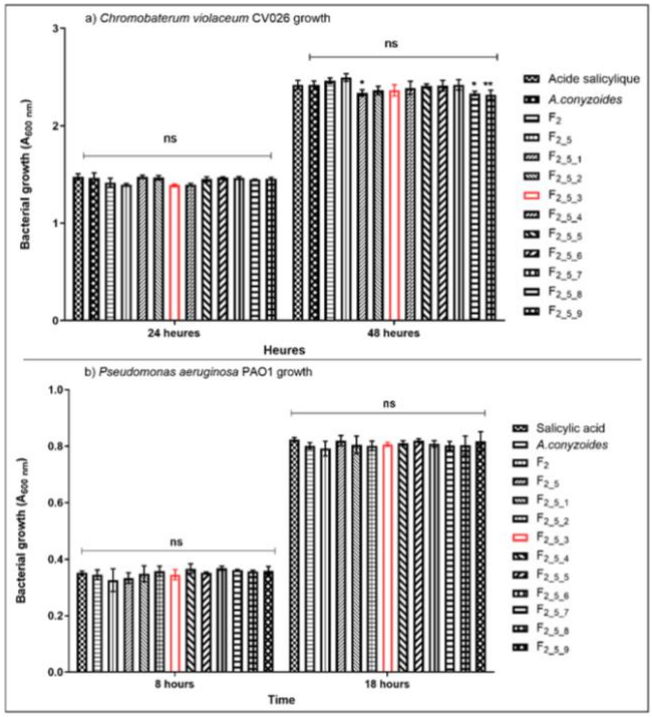

Fig. 4: Effects of Subfractions on C. violaceum CV026 growth (a) and P. aeruginosa PAO1growth (b). Statistically significant differences (*) at P<0.05, ns: not significant

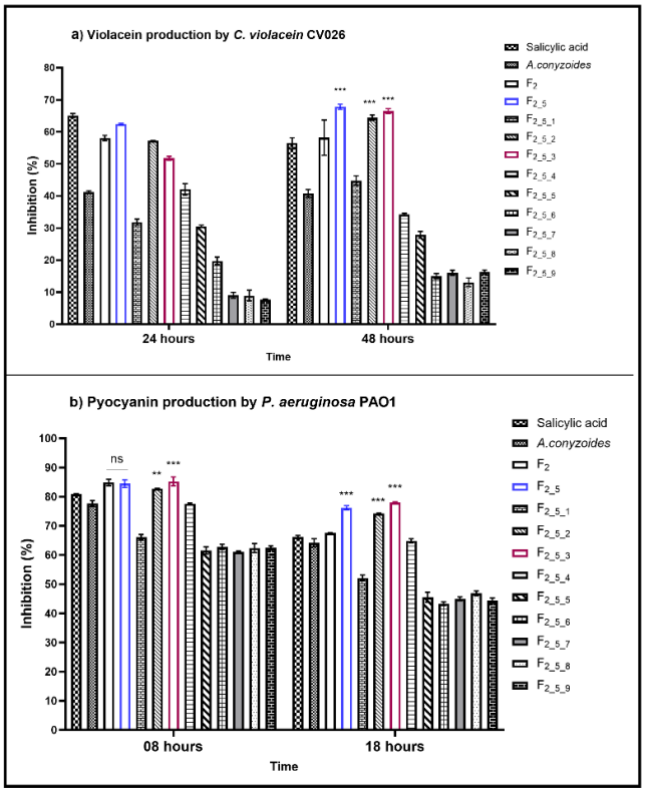

Fig. 5: Effects of Subfractions from the second flash chromatography on C. violaceum CV026 (a) and P. aeruginosa PAO1 (b). Statistically significant differences at P<0.001 (***), P<0.01 (**), P<0.05, ns: not significant

Phytochemical Content and Antioxidant Capacity

Total phenolic compound content in the last sub-fractions varied from 100.77 to 37.23 mg/g GAE, corresponding to fractions F2-5-5 and F2-5-7, respectively (Table 1). The results reveal a significant difference in total polyphenol content between the parent fraction F2-5 (555.87 ± 2.4 mg/g GAE) and its sub-fractions (F2-5-1 to F2-5-9). The parent fraction has a substantially higher polyphenol concentration compared to all its sub-fractions, suggesting a possible dilution effect or selective separation of phenolic compounds during fractionation.

