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Targeting multi-drug-resistant ESKAPE pathogens: antibacterial, antioxidant, cytotoxicity, and metabolic profiling of selected Cameroonian plants

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Abstract

Background Different parts of *Allanblackia floribunda, Calotropis procera, Hymenocardia acida, Irvingia gabonensis, Newbouldia laevis*, and *Xylopia acutiflora* have been used traditionally across different parts of Cameroon to overcome infectious diseases, especially pneumonia. Hence, this study investigated the antibacterial potential of six Cameroonian medicinal plants against selected ESKAPE pathogens (*Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Escherichia coli, Pseudomonas aeruginosa)* as well as *Streptococcus pneumoniae*.

Methodology The inhibitory activities of different extracts from six Cameroonian plants against the pathogens were evaluated by determining their MICs. Subsequently, the top four bioactive plant extracts were assessed for antibiofilm activity, time-kill kinetics, cytotoxicity (Raw and Vero cell lines), as well as antioxidant activities. Finally, the effect of the most potent extract, viz. ethanolic extract of *Xylopia acutiflora* steam bark, on bacterial morphology was elucidated through scanning electron microscopy while its phytochemical composition was profiled using liquid chromatography–mass spectrometry (LC–MS).

Results Twenty-two out of the 32 prepared extracts showed significant antibacterial activity, with MICs varying from 31.5 to 1000 µg/mL. The ethanolic, methanolic, and hydroethanolic extracts from *Xylopia acutifolia* and ethanolic extract from *Colotropix procera* exhibited broad-spectrum activity, inhibiting and eradicating bacterial biofilm. Furthermore, the extract from *X. acutifolia* was shown to be the most effective scavenger against DPPH (IC_{50}; 83.79 ± 1.92 µg/mL) and FRAP (IC_{50}; 22.89 ± 1.36 µg/mL) radicals, while *C. procera* extract was the most effective against ABTS (IC_{50}; 67.95 ± 1.83 µg/mL). The extracts were demonstrated to possess low cytotoxicity on both Raw and Vero cell lines. In addition, SEM revealed that *X. acutifolia* elicited cell membrane rupture and consequently cytoplasm leakage in *E. coli* and *P. aeruginosa*. Twenty-four different compounds were detected in the *X. acutifolia* extract via LC–MS analysis, and it was hypothesized that the recorded bioactivity in the extract might be ascribed to these compounds.

Conclusion Results from this study have scientifically validated the ethnomedicinal uses of the six Cameroonian plants as therapeutics for infections with *X. acutiflora* ethanolic extract displaying the highest bioactivity. Thus, there is the need for further investigations into phytochemicals from these plants as they could serve as important sources of novel antioxidants and antimicrobial agents.

Keywords Antibacterial, Antibiofilm, Cytotoxicity, ESKAPE pathogens, Phytochemical, Multi-drug resistance

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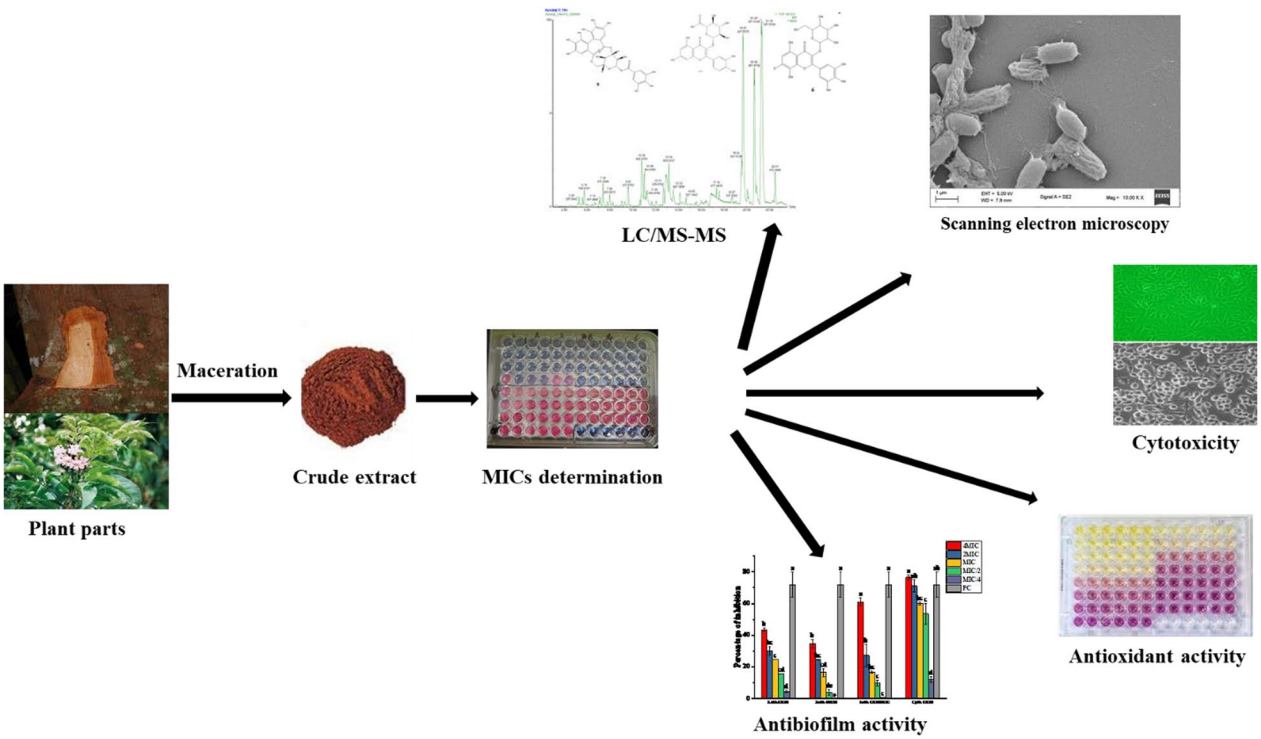
Graphical abstract



Background

The indiscriminate consumption of antibiotics, especially antibacterials, has culminated into a loss in the effectiveness of many of these drug compounds against various pathogens, leading to a rise in the prevalence of drug-resistant microbes [1, 2]. The multi-drug-resistant ESKAPE bacterial group, viz. *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter* spp., are responsible for most of the common fatal infections constituting a major public concern [3]. Consequently, the World Health Organization (WHO) has prioritized these notorious bacteria in its global surveillance of antibiotic resistance [4]. Efforts to develop new antimicrobial medications have accelerated in the last two decades to mitigate the deleterious effects of these ESKAPE pathogens and other antibiotic-resistant strains [5].

