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Anti-motilities and anti-biofilm effects of *Ageratum conyzoides* L. methanol extract

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Background: Infectious diseases are one of the leading causes of death worldwide because of antibiotic resistance. *Ageratum conyzoides* is one of the antimicrobial medicinal plants that is being used to fight various multi-resistant pathogenic bacteria in Burkina Faso.

Aim: The aim was to promote safe medicinal use of *A. conyzoides* by highlighting the anti-biofilm and anti-motility effects of its methanol extract.

Setting: The study was conducted at the *Université Joseph KI-ZERBO*, Ouagadougou, Burkina Faso.

Methods: The antibacterial activities of methanol extract were evaluated by evaluating swimming, swarming and twitching motilities performed in an agar medium. The anti-biofilm effect was conducted in microtiter plates using the crystal violet method. The antioxidant and enzyme inhibition activities were evaluated using 2,2-diphényl-1-picrylhydrazyl; 2,2'-azino-bis (3-éthylbenzothiazoline-6-sulfonic acid), Ferric Reducing Antioxidant Power and conducting lipoxygenase test.

Results: From the study, 100 µg/mL and 200 µg/mL of extract presented significant inhibition of *P. aeruginosa* and *E. coli* swarming motility but did not exhibit a significant effect on *P. aeruginosa* swimming and *E. coli* twitching motilities. The extract was effective in reducing biofilm formation in a concentration-dependent manner without affecting bacterial growth. In addition, the extract showed some capabilities to inhibit lipoxygenase activity and exhibit antioxidant potential, which could contribute to the control of oxidative stress-related diseases.

Conclusion: From this study the anti-biofilm and anti-motility potential of the *A. conyzoides* extract provided the experimental background for the further development of antibacterial drugs.

Contribution: This study provided additional scientific evidence to support the use of *A. conyzoides* in traditional medicine against bacterial infections.

Keywords: *Ageratum conyzoides*; antioxidant; biofilm; motility; swimming; swarming, twitching; *Pseudomonas aeruginosa*; *Escherichia coli*.

Introduction

Infectious diseases are one of the leading causes of death worldwide (Naghavi et al. 2024; WHO 2020). In Burkina Faso, infectious diseases rank as the third leading cause of death in the country, after malaria and malnutrition diseases. In 2014, the mortality (8.2%) and the case fatality rate (23.4%) in hospitals were significantly greater than acute malaria (6.8%). In 2016, these infectious diseases were the second leading cause of maternal and child mortality in hospitals (ThinkWell 2020).

Antibiotics and antiviral drugs are commonly used to treat these various infectious diseases. The use of antibiotics has successfully treated bacterial infections, thus saving lives, and improving the health of many patients in the world (Micoli et al. 2021). They contributed to a reduction in worldwide mortality from 216% deaths in 1950 to 39% deaths in 2017 (Browne et al. 2021; Burstein et al. 2019). Since then, antibiotic consumption has increased significantly. In 2018, global antibiotic consumption was 46% greater than in the 2000s (Browne et al. 2021). In 2015, Burkina Faso used up 52.797 billion Communauté Financière Africaine dans les pays de l'Union Economique et Monétaire Ouest Africaine (CFA) francs for importing antimicrobials, that is, almost 50% of all pharmaceutical imports

(Ministère de la santé 2017). As a result, the national average rate of antibiotic prescription in health facilities increased from 75.27% in 2010 to 83.2% in 2017 (Sana et al. 2019). This rate is higher than the WHO standard (≤ 30%).