Among the sub-fractions, F2-5-4 (100.05 ± 0.02 mg GAE/g) and F2-5-5 (100.77 ± 0.09 mg GAE/g) had relatively high polyphenol content, although still significantly lower than those of the parent fraction. In contrast, F2-5-7 (37.23 ± 0.2 mg/g GAE) and F2-5-9 (39.82 ± 0.29 mg GAE/g) exhibit significantly lower polyphenol contents. While no significant differences were observed among the values of fractions F2-5-2, F2-5-3, F2-5-4, and F2-5-5, these levels were found to be five times lower than that of the parent fraction F2-5. Thus, the significantly higher polyphenol content in the parent fraction F2-5 compared to its subfractions indicates that the fractionation process led to an uneven distribution of phenolic compounds, with a preferential enrichment in some subfractions and a notable reduction in others. The highest flavonoid content was recorded in fraction F2-5-3. Moreover, flavonoid levels were not determined in some fractions (F2-5-5, F2-5-6, F2-5-7, F2-5-8, F2-5-9). The antioxidant capacity, assessed through DPPH radical scavenging, ranged from 12.16 to 42.12 µg/mL (Table 1) The highest activities, corresponding to the lowest IC50 values, were observed in fractions F2-5-4 (12.16 ± 0.38 µg/mL) and F2-5-3 (14.36 ± 3.76 µg/mL). No significant difference was found between the fraction and sub-fraction regarding ABTS antioxidant capacity, as values ranged from 0.407 to 0.914 mmol TE/g. In this test, all concentration values were clearly below 1 mmol TE/g. However, fraction F2-5-3 (0.91 ± 0.01 mmol TE/g) exhibited a higher activity compared to the parent fraction F2-5 (0.90 ± 0.01 mmol TE/g). The results obtained from the FRAP assay were similar to those of the ABTS method, with values ranging from 0.60 ± 0.02 to 1.52 ± 0.08 mmol AAE/g. In terms of their ability to reduce Fe^{3+} to Fe^{2+}, the fractions exhibited the following decreasing order of activity: F2-5-2 > F2-5-3 > F2-5-5 > F2-5-6 > F 2-5-4.

Table 1: Total polyphenol, flavonoid content, and antioxidant activity of the fractions

	Content		Antioxidant capacity		
	Total Polyphenols (mg/g GAE)	Total Flavonoids (mg/g QE)	DPPH IC_{50} (µg/mL)	ABTS (mmol TE/g)	FRAP (mmol AAE/g)
F_{2-5}	555.87±2.4	103.62±3.2	23.08±2.25	0.90±0.01	1.22±0.72
F_{2-5-1}	64.77±0.7^{a}	3.28±0.2^{a}	*ND*	0.41±0.01^{a}	*ND*
F_{2-5-2}	99.27±0.0^{b}	10.31±0.3^{b}	28.54±1.23^{a}	0.66±0,01^{b}	1.52±0.08^{a}
F_{2-5-3}	99.94±0.0^{b}	16.82±0.2^{c}	14.36±3.76^{b}	0.91±0.01^{c}	1.05±0.22^{b}
F_{2-5-4}	100.05±0.0^{b}	14.079±0.3^{d}	12.16±0.38^{b}	0.79±0.01^{d}	0.60±0.02^{c}
F_{2-5-5}	100.77±0.1^{b}	*ND*	40.33±0.36^{c}	0.77±0.00^{e}	0.77±0.05^{d}
F_{2-5-6}	87.32±4.2^{c}	*ND*	42.12±0.52^{c}	0.74±0.00	0.68±0.04^{e}
F_{2-5-7}	37.23±0.2^{d}	*ND*	*ND*	0.71±0.11	*ND*
F_{2-5-8}	63.55±0.5^{a}	*ND*	*ND*	0.66±0.01	*ND*
F_{2-5-9}	39.82±0.3^{e}	*ND*	*ND*	0.49±0.07	*ND*

Results are expressed as the mean (± standard deviation) of three independent experiments. Values with the same letter are not significantly different (p≤0.05). ND = not determined, due to insufficient quantity of sample material.