Infectious bacteria have been established to exhibit their pathogenicity via the generation of a range of reactive nitrogen and oxygen species—such as O_2^-, H_2O_2, and HO^-, NO^-, NO^+, RS–NO, and dinitrosyl iron complexes—which elicit oxidative damage in host cells [6]. Hence, there is a pertinent need for antibiotic compounds with concomitant antioxidant activity to mop up free radicals and restore homeostatic balance in host cells. In this sense, plants, which naturally produce a diverse range of compounds as defenses against microbes, insects, and herbivores, remain one of the most valuable resources in the search for novel antibiotics [7]. These plant compounds, known as phytochemicals, are typically produced in response to disease or environmental stress, nutrition, reproduction, and natural plant aesthetics [8]. Interestingly, a significant number of these phytocompounds have been demonstrated to possess numerous bioactivities including but not limited to anthelminthic, antioxidant, anti-hypertensive, antimicrobial, anti-insecticidal, gut-modulating, hepatoprotective, radical scavenging, and nephroprotective activities, which have bestowed on them important roles in human and animal health [9].

More than three-fourth of the global population are dependent on plant resources for healthcare, with even higher reliance in developing countries due to the affordability, accessibility, lower side effects, and indigenous knowledge of the selective plant efficacy against a specific disease [10, 11]. Hence, herbal plants are integral to traditional medicine practices, and their therapeutic potential

is increasingly recognized in modern pharmacology. However, thorough assessments of the biological activities, toxicities, and biochemical composition of plant materials are required to promote their potential as sources of drug compounds, including antimicrobial agents [12]. It was estimated that 25% of conventional medicines are of plant origin despite the fact that less than 15% of plant species have been investigated for their medical use, hence opening a wide window of opportunities for drug discovery [13]. Plant selection is one of the most important steps in pharmacology, and plants with remarkable ethnomedicinal histories are believed to possess the most potential for drug discovery [14].

The flora of Cameroon is rich in herbal medicines that have served various therapeutic purposes since time immemorial [15]. The plant selection was based on their ethnomedicinal uses in different parts of Cameroon as therapeutics for ailments and diseases that have since been linked to various bacterial pathogens. For instance, *Allanblackia floribunda* is a commonly used traditional plant in Cameroon to relieve many symptomatic diseases such as asthma, bronchitis, cough, dysentery, hypertension, respiratory tract infections, stomach pains, as well as toothache [16, 17]. *Irvingia gabonensis* was also noted to attenuate symptoms accruing from asthenia, diarrhea, dysentery, inflammation, and yellow fever among others [18]. Similarly, *Hymenocardia acida* is used traditionally to treat conditions such as abscesses, diabetes, diarrhea, dysentery, eye infections, skin diseases, syphilitic sores, pains, and pulmonary infections [19]. *Newbouldia laevis* is used to treat asymptomatic diseases like convulsion, epilepsy, inflammation, and pain [20], diabetes, malaria, and pile [21], while *Xylopia acutiflora* is known for treating febrile pains and pneumonia [22]. The ethnopharmacological importance of these selected medicinal plants highlights their potential as valuable sources of antibacterial agents, thus opening a new vista for drug research and development. Therefore, this study aimed to validate the ethnopharmacological use of six Cameroonian medicinal plants and highlight their potential as sources of lead antibacterial compounds. In this regard, the *in vitro* antibacterial potential of the plants on six pathogenic bacteria (five ESKAPE pathogens, viz. *Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Escherichia coli, Pseudomonas aeruginosa*, and one non-ESKAPE pathogen, *Streptococcus pneumoniae*), their antioxidant properties as well as their cytotoxic activities were investigated.

Materials and methods

Materials

Plants

Six medicinal plants, viz. *Allanblackia floribunda* Oliv. (Clusiaceae) (1380/HNC), *Calotropis procera* (Aiton) Dryand (Apocynaceae) (7808/SRF/Cam), *Irvingia gabonensis Baill.* (Irvingiaceae) (28,054/HNC), *Newbouldia laevis* (P.Beauv.) Seem. (Bignoniaceae) (29,469/HNC), *Hymenocardia acida* Tul. (Euphorbiaceae) (50,114/HNC), and *Xylopia acutiflora* (Dunal) A.Rich (Annonaceae) (28,718 SRF/Cam), were collected in January 2022 from the Bandounga forest, Tonga, Cameroon. The plant species were all identified botanically, and the voucher specimens were all deposited at the Cameroon National Herbarium. Furthermore, the botanical names of the plants used in this study were validated through "The World Flora Online" (http://www.worldfloraonline.org/), and they adhered to the latest revision as of September 10, 2024.

Chemicals and reagents

Ascorbic acid, resazurin, and ciprofloxacin were supplied from Sigma Aldrich. All other chemicals were of analytical grade and purchased from validated suppliers.

Bacterial strains and cell lines

The bacterial strains: *Acinetobacter baumani* NR 13374 (AC NR 13374); *Pseudomonas aeruginosa* NR 48982 (PA NR 48982); *Klebsiella pneumoniae* NR 41897 (KP NR41897), were from BEI Resources (Biodefense and Emerging Infections Research Resources Repository); *Escherichia coli* ATCC 25922; *Staphylococcus aureus* ATCC 43300 from American Type Culture Collection (ATCC); and *Streptococcus pneumoniae* isolate (Spi) from Centre Pasteur du Cameroun (CPC), Yaoundé, Cameroon. Mueller–Hinton (MH) agar slant was used to maintain the bacterial cultures at 4 °C throughout the study. The macrophage cell line Raw 264.7 and Vero cell lines ATCC CRL 158 were obtained from CPC.