The overuse of antibiotics in human and animal health has its effect on socioeconomic and environmental conditions. It has allowed the emergence, development and spread of antibiotic-resistant bacteria (Ahmed et al. 2024; WHO 2021). The effectiveness of antibiotics is questioned. Over 90% of *Salmonella* spp and *Escherichia coli* isolates in 2012–2013 were reported to be resistant to first-generation antibiotics (Maltha et al. 2014). Recently, data from the Health Department in 2018 and 2019 reported that *E. coli* resistance level to penicillin and sulphonamides was 80% in both years (Ministère de la santé 2019). *Klebsiella* spp. resistance to quinolones was around 50%. *Acinetobacter baumanii* resistance to ticarcillin and imipenem was 64.6% and 17.7% respectively, while *Pseudomonas aeruginosa* resistance to ticarcillin ceftazidime and imipenem was 100% (Ministère de la santé 2018, 2019).

The practice of using medicinal plants to treat bacterial infections is widespread in Burkina Faso (Zizka et al. 2015). Plant-derived compounds could provide an effective and efficient approach against pathogenic bacteria (Gautam et al. 2023). Flora in Burkina Faso offers a wide range of plants from which antibacterial compounds can be extracted. *Ageratum conyzoides* is an annual herbaceous plant with a long history of traditional medicinal uses (Nacoulma 1996; Zizka et al. 2015). This plant is well-known for its antimicrobial properties. It is known for the treatment of various conditions, such as burns, wounds and chronic infected wounds.

The aim of the study was focused on the promotion and enhancement of traditional medicine in Burkina Faso specifically to provide more scientific evidence in support of the use of *A. conyzoides* in traditional medicine against bacterial infections.

Research methods and design

Plant materials and extraction

The whole plant was collected at Gamplela (Ouagadougou, Burkina Faso). The voucher specimen has been registered at the National Herbarium of Burkina Faso under the code 8755. The plant samples were air-dried, ground to powder and kept tightly closed in glass containers until the extract process was performed. The methanolic maceration at 37°C was carried out. The extract was concentrated by using Rotavapor and stored at 4°C for further experimentation process.

Bacteria strains, media and chemicals

Two bacterial strains were used in this study: *Pseudomonas aeruginosa* PAO1, from the Plant Biotechnology Laboratory (LBV) of the Université Libre de Bruxelles (Belgique) and *Escherichia coli* ATCC 25922, from the bacteriology laboratory of the Centre Muraz, Bobo Dioulasso, Burkina Faso. Both strains were maintained in Lauria-Bertani (LB) broth (10 g/L tryptone, 5 g/L yeast extract and 5 g/L NaCl) and LB agar before being inoculated into the motility test. Media and chemicals used, acetic acid, crystal violet, glucose, yeast extract, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), aluminium trichloride, nutrient agar and LB culture medium were obtained from Sigma-Aldrich, Germany.

Anti-motility assay

Anti-motility assays were conducted in glass Petri dishes (75 mm diameter) by using 50, 100 and 200 µg/mL of methanolic extract. The swarming medium was composed of 8 g/L of LB, 0.5% agar (wt/vol) and D-glucose (5 g/L). The swim and twitch media used were LB broth supplemented with 0.3% and 1.0% (w/v) agar, respectively (O'May & Tufenkji 2011). Two different concentrations of 10 mg/mL extract were first prepared in DMSO 100% and 50%. For the assays, 200 µL of 10 mg/mL extract (in DMSO 100%) were added to 40 mL and 20 mL in agar medium in order to obtain the respective final concentrations of 50, 100 µg/mL (in DMSO 1%). Then 400 µL of 10 mg/mL extract (in DMSO 50%) was added to 20 mL agar medium in order to obtain a final concentration of 200 µg/mL extract (in DMSO 1%). The DMSO 1% (200 µL DMSO 100% in 20 mL agar medium) has been used as a negative control. A total of 20 mL of each agar medium (with control or extracts at different final concentrations) were aliquoted into Petri dishes. Finally, 20 mL of each agar medium (containing the control or the extracts at different final concentrations) were poured onto the Petri dishes and dried overnight at room temperature.