HPTLC Profile of the Sub-Fractions

The HPTLC analysis of the A. conyzoides methanol extract, F2 fraction, F2-5 sub-fraction and its nine (09) sub-fractions (F2-5-1 to F2-5-9) has detected the presence of phenolic acids (dark spot under UV 254 nm/Figure 6A and blue spot under UV 366 nm/Figure 6B), flavonoids (yellow spots/Figure 6B), and chlorophyll pigments (red zone, Figure 6B). Under UV light at 366 nm, A. conyzoides methanol extract (UE102), fraction F2, as well as subfractions F2-5, F2-5-1, F2-5-2, F2-5-3 and F2-5-4, predominantly contained phenolic compounds (blue spot). In addition, in fractions F2-5-3 and F2-5-4, less chlorophyll (red zone, Figure 6B) was observed than in other fractions, but a significant phenolic compound was detected:

- 03 groups of intense phenolic compounds (blue spot, 0.05 < Rf < 0.2)

- 01 group of flavonoids in fraction $F_{2\text{-}5\text{-}2}$ (yellow spot, Rf = 0.1)
- group of flavonoids in fraction $F_{2\text{-}5\text{-}3}$ (yellow spot, 0.1 < Rf < 0.3)
- 2 group of flavonoids in fraction $F_{2\text{-}5\text{-}4}$ (yellow spot, 0.2 < Rf < 0.3)

The high phenolic compound found in fractions F2-5-3 and F2-5-4, during chromatography analysis, may account for their strong anti-QS activity of these fractions. Due to the low mass of the bioactive subfraction, no more fractionation was possible to isolate and purify the bioactive molecules. Therefore, a chemometric approach using PCA was performed on the extracts and bioactive fractions in order to investigate the patterns and potential relationships between their phytochemical composition and anti-QS activities.

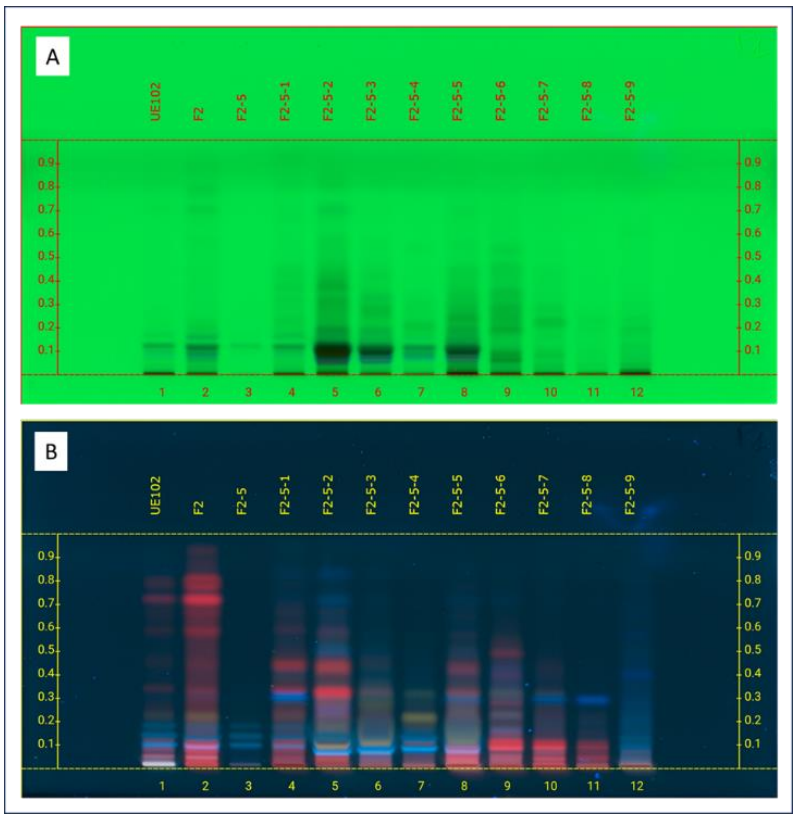

Fig. 6: Thin-Layer chromatography profile of extract and subfractions

1= UE102 (A. conyzoides methanol extract); 2= F2; 3=F2-5; 4=F2-5-1; 5=F2-5-2; 6=F2-5-3, 7=F2-5-4; 8=F2-5-5; 9=F2-5-9, 10=F2-5-7; 11=F2-5-8; 12=F2-5-9. Elution system: Hexane: ethyl acetate (60/40, v/v). A) UV 256 nm. B) Revelation with PEG 400 reagent, UV 365 nm.