Methods

Plant extraction

The parts of the selected plants, viz. stem barks and leaves selected based on their ethnopharmacological used, were air-dried, powdered, and subsequently extracted by maceration using different solvents: water, hydro-ethanol (70/30 v/v), ethanol, methanol, and dichloromethane. Briefly, 50 g of the pulverized samples was submerged in 500 mL of each solvent, stirred, and gently agitated at 25 ± 2 °C for 48 h. Whatman® qualitative filter paper, Grade 1, was used to filter the resulting solutions, concentrated with rotavapor (Rotavapor ® R—100, Büchi, Switzerland) at 40 °C, and finally air-dried to remove residual solvents. The plant extracts were refrigerated (4 °C) until subsequent analysis.

Antibacterial activity

Minimum inhibitory concentrations (MICs) of plant extracts The MICs of the extracts were assessed using the microdilution method according to Nguena-Dongue., et al. [18]. Crude extract preparation was done in concentrations of 15.62–1000 µg/mL; afterward, 0.1 mL of standardized bacterial suspension (10^6 CFU/mL) was added to achieve final volumes of 0.2 mL. Ciprofloxacin (0.039–5 µg/mL), inoculated media, and sterile media served as the positive, negative, and sterility controls, respectively. The microplates were incubated at 37 °C for 24 h; subsequently, 20 µL of resazurin (0.15 mg/mL) was added as the indicator. The MIC was defined as the lowest plant extract concentration that resisted a change from blue to pink coloration. The most active extracts were selected for subsequent experiments.

Time-kill kinetics

This was carried out according to Klepser et al. [23] with some modifications; ciprofloxacin served as the positive control. Briefly, 100 µL of MH broth containing plant extracts at 8 MIC was introduced in the first well of 96-well microplates, followed by serial dilution. Subsequently, 100 µL of bacterial suspension (1×10^6 CFU/mL) was introduced in individual wells (except sterility control wells) and incubated at 37 °C for 0, 2, 4, 6, 8, 12, and 24 h. Optical densities at 630 nm were taken after each incubation period using a microplate reader.

Biofilm inhibition and eradication

Initially, the biofilm produced by each test organism was quantified according to Nguena-Dongue et al. [18], to identify the best conditions for biofilm production of each strain. Subsequently, the 50% minimum biofilm eradicative concentration ($MBEC_{50}$) and minimum biofilm inhibitory concentration ($MBIC_{50}$) of the selected extracts were evaluated by broth microdilution methods. Briefly, four twofold dilutions of the samples prepared at 8 MIC in Brain Heart Infusion (BHI) broth supplemented with 1% glucose (*P. aeruginosa*) and BHI broth (*E. coli*) were done in a microplate. Afterward, 0.1 mL inoculum (2×10^6 CFU/mL) was added in all the wells except sterility control wells. Ciprofloxacin served as the positive control. Microplates were kept for 24 h (*E. coli*; 37 °C, *P. aeruginosa*; 25 °C) and then treated with the indicator dye (crystal violet); finally, the absorbances at 590 nm were recorded [24]. For the determination of $MBEC_{50}$, the biofilms of each strain were first formed under the same conditions for 24 h before being exposed to different concentrations of the extracts (4MIC to MIC/4) and re-incubated for another 24 h.

Bioactive constituents and antioxidant properties

Preliminary phytochemical screening The standard colorimetric methods described by Harborne [25] were adopted to evaluate flavonoids, saponins, phenolic compounds, triterpenes, and tannins presence in the selected plant extracts.

Quantification of phenolic, flavonoid, and tannin contents The total phenolic, flavonoid, and tannin content of the selected plant extracts was quantified according to Singleton and Rossi [26], Zhishen et al. [27], and Bainbridge et al. [28], respectively.

Antioxidant activity assays The anti-DPPH activities of the promising extracts were evaluated according to Sharma and Bhat, [29], and the 50% radical scavenging activity (RSA_{50}) was computed accordingly. Subsequently, the RSA_{50} was used to compute the 50% effective concentration ($EC_{50} = RSA_{50}/[DPPH\cdot]$) and antiradical power ($AP = 1/EC_{50}$). The $ABTS^{+}\cdot$ cation discoloration test was also carried out [30], while the RSA_{50}, $EC_{50,}$ and AP were recorded as done for the DPPH assay. The ferric iron-reducing activity of the selected extracts was evaluated according to Moffatt et al. [31] with slight modifications, and the 50% inhibitory concentration (IC_{50}) of Fe^{3+} was determined accordingly.

Cytotoxicity activity of plant extracts

Cytotoxicity on Vero and Raw cell lines was assessed using the resazurin viability cell method [32]. Briefly, cell suspensions (100 µL) were pipetted into a 96-well microplate at 10^4 cells/well and incubated at 37 °C for 24 h for adhesion. Subsequently, non-adherent cells were eliminated by emptying the wells, and 100 µL of new medium containing the extracts (various concentrations) was introduced and incubated in CO_2 incubators for 48 h at 37 °C. The culture medium, 10 µM podophyllotoxin, and cells without inhibitor served as the sterility control, positive control, and negative control, respectively. Afterward, 10 µL of resazurin (0.15 mg/mL) was added to the wells and kept for 4 h at 37 °C, 5% CO_2. Fluorescence was then recorded at 530 nm (excitation wavelength) and 590 nm (emission wavelength). The percentages of cell inhibition were evaluated based on obtained optical densities and used to plot dose–response curves from which the 50% cytotoxic concentration (CC_{50}) was calculated. The most active and least toxic extract was selected for the rest of the study.