Bacteria (10^7 CFU/mL) were point inoculated as 5 µL aliquot of an overnight culture, with a sterile loop and incubated for 18 h (37 °C/PAO1 and 30 °C/*E. coli*). Motility was estimated by measuring the circular turbid area formed by bacterial cells moving from the point of inoculation. Overall, three independent experiments were performed. Each experiment was then repeated using three independent cultures. Images from the independent experiments were examined after 18 h.

Anti-biofilm formation assay

Biofilm formation was analysed by using crystal violet according to the method described by O'Toole and Kolter (1998). Briefly, bacterial strains culture supplemented with extract (50 µg/mL, 100 µg/mL and 200 µg/mL) were grown for 18 h. After growth, the strains were rinsed with water and then fixed with 99% methanol. The methanol was washed off after 15 min. Crystal violet (0.1%) was added to each well (2 mL/well) for 30 min. The biomass of attached cells (biofilm) was quantified by solubilisation of the dye in acetic acid (33%).

Antioxidant assay

The antioxidant (DPPH, ABTS and FRAP) activities of the plant extract were evaluated as described by Compaore et al. (2016). The anti-DPPH ability was expressed as sample concentration scavenging 50% of DPPH

radicals (IC_{50}). The ABTS radical cation decolourisation assay was expressed as mg Trolox ($R^2 = 0.998$) equivalent per gram of extract. The evaluation of the reducing power FRAP was expressed as mmol ascorbic acid ($R^2 = 0.9996$) equivalent per gram of extract (mmol EAA/g extract). Quercetin, gallic acid and ascorbic acid were used as reference substances.

Anti-inflammatory assay

The anti-inflammatory assay was carried out by evaluating the lipoxygenase inhibition effect. The inhibitory activity of the methanolic extract was investigated by using the test developed by Malterud and Rydland (2000). This reaction medium was a mixture of 100 µL of extract prepared in borate-methanol buffer (1%) and 400 µL of 15-LOX (167 U mL^{-1}). The inhibitory percentage was determined.

Data analysis

The data were expressed as means (± s.d.). GraphPad Prism 9.3.1471 software was used for statistical analysis (GraphPad software Inc., San Diego, CA, USA). One-way ANOVA followed by Bonferroni test, a $p \leq 0.05$ was considered statistically significant.