Chemometrics Analysis Using Principal Component Analysis (PCA)

The Near Infrared (NIR) signals (4000 cm-1 to 10000 cm-1) recorded for the A. conyzoides powder, its methanolic extract (totum), fractions F2, F2-5, as well as the sub-fractions F2-5-2, F2-5-3 and F2-5-4, which exhibited higher biological activity than the parent fraction F2-5. Principal component analysis (PCA) of A. conyzoides powder, totum/extract and fractions indicates that axis 1 (55.6 %) and axis 2 (34.2 %) represent 89.8 % of variability. Indeed, according to axis 1, we distinguish the powder and fractions F2-5-2 and F2-5-4 on the one hand and the totum and fractions F2 and F2-5-3 and F2-5 on the other hand (Figure 7). Meanwhile, according to axis 2, there was a correlation between AC powder, fractions F2-5-2 and F2-5-4 on one side and on the other side, there was a correlation between totum/extract, fractions F2-5-3, F2-5 and F2.

It can be noted from this PCA that the F2-5-2/F2-5-4 fractions share common properties with A. conyzoides powder. From the PCA results it will be noted that the totum (complete extract) is an intermediate position between the fractions.

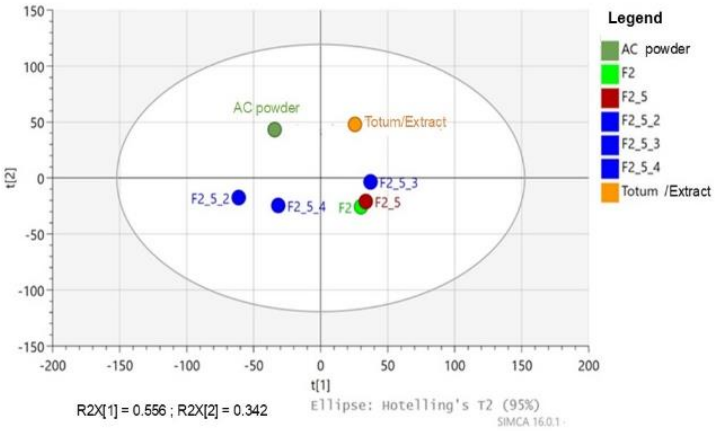

Fig. 7: Principal Component Analysis of A. conyzoides, totum and fractions

Discussion

Medicinal plants are a valuable resource of bioactive compound due to their richness in therapeutic properties and bioactive antimicrobial compounds bioassay-guided fractionation of the A. conyzoides methanol extract, successfully led to the isolation of a bioactive subfraction, F2-5-3 [43, 29]. This subfraction effectively inhibited QS-regulated phenotypes, including violacein and pyocyanin production in C. violaceum CV026 and P. aeruginosa PAO1 respectively. Indeed, pyocyanin production, a QS-regulated virulence factor is a key contributor to chronic wound and burn infections [44-45]. Pyocyanin, is a redox-active virulence factor, interferes with various cellular processes, specifically iron acquisition, due to its ability to extract iron from transferrin and promote the redox cycle [46]. This leads to intracellular accumulation of reactive oxygen species and contributes to cytotoxicity [44]. Therefore, the inhibition of pyocyanin production could contribute to the reduction of cellular cytotoxicity [47]. The exogenous addition of C6-HSL in C. violaceum CV026 cultures induces violacein production, suggesting that A. conyzoides compounds interfere with QS by inhibiting the C6-HSL signal response [48-49].