Scanning electron microscopy

The action of the ethanolic extract of *X. acutifolia* stem bark on *E. coli* and *P. aeruginosa* morphology was

accessed using scanning electron microscopy (SEM). Overnight cultures of *P. aeruginosa* and *E. coli* were calibrated at 10^8 CFU/mL in 2 mL PBS buffer (pH 7.2) and centrifuged (7000 ×g for 5 min), and the pellets were rinsed twice with PBS. The bacterial cells were treated with 1 mL of the *X. acutifolia* extract (2.5 mg/mL) and incubated at 37 °C for 2 h while *P. aeruginosa* and *E. coli* suspensions without treatment served as controls. Subsequently, glutaraldehyde (2.5%) was used to fix bacterial lysates at 4 °C for 6 h and dehydrated in a graded ethanol series. The collected pellets were adhered to polished silicon wafers (10 ×10 mm) and dried for 24 h at 25 °C [33]. Images of the gold-coated samples were then captured using an S–3000 N scanning electron microscope (Hitachi, Tokyo, Japan).

LC–MS of Xylopia acutifolia stem bark ethanolic extract

For liquid chromatography–mass spectrometry (LC–MS) analysis, a Waters Acquity Ultra-Performance Liquid Chromatography (Milford, MA, USA) system coupled with an Acquity PDA detector. The extract was injected at a volume of 1 µL, and chromatographic separations were performed on a Waters UPLC BEH C18 column. The mobile phases used were water (A) and acetonitrile (B). The chromatography was implemented in the following gradient: gradient: 95% A, 5% B at 0.5 min; 56% A, 44% B at 20 min; 0% A, 100% B at 21 min; and 95% A, 5% B at 23 min. Each chromatography was run for 26 min with a flow rate of 350 µL min^{-1}. The conditions for MS analysis were positive mode with a cone and capillary voltages of 15 V and 3.0 kV, respectively, and 6 V low energy was utilized for general data acquisition [34]. Peak alignments were exported to MSFinder 3.5 to facilitate compound annotation by the alignment of the m/z ions (MS2 spectra) with those generated by in-silico fragmentation against a host of in-built database libraries [34].

Statistical analysis

Statistical analyses were conducted using the ANOVA (one-way analysis of variance) of Origin Pro 2024. The differences between the means were compared by the Tukey test at $p \leq 0.05$, and the results were expressed as mean ± standard deviation. All experiments were carried out in at least three replicates.

Results

Extraction yield

The solvent extraction of the selected medicinal plants showed that the extraction yield varied with the plant, the part, and the solvent used, with values ranging from 7.08 to 47.81% (Table 1). The stem bark of *I. gabonensis* recorded the highest yields of all the plant parts studied, with the aqueous extract giving the highest performance (47.81%). On the other hand, the yield of *A. floribunda* stem bark was found to be the lowest among the extracted plant parts. It was also observed that hydroethanol (70/30 v/v) was the best solvent for extraction across all the plant parts.

Antibacterial activity of plant extracts

Minimum inhibitory concentration

The MICs of the extracts from *A. floribunda*, *C. procera*, *H. acida*, *I. gabonensis*, *N. laevis*, and *X. acutiflora* that showed activity against at least one of the bacterial strains were recorded to be between 31.5 and 1000 µg/mL (Table 2). The *X. acutifolia* stem bark ethanolic, methanolic, and hydroethanolic extracts as well as the *C. procera* stem bark ethanolic extract displayed the broadest spectra of activity against five of the six tested bacterial strains (83.33%). However, the methanolic extract of *I. gaboneines* stem bark (IgSb MtOH) exhibited the lowest MIC against *S. aureus* ATCC 43300 and *S. pneumoniae*. Based on the broad antibacterial spectrum, the ethanolic, methanolic, and hydroethanolic (70%) extracts of *X. acutifolia* stem bark (XaSb EtOH, XaSb MtOH, and XaSb EtOH/H_2O), and the ethanol extract of *C. procera* stem bark (CpSb EtOH) were selected for further experiments.

Time-Kill kinetics

XaSb EtOH, XaSb MtOH, XaSb EtOH/H_2O, and CpSb EtOH were selected to evaluate the time-dependent activity on *E. coli* ATCC 25922. Without any extract treatment, the growth curve presented by *E. coli* can be differentiated in lag, exponential, and stabilization phases (Fig. 1). XaSb EtOH (at 2 MIC and 4 MIC), CpSb EtOH (2 MIC and 4 MIC), and XaSb EtOH/H_2O (4 MIC) completely inhibited the growth of *E. coli* ATCC 25922 with no significant difference ($P > 0.05$) when compared to Ciprofloxacin tested, thus demonstrating their bactericidal effect on *E. coli*. Moreover, *E. coli* under the effect of the subinhibitory concentration of the tested samples presented an extended lag phase with a lower intensity than the control. These results show that *C. procera* and *X. acutifolia* extracts inhibited the growth of *E. coli* in a dose–response manner.

XaSb EtOH (2 MIC and 4 MIC), XaSb MtOH (2 MIC and 4 MIC) XaSb EtOH/H_2O (4 MIC), and CpSb EtOH (2 MIC and 4 MIC) completely inhibited the growth of *P. aeruginosa* NR 48982 showing a bactericidal effect similar to ciprofloxacin (MIC) (Fig. 2). Furthermore, *P. aeruginosa* treated with the subinhibitory concentration of the tested samples resulted in a longer lag phase and lower intensity compared to the control. These results show that the extract of *X. acutiflora* and *C. procera* also