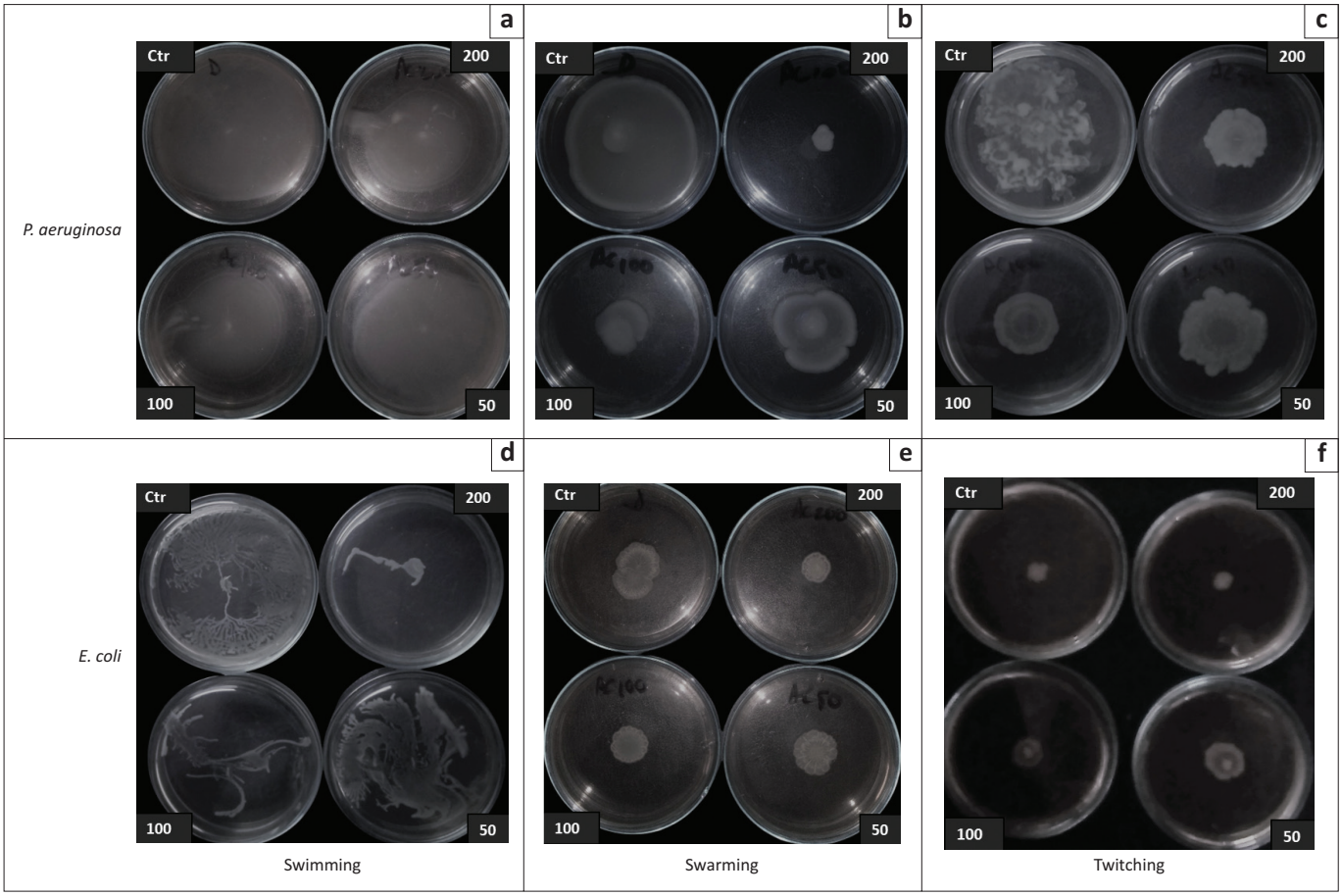
Ethical considerations

This article followed all ethical standards for research without direct contact with human or animal subjects.

Results and discussion

Antimotility assay

Methanol extract did not inhibit *P. aeruginosa* swimming at 200, 100 and 50 µg/mL (Figure 1a). Visually, swimming of *E. coli* was significantly reduced at 200 µg/mL. This effect was concentration dependent and *E. coli* was more sensitive than *P. aeruginosa*. As regards swarming motility, *P. aeruginosa* has formed tendrils like a clover leaf moving outward from the point of bacterium inoculation (Figure 1c and d), with continuous branching as the bacterium moved away from the negative control dish centre (Figure 1c). The same pattern was observed with *E. coli* swarm (Figure 1d); however, *E. coli* formed dendritic arborescent projections from the centre. This arborescent that was either weak or abashed was found to produce more dendritic clusters around the edges of the plate. This arborescent dendritic form was in disarray (Figure 1d). Interestingly, in the presence of the extract, *P. aeruginosa* was able to grow but in small-diameter (Figure 1c). This characteristic motility agrees with previous



Source: Compaoré, E., 2024, 'Interet médicinal de Ageratum conyzoides dans la lute contre la resitance bactérienne: interference avec le quorum sensing et investigation phytochimique', PhD Thesis; Jospeh ki-Zerbo University, Burkina Faso