The HPTLC performed on A. conyzoides methanol extract and its fractions showed the presence of phenolic acids and flavonoids, especially in the last bioactive fraction F2-5-3. The total phenolic content varied throughout the fractionation process, with a significant concentration observed in bioactive fraction F2-5-3 of A. conyzoides.

This result can be support by principal component analysis (PCA) which show a separation between the fractions, the initial powder (AC powder) and the crude methanolic extract (Totum/Extract). This separation may reflect a variation in chemical composition related to the extraction and fractionation process. Each fraction appears to have a distinct molecular profile, which may explain the value of fractionation for the isolation of specific compounds. Furthermore, methanol extraction may alter the chemical composition of A. conyzoides powder by affecting certain compounds, such as polyphenols, as well as by inducing chemical transformations, for example, through the degradation of molecules sensitive to heat or pH. These effects could explain why the phytochemical profiles differ between the crude powder and the extract. Several studies, like those of Antony and Farid; Lee et al., support our findings by highlighting how factors like pH, solvent choice, and temperature during extraction can impact the chemical makeup of the resulting fractions [50-51]. Moreover, principal component analysis reinforces the idea that the processes of extraction and fractionation help concentrate bioactive compounds that play key roles in inhibiting quorum sensing and boosting antioxidant activity. Furthermore, the chemical variability exhibited by PCA would be consistent with biological assays, highlighting how changes in chemical profiles correspond to functional activity. Therefore, PCA not only distinguishes chemical proximity but also provides a framework to link phytochemical diversity, extraction methodology, and biological efficacy in A. conyzoides preparations.

Phenolic compounds are major natural antioxidants acting as effective free radical scavengers [52, 53]. The DPPH, FRAP, and ABTS assays have shown significant antioxidant activity in the F2-5-3 fraction. Phenolic acids and flavonoids are likely the main contributors to this activity. [54]. The presence of these compounds in subfraction F2-5-3 may help reduce oxidative stress induced by pyocyanin, potentially leading to the downregulation of QS-related genes expression such as rhlR, rhlI, lasR and lasI.

Beyond their antimicrobial and antioxidant properties, phenolic acids and flavonoids have previously been reported as potent bacterial quorum-sensing inhibitors [11, 32, 55-57]. Their mechanism of action primarily involves disrupting the synthesis of QS mediators and their receptors [14]. Flavonoids group such as flavones, flavonols, isoflavones, and C3-substituted flavonols, have been reported in earlier studies to inhibit LuxR receptors via structural mimicry of AHL autoinducers and blockade of protein binding sites [58-60]. Specifically, Catechin, quercetin, kaempferol, naringenin, apigenin, baicalin and several other flavonoids have been shown to interfere with QS in P. aeruginosa PAO1 and C. violaceum CV026, either by direct modulation of the target promoter transcription or by an inhibition of virulence factor production [11, 45, 16-17].

Overall, these findings provide more evidence that the medicinal plants from Burkina Faso are rich in anti-QS molecules. The research into these compounds remains essential to significantly enhance our therapeutic arsenal with innovative anti-infective agents in the fight against pathogenic bacteria. A. conyzoides presents a promising source of anti-quorum-sensing molecules that could serve as alternatives to conventional therapies. Further molecular investigation is needed, starting with compound identification, to elucidate the precise mechanisms of action.

Conclusion

This study highlights the potential of bioactive compounds from A. conyzoides as QS inhibitors, which in turn presents promising prospects for innovative therapeutic strategies. Although, bioassay-guided fractionation did not allow for the complete isolation of pure bioactive compounds, the enriched subfraction F2-5-3 showed significant anti QS activity. Future studies are needed to elucidate the molecular interactions of chemical compounds involved in the QS system. In an era where antibiotics alone are insufficient against persistent biofilm-associated infections, identifying molecules that act synergistically with existing treatments becomes imperative.

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Author’s Contributions

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Lassané Ouedraogo: Methodology Formal analysis, Review and Editing.
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Ablassé Rouamba: Supervision, Ressources.
Vincent Ouédraogo: Conceptualization, Writing-Editing.
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Moussa Compaoré: Formal analysis, Revision.
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Ethics

The article is an original and includes unpublished material. The corresponding authors assures that all other authors have read and approved the manuscript and that no ethical issues are involved.

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