Table 1 Extraction yields of the studied plants

Plants species	Harvested part	Solvent	Code	Extraction yield (%)
Allanblackia floribunda Oliv. (1380/HNC)	Stem bark	Ethanol	AfSb EtOH	4.90 ± 0.30
		Methanol	AfSb MtOH	4.79 ± 0.23
		Hydro-ethanol*	AfSb EtOH/H_2O	2.24 ± 0.15
		Water	AfSb H_2O	3.35 ± 0.40
		Dichloromethane	AfSb DCM	5.08 ± 0.22
		Dichloromethane/ methanol (50% v/v)	AfSb DCM/MtOH	7.58 ± 0.32
Calotropis procera (Aiton) Dryand (7808/SRF/Cam)	Stem bark	Ethanol	CpSb EtOH	26.92 ± 1.50
		Methanol	CpSb MtOH	13.26 ± 0.90
		Hydro-ethanol*	CpSb EtOH/H_2O	17.57 ± 0.56
		Water	CpSb H_2O	13.00 ± 1.30
Irvingia gabonensis Baill. (28,054/HNC)	Stem bark	Ethanol	IgSb EtOH	35.24 ± 2.80
		Methanol	IgSb MtOH	39.43 ± 1.90
		Hydro-ethanol*	IgSb EtOH/H_2O	43.62 ± 3.40
		Water	IgSb H_2O	47.81 ± 2.50
Newbouldia laevis (P.Beauv.) Seem. (29,469/HNC)	Leaves	Ethanol	NIL EtOH	11.48 ± 0.80
		Methanol	NIL MtOH	13.09 ± 2.30
		Hydro-ethanol*	NIL EtOH/H_2O	12.56 ± 1.30
		Water	NIL H_2O	9.9 ± 0.54
	Stem bark	Ethanol	NISb EtOH	11.87 ± 1.23
		Methanol	NISb MtOH	10.98 ± 0.98
		Hydro-ethanol*	NISb EtOH/H_2O	14.03 ± 1.72
		Water	NISb H_2O	4.97 ± 0.24
Hymenocardia acida Tul. (50,114/HNC)	Leaves	Ethanol	HaL EtOH	11.4 ± 2.14
		Methanol	HaL MtOH	8.81 ± 0.32
		Hydro-ethanol*	HaL EtOH/H_2O	12.98 ± 1.54
		Water	HaL H_2O	33.92 ± 4.01
	Stem bark	Ethanol	HaSb EtOH	12.09 ± 2.30
		Methanol	HaSb MtOH	15.01 ± 0.99
		Hydro-ethanol*	HaSb EtOH/H_2O	7.08 ± 0.48
		Water	HaSb H_2O	6.58 ± 0.67
Xylopia acutiflora (Dunal) A.Rich (28,718 SRF/Cam)	Stem bark	Ethanol	XaSb EtOH	13.93 ± 2.25
		Methanol	XaSb MtOH	13.90 ± 1.45
		Hydro-ethanol*	XaSb EtOH/H_2O	12.85 ± 0.98
		Water	XaSb H_2O	33.17 ± 3.30

*Hydro-ethanol (70/30 v/v)

inhibited the growth of *P. aeruginosa* in a time and dose–response manner.

All the extracts at different tested concentrations inhibited the growth of the *S. pneumoniae* isolate demonstrated by a longer lag phase and a lower concentration than the growth curve of *S. pneumoniae* isolates without inhibitor (Fig. 3). In general, the effect of different tested plant extracts on *S. pneumoniae* isolate was similar before 8 h of incubation after re-emergence of the bacterial growth (MIC, MIC/2, and MIC/4) was observed, and the constancy or decrease in optical density (2 MIC and 4 MIC). Moreover, there were no significant differences between the activity of CpSb EtOH, XaSb MtOH, XaSb EtOH/H2O at 4 MIC, and the ciprofloxacin at its MIC.

Antibiofilm activity

The incubation temperature, incubation time, culture medium, and glucose concentration were observed to significantly affect the biomass of biofilm produced by *E. coli* and *P. aeruginosa.* BHI supplemented with glucose 1% at 25 °C was found to be the best condition for forming biofilm by *P. aeruginosa* (Supplementary file; Fig. S1. A and B) while *E. coli* produced the most biofilm on BHI supplemented with 1% glucose at 37 °C (Supplementary

Table 2 Antimicrobial activity of plant extracts

Extracts	Minimal inhibitory concentrations (MICs) (µg–mL)					
	SA ATCC 43300	PA NR48982	KP NR 41897	EC ATCC 25922	AC NR13374	Spi
XaSb EtOH	250	250	–	500	1000	250
XaSb MtOH	1000	500	–	500	500	250
XaSb EtOH–H_2O	250	500	–	500	1000	250
XaSb H_2O	–	–	1000	–	–	–
NlSb EtOH	250	–	–	1000	–	1000
NlSb MtOH	500	–	–	1000	1000	1000
IgSb EtOH	125	–	–	–	–	125
IgSb MtOH	31.5	–	–	–	–	31.5
IgSb EtOH–H_2O	250	–	–	–	–	250
IgSb H_2O	–	–	–	–	–	500
CaSb MtOH	–	–	–	–	1000	–
CaSb EtOH–H_2O	–	–	–	–	500	–
CaSb H_2O	500	1000	–	–	250	500
CpSb EtOH	–	500	1000	500	500	500
CpSb MtOH	–	1000	–	–	–	–
HaL EtOH	125	–	–	–	–	125
HaL MtOH	250	–	–	–	1000	250
AfSb EtOH	–	250	–	–	–	–
AfSb MtOH	–	500	1000	–	–	–
AfSb EtOH–H_2O	–	250	1000	–	–	–
AfSb DCM	–	1000	1000	–	–	–
AfSb DCM–MtOH	–	500	1000	–	–	–
Ciprofloxacine	0.078	0.031	0.078	0.031	0.078	0.156

SA ATCC 43300: *Staphylococcus aureus* ATCC43300; PA NR48982: *Pseudomonas aeruginosa* NR48982; KP NR41897: *Klebsiella pneumoniae* NR41897; EC ATCC25922: *Escherichia coli* ATCC25922; AC NR13374: *Actinetobacter baumani* NR13374; Spi: *Streptococcus pneumoniae* isolate; –: MICs greater than 1000 µg/mL

file; Fig S1. C and D) after 24 h of incubation. The same conditions were also used to assess the antibiofilm activity of the selected extracts against *P. aeruginosa* and *E. coli.*

XaSb EtOH, XaSb MtOH, XaSb EtOH/H2O, and CpSb EtOH showed significant antibiofilm activities against *E. coli* with an inhibition and eradication percentage varying from 2.54 to 77.88% and 5 to 67.49%, respectively (Fig. 4). XaSb EtOH, XaSb MtOH, and XaSb EtOH/H2O at 4 MIC and all the tested concentrations of CpSb EtOH inhibited more than 50% of *E. coli* biofilm formation. In addition, there was no significant difference in the inhibitory effect between all the tested concentrations of CpSb EtOH and ciprofloxacin used as positive control (Fig. 4 A). However, XaSb EtOH and XaSb MtOH (4 MIC and 2 MIC) and XaSb EtOH/H_2O (4 MIC, 2 MIC, and MIC) and all the tested concentrations of CpSb EtOH eradicated more than 50% of the biofilm formed by *E. coli* with no significant difference with ciprofloxacin (Fig. 4 B).