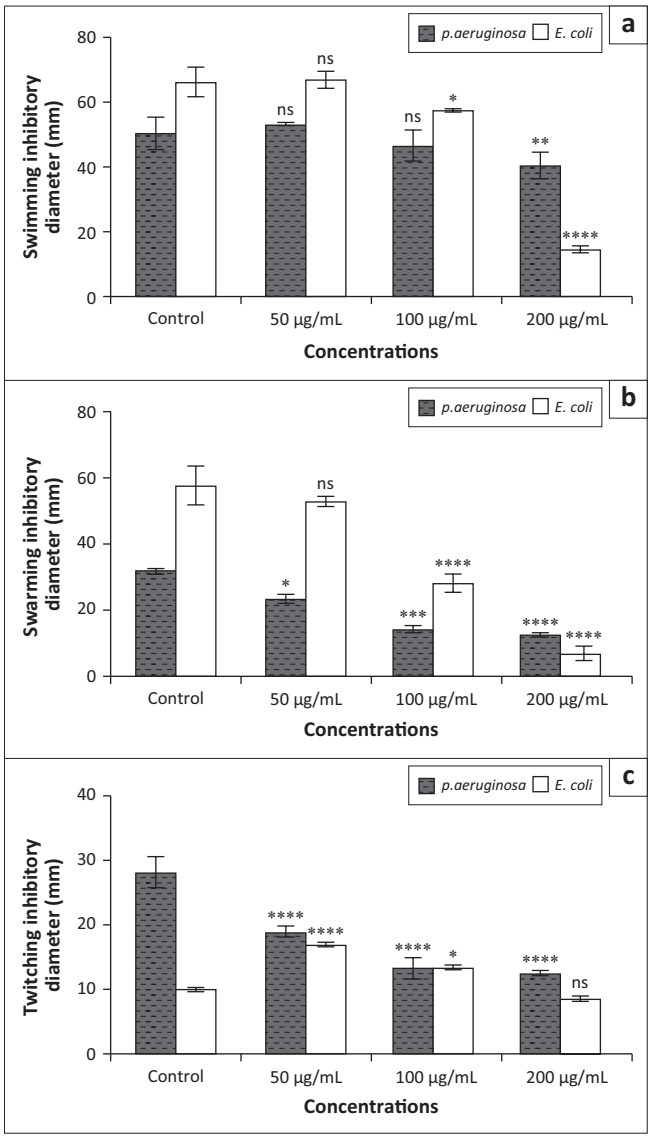
Note: Numbers 50, 100, 200, indicate concentration in mg/mL.

Ctr, control.

FIGURE 1: Colonies tendril and dendritic projection. (a,b) Swimming; (c,d) Swarming; (e,f) Twitching.

P. aeruginosa and *E. coli* swarming motility effects reported by some workers using other plant extracts (Magnini et al. 2021; O'May & Tufenkji 2011; Partridge 2022). At 100 µg/mL and 200 µg/mL, *P. aeruginosa* swarming motility appeared to be completely disrupted, as the inoculated aliquots showed no distinct tendril-like growth, giving small swarming areas with smooth edges. The *E. coli* swarm motility appeared to be reduced with shorter arborescence at 200 and 100 µg/mL (Figure 1D). The *P. aeruginosa* twitching motility effects decreased progressively as the extract concentration increased (Figure 1E). In contrast, no reduction was observed on *E. coli* twitching motility diameter. Therefore, its motility was unaffected in all extract concentrations (Figure 1F). Overall, the *A. conyzoides* extract blocked the swarming motility of both bacterial strains but did not block *P. aeruginosa* swimming and *E. coli* twitching motilities.

Methanol extract (200 µg/mL) significantly inhibited the swimming motility of *E. coli* with 79.7% inhibition rate (Figure 2A) and moderately inhibited *P. aeruginosa* POA1 swimming (19.8% inhibition). Consequently, *E. coli* was more sensitive than *P. aeruginosa* POA1 at 200 µg/mL. Insignificantly, *P. aeruginosa* swimming diameters were inhibited at 100 µg/mL and 50 µg/mL. Interestingly, there was a positive correlation between the swarming inhibition and the extract concentration (Figure 2B). *Pseudomonas aeruginosa* swarming motility inhibition was recorded with 60.6% ± 0.04, 55.0% ± 0.11 and 27.0% ± 0.14% at 200, 100 and 50 µg/mL, respectively. *Escherichia coli* swarming motility inhibition was 87.7% ± 0.21; 51.2% ± 0.27 and 8.4% ± 0.14 in the concentration distribution. Lastly, the twitching inhibitory diameter in Figure 2C shows that only *P. aeruginosa* twitching was significantly reduced. Its inhibition rates were changed from 33.7% ± 0.09, 52.8% ± 0.17 to 55.5% ± 0.08 for 50 µg/mL, 100 µg/mL and 200 µg/mL, respectively. Previously, it was demonstrated that swimming and swarming motilities were dependent on the flagella of bacteria and twitching motility was controlled by the type of pili, so the anti-motility effect of extract could be because of its interaction with flagella or pili (Nakamura & Minamino 2019; Partridge 2022).



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Note: Results are expressed as mean ± s.d. $n = 3$; *** indicates $p < 0.001$, ns: non significative compared to untreated control.

FIGURE 2: Colonies diameter inhibition zones. Each experiment was conducted using three independent cultures and representative values are shown.

Biofilm formation

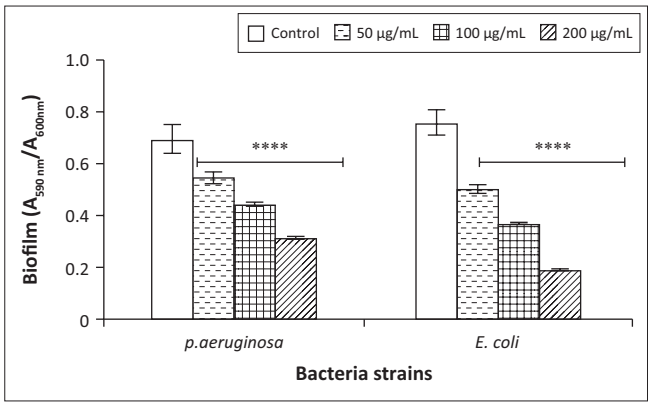
The antibiofilm effect that was showed in the inhibition of *P. aeruginosa* biofilm was significantly pronounced (Figure 3) at 200 µg/mL (54.7%) and 100 µg/mL (36.2%). Similar observations were found in *E. coli* anti-biofilm formation (75.3% and 51.3% at 200 and 100 µg/mL, respectively). Remarkably, the extract had a stronger impact on *E. coli* biofilm formation than *P. aeruginosa* biofilm. Moreover, the biofilm formed by *E. coli* was reduced even at the lowest concentration of 50 µg/mL (33.8%) in contrast to the biofilm formed by PAO1 (21.5%). It was noticed that more than > 50% inhibition was recorded for both strains at 100 µg/mL. There was no significant effect on the growth of planktonic cells of *P. aeruginosa* and *E. coli* strains (data did not show) suggesting that the anti-biofilm effect (Figure 2) as well as anti-motility effects (Figure 1) were not related to a decrease in bacteria viability. Overall, the methanol extract of *A. conyzoides* was found to significantly affect swimming (*E. coli*), swarming (*P. aeruginosa* and *E. coli*) and twitching (*P. aeruginosa*) motilities. The biofilm formed was then significantly inhibited in these two bacterial pathogens.

Many bacterial species, such as *P. aeruginosa*, *E. coli* and *Staphylococcus* spp., are natural biofilm-formers (Cangui-Panchi et al. 2022). The chronic infections because of biofilm-forming bacteria are generally associated with persistent inflammation and tissue damage (Sharma et al. 2023). In particular, chronic pseudomonas and *E. coli* infections are difficult to treat and persist even after antibiotic treatment or host immune and inflammatory responses. These pathogens enclosed in a biofilm are more resistant to antibiotics than planktonic cells (Sharma et al. 2023). So, the disruption of the bacteria motility and biofilm formation is a promising

mitigation of bacterial pathogenesis (Araújo et al. 2024). The presence of extract could therefore contribute to the exposure of cells to the antibiotic's bactericidal action.