The biofilm inhibition and eradication percentage of XaSb EtOH, XaSb MtOH, XaSb EtOH/H_2O, and CpSb EtOH on *P. aeruginosa* was accessed, and the obtained results are illustrated in Fig. 5 A and B. All the extracts inhibited and eradicated the biofilm formed by *P. aeruginosa* with a percentage varying from 1 to 76.6% and 3.71 to 69.29%, respectively. XaSb EtOH/H_2O (4 MIC) and CpSb EtOH (4 MIC and MIC) inhibited more than 50% of the biofilm with no significant difference with ciprofloxacin (Fig. 5A). In addition, no significant difference was observed in the eradication effect of the XaSb EtOH/H_2O and CpSb EtOH at 4 MIC, and ciprofloxacin (Fig. 5 B).

Evaluation of phytochemical contents

Qualitative screening of the secondary metabolites of the selected plant extracts

The preliminary phytochemicals screening of XaSb EtOH, XaSb MtOH, XaSb EtOH/H_2O, and CpSb EtOH showed the presence of flavonoids, phenolic compounds, triterpenes, and tannin. Saponin was found in all the selected plant extracts apart from XaSb EtOH (Table 3). However, alkaloids were present in XaSb EtOH, and XaSb MtOH only in trace amounts.

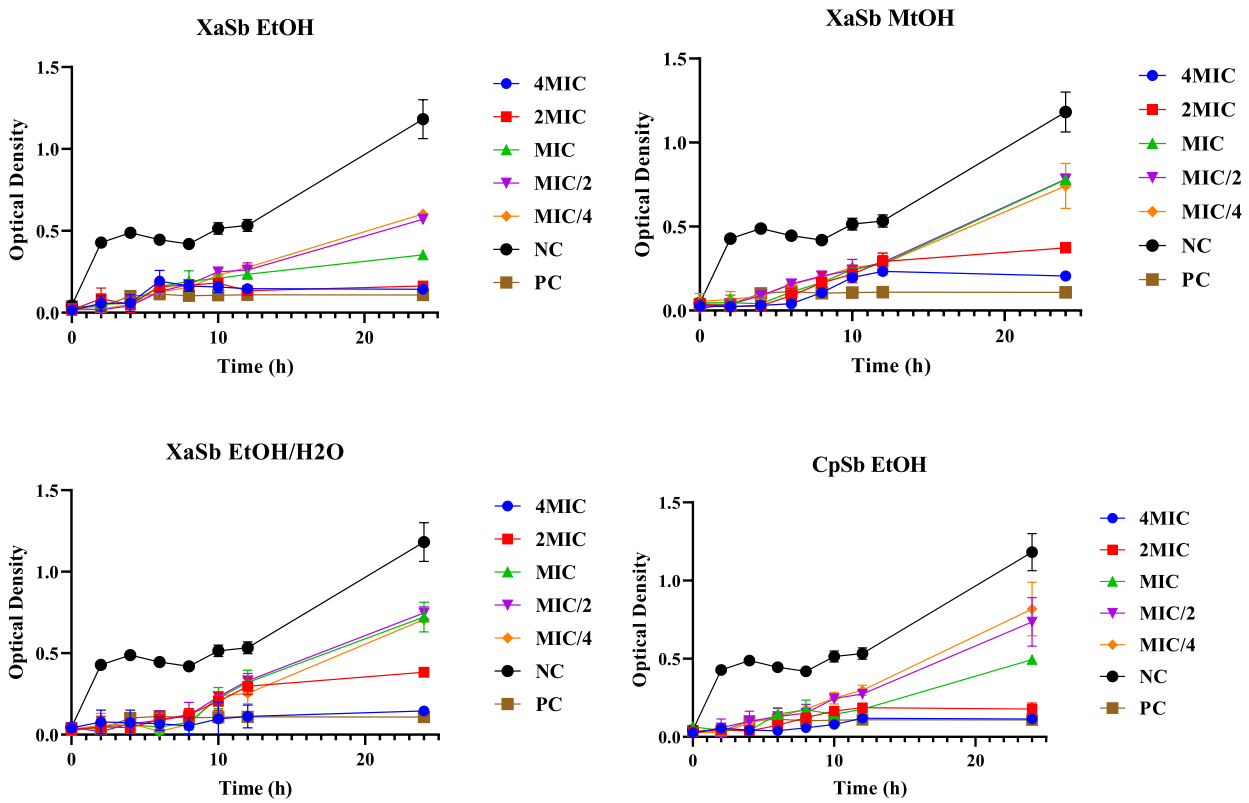


Fig. 1 Time-kill curve of *E. coli* ATCC 25922 following exposures to various concentrations of the ethanolic, methanolic, and hydroethanolic extract of *X. acutifolia* stem bark (XaSb EtOH, XaSb MtOH, and XaSb EtOH/H_2O, respectively) and the ethanolic extract of *C. procera* stem bark (CpSb EtOH); NC: negative control; PC: positive control

Total phenolic compounds, flavonoids, and condensed tannin contained in the selected plant extracts

The quantitative analysis of XaSb EtOH, XaSb MtOH, XaSb EtOH/H2O, and CpSb EtOH revealed a wide variation in the distribution of secondary metabolites from 1115.85 ± 22.63 to 1717.17 ± 70.78 mg GAE/g E, 222.13 ± 15.88 to 541.28 ± 64.73 mg QUE/g E and 121.37 to 171.75 ± 36.52 mg CE/g E for the total phenolics, flavonoid, and tannin content, respectively (Table 4). The highest amount of phenolic compound and flavonoid was obtained with XaSb EtOH. In contrast, similar results were observed in the condensed tannin content of all the tested plant extracts.