Antioxidant and anti-lipoxygenase assay

The extract showed a good anti-free radical potential against both DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ (Table 1). The concentration of methanolic extract IC_{50} was 25.4 ± 0.5 µg/mL. The extract showed a reducing activity of ferric ion Fe $^{3+}$ to ferrous ion Fe^{2+} (0.95 ± 0.52 mmol EAA/g) but was lower than those of quercetin (3.56 ± 0.07 mmol AAE/g) and gallic acid (6.43 ± 0.08 mmol/g). Previous work has shown that the methanol extract of *A. conyzoides* had substantial antioxidant activity by scavenging DPPH free radicals (IC_{50} = 46.01 ± 2.23 µg/mL) (Nasrin 2013). The IC_{50} value in our study was relatively two-fold smaller. This suggests a conceivable safe and promising use of the *A. conyzoides* methanol extract as a potential antioxidant. The extract reduces iron III (Fe^{3+}) to iron II (Fe^{2+}) because of natural antioxidants such as flavonoids, phenols and terpenoids, which are electron donors to Fe^{3+}. These phytochemicals are involved in redox reactions, in which ferric iron is reduced to ferrous iron. The reduction of Fe^{3+} to Fe^{2+} by the extract could therefore improve the absorption of iron into the body and so prevent iron deficiency and diseases related to oxidative stress. This antioxidant activity of the extract would provide evidence for the lipoxygenase inhibitory power (50% inhibition) at 150 µg/mL ± 5.7 µg/mL. This inhibitory power presented by the extract could be explained by its capacity to reduce the iron III to iron II within the structure of the enzyme (Dobbelaar et al. 2021).



Source: Compaoré, E., 2024, 'Interet médicinal de Ageratum conyzoides dans la lute contre la resitance bactérienne: interference avec le quorum sensing et investigation phytochimique', PhD Thesis; Jospeh ki-Zerbo University, Burkina Faso

****, Significantly different compared to DMSO treatment ($p < 0.0001$).

FIGURE 3: Anti-biofilm formation effect.

TABLE 1: Antioxidant activity and lipoxygenase inhibition effect.

Samples	DPPH: IC_{50} (µg/mL)	ABTS: (mmol TE/g)	FRAP: (mmol AAE/g)	Lipoxygenase: IC_{50} (µg/mL)
Methanolic extract	25.4 ± 0.500	1.007 ± 0.01	0.95 ± 0.52	150 ± 5.70
Quercetin	8.87 ± 0.080	85.39 ± 1.32	3.56 ± 0.07	2.50 ± 0.12
Gallic acid	4.96 ± 0.026	86.40 ± 4.61	6.43 ± 0.08	Non determinated

Source: Compaoré, E., 2024, 'Interet médicinal de Ageratum conyzoides dans la lute contre la resitance bactérienne: interference avec le quorum sensing et investigation phytochimique', PhD Thesis; Jospeh ki-Zerbo University, Burkina Faso

Note: Values are expressed as mean values ± s.d. (n = 6 independent experiments).

DPPH: 2,2'-Diphenyl-1-picrylhydrazyl; ABTS: 3-ethylbenzothiazoline-6-sulfonic acid. FRAP: Ferric reducing antioxidant: IC_{50}: Intermaximal inhibitory concentration. mmol EAA/g: Millimol ascorbic acid equivalent/gram. mmol TE/g: millimol trolox equivalent/gram.