Antioxidant activities of the selected plant extracts

All the selected plant extracts scavenged the DPPH radical with the RSA_{50} varying from 370.7 ± 2.57 to 83.79 ± 1.92 µg/mL except for the CpSb EtOH (Table 5). XaSb MtOH showed the best anti-DPPH radical activity with the RSA_{50} of 83.79 ± 1.92 µg/mL. In addition, the selected plant extracts demonstrated a sustainable anti-ABTS activity with the RSA_{50} ranging from 76.9 ± 1.89 to 144.2 ± 2.16 µg/mL. The most active extract was CpSb EtOH (RSA_{50} = 76.9 ± 1.89 µg/mL). However, no significant difference ($p > 0.05$) was observed between the ABTS antiradical activities of XaSb MtOH, XaSb EtOH/H_2O, and ethanol extract of CpSb EtOH. Moreover, all the tested plant extracts showed ferric-reducing ability with the IC_{50} varying from 22.89 ± 1.36 to 130.5 ± 2.12 µg/mL. XaSb EtOH was the most active with an IC_{50} of 22.89 ± 1.36 µg/mL. Furthermore, no significant difference (at $p > 0.05$) was detected between the reducing antioxidant power activity of CpSb EtOH and the gallic acid.

Cytotoxicity of selected plant extracts

The CC_{50} of the tested plant extracts (Table 6) ranged widely, from 14.52 ± 2.01 to 353.75 ± 2.33 µg/mL (Raw 264.7 cells) and 141.35 ± 1.34 to 550.50 ± 1.13 µg/mL (Vero cells). According to the National Institute of Cancer in 2001, a substance is considered to be cytotoxic if it has a CC_{50} of less than 30 µg/mL (Senthilraja & Kathiresan, 2015). Thus, from the data obtained in this study, it can be observed that all the extracts were non-toxic on the tested cell lines, except CpSb EtOH which exhibited toxicity against the Raw cell line with a CC_{50} of 14.52 ± 2.01 µg/mL.

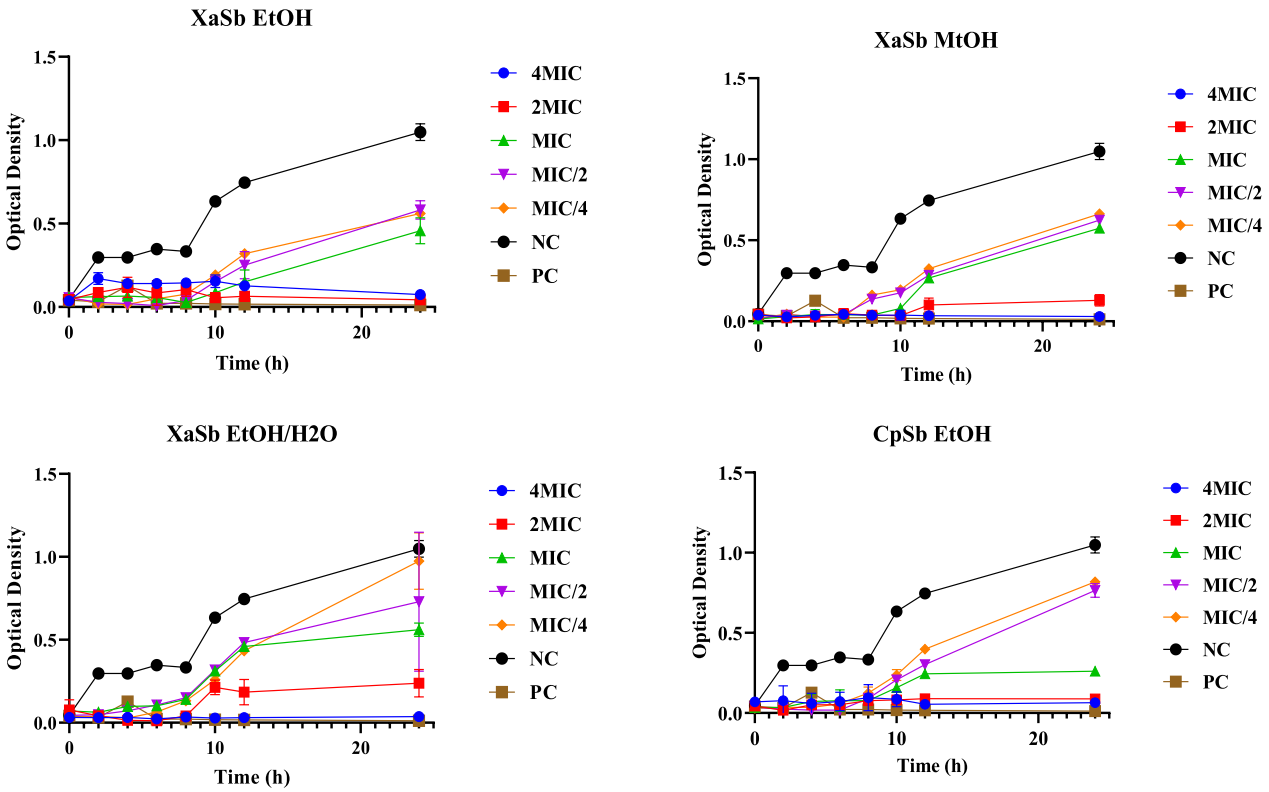


Fig. 2 Time-kill curve of *P. aeruginosa* NR 48982 following exposure to various concentrations of the ethanolic, methanolic, and hydroethanolic extract of *X. acutifolia* stem bark (XaSb EtOH, XaSb MtOH, and XaSb EtOH/H_2O, respectively) and the ethanolic extract of *C. procera* stem bark (CpSb EtOH); NC: negative control; PC: positive control

Effect of the ethanolic extract of *X. acutifolia* stem bark (XaSb EtOH) on bacterial cell morphology

The ability of XaSb EtOH to destabilize the structure of *E. coli* and *P. aeruginosa* was further elucidated using electron microscopy. The micrographs of *E. coli* (Fig. 6B) and *P. aeruginosa* (Fig. 6D) treated with 10 ×MICs of XaSb EtOH revealed a significant decrease in the number of cells when compared to the untreated controls (Fig. 6A, C, respectively). In addition, pronounced destruction of a fundamental cellular structure as a consequence of the rupture and release of the cytoplasm into the surrounding environment can be observed in the treated cells (Fig. 6B, D) compared to the control (Fig. 6A, C). The control cells of *E. coli* and *P. aeruginosa* without the extract treatment had smooth and intact cell surfaces (Fig. 6A, C, respectively). These results confirm that the bactericidal effect of XaSb EtOH can be related to the cell membrane rupture and consequently cytoplasm leakage.