Oxidative stress was an additional factor that induced biofilm overgrowth (Singh et al. 2021). Under oxidative stress, the overproduced biofilms protect the bacteria from oxidative radicals. The extract showed a potent ability to scavenge free radicals (DPPH$^{\bullet+}$ and ABTS$^{\bullet+}$) and caused the reduction of Fe^{3+} to Fe^{2+} involved in maintaining biofilm matrix integrity. This relationship between biofilm and reducing power activity has been reported in several studies (Lin et al. 2012; Oh et al. 2018; Soldano et al. 2020). Earlier studies have shown that iron acquisition was necessary for biofilm formation by *P. aeruginosa* and *E. coli* (Hancock, Ferrières & Klemm 2008; Kang & Kirienko 2018). The iron is essential for swimming, swarming, twitching motilities and biofilm growth in these species (Frick-Cheng et al. 2024; Kang & Kirienko 2018). It affects the flagella, pili and other adhesive structures involved in biofilm formation. In the biofilm formation process, *P. aeruginosa* and *E. coli* use iron in the ferric form (Fe^{3+}) (Hancock et al. 2008; Kang & Kirienko 2018). *Pseudomonas aeruginosa* secretes siderophores such as pyoverdine to bind ferric iron (Fe^{3+}) and facilitate its transfer into the cell (Ghssein & Ezzeddine 2022). *Escherichia coli* also uses siderophores such as enterobactin to scavenge (Fe^{3+}) (Tsylents et al. 2024). Hence, the reduction of Fe^{3+} to Fe^{2+} could disrupt iron availability, and therefore limit biofilm formation and stability. Iron chelators therefore disrupt the structure of the biofilm and facilitate the removal of bacteria by antibiotics.

Lipoxygenases are an important group of enzymes in the inflammatory and immune responses to bacterial infections (Amoah et al. 2024). Their over-expression favours the development of inflammation-related diseases. Inhibition of lipoxygenase activity may be beneficial in reducing the seriousness of *P. aeruginosa* infection. *Pseudomonas aeruginosa* has been reported to secrete a functional lipoxygenase (15-LOX) that promotes the invasion and persistence of *P. aeruginosa* in lung tissue (Amoah et al. 2024; Morello et al. 2019). Therefore, interference with lipoxygenase may contribute to reduced lung pathogenesis triggered by this antibiotic-resistant pathogen.

The potency shown by the *A. conyzoides* methanol extract may be related to some of the bioactive compounds found in the extract. Our previous work conducted on the anti-quorum sensing potential of this methanol extract has identified some flavonoids and phenolic compounds such as gallic acid, vanillic acid, ellagic acid, sinapic acid and quercetin (Compaoré et al. 2022). These natural products seem to be promising agents that could provide new strategies against biofilm-associated infections. Anti-motility and anti-biofilm effects are not a straightforward solution against antibiotic resistance. In the same way, the anti-QS effect of our previous study cannot remove all the persistent bacteria but instead allows them to be more accessible to the immune system.

Conclusion

This work constituted the first report on the antibacterial properties of *Ageratum conyzoides* against the mobility of multidrug-resistant *P. aeruginosa* and *E. coli*. From the findings, the traditional use of *A. conyzoides* in wound treatment and disinfection appears justified in preventing and combating infections caused by multidrug-resistant pathogens such as *P. aeruginosa* and *E. coli*. *Ageratum conyzoides* methanolic extract provided a starting point for further characterisation of anti-motility and anti-biofilm compounds, which may decrease the risk of bacterial antibiotic resistance, to prevent *P. aeruginosa* and *E. coli* biofilm related infections. Our finding could help to promote the optimal and safe use of *A. conyzoides* as an antimicrobial and antioxidant. This study also opened the perspectives of a research on molecules able to inhibit the bacterial motility and the formation of biofilm.

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Competing interests

The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article.

Authors' contributions

E.C. was involved in conceptualisation, methodology, formal, analysis and writing the draft. M.C. was responsible for methodology, formal analysis, data curation, writing, revision and editing. V.O. was responsible for conceptualisation, formal analysis, co-supervision, writing and editing. A.R. was responsible for co-supervision, and resources. A.B. was responsible for writing, revision and resources. M.R.D. was responsible for conceptualisation, methodology, writing and revision. M.K. was responsible for supervision, validation and funding acquisition.

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Data availability

All data generated or analysed during this study are included in the article.

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