Chemical characterization of the ethanolic extract of Xylopia acutifolia stem bark

The chemical components of the ethanolic extract of *X. acutifolia* stem bark were identified using LC–MS/MS analysis (Table 7), with their chromatographic profile in Supplementary file, Fig. 2. The identified compounds belonged to diverse subclasses such as phenolic, flavonoid, tannin, terpene, fatty acid, quinic acid, psoralens, and iridoid-O-glucoside. The most predominant compounds were found to be kelampayoside A, coniferyl alcohol, 8-iso- 15-keto-PGE2, eschweilenol C, (Z)− 3-Hexenyl beta-D-glucopyranoside, gentiobiosyl 2-methyl- 6-oxo- 2E,4E-heptadienoate, jasminoside I, seguinoside F among other. Furthermore, the chemical structures of some of the compounds have been reported for their antimicrobial and antioxidant activities (kelampayoside A, coniferyl alcohol, 8-iso- 15-keto-PGE2, eschweilenol C, vanillic acid, luteone, gentisic acid, hibiscitrin, and corilagin) (Fig. 7).

Discussion

The Cameroonian plants investigated in this study were selected based on their age-long ethnomedicinal importance in the traditional treatment of various infections in different parts of the country. However, the phytochemistry of the plants has not been well elucidated, hence the need for this study to provide a scientific basis for their efficacy. The extraction of different parts of the plants was done using the commonly used

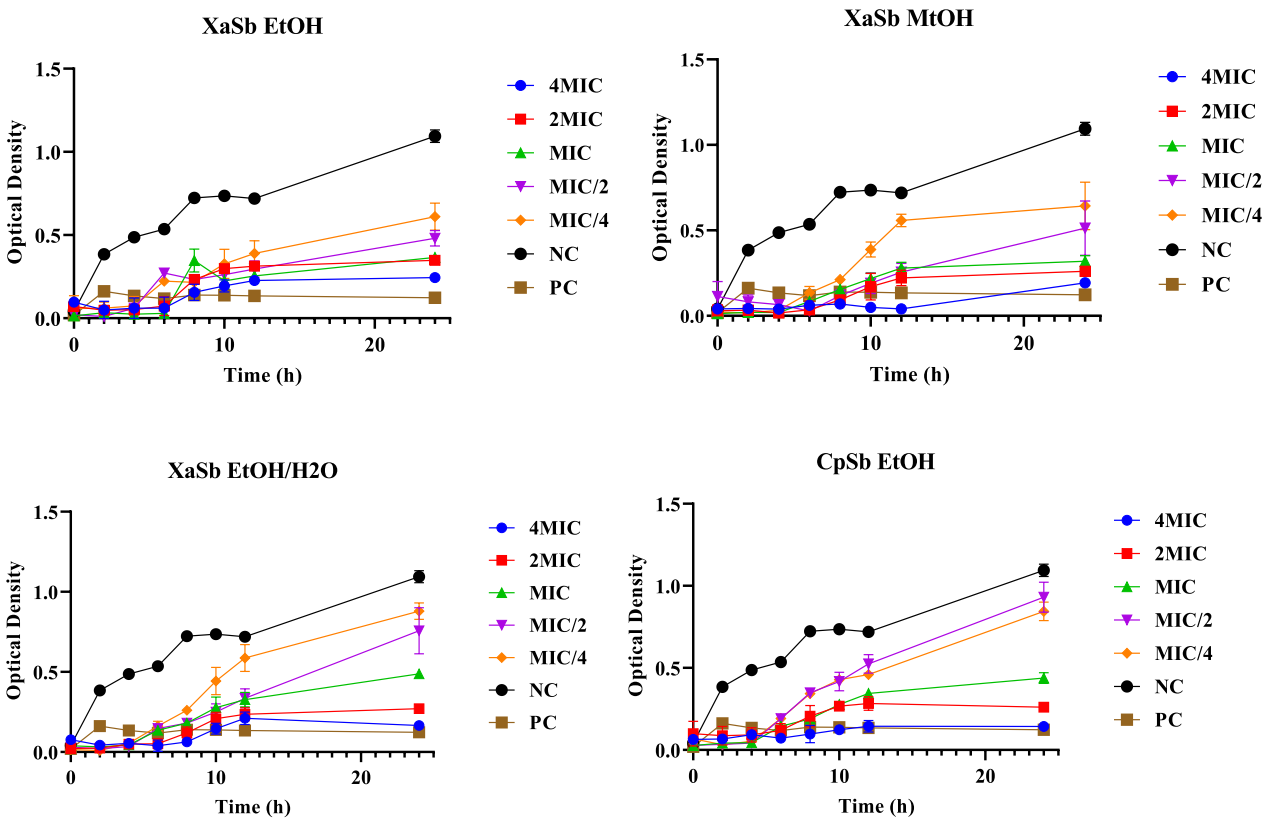


Fig. 3 Time-kill curve of *S. pneumoniae* following exposure to various concentrations of the ethanolic, methanolic, and hydroethanolic extract of *X. acutifolia* stem bark (XaSb EtOH, XaSb MtOH, and XaSb EtOH/H2O, respectively) and the ethanolic extract of *C. procera* stem bark (CpSb EtOH); NC: negative control; PC: positive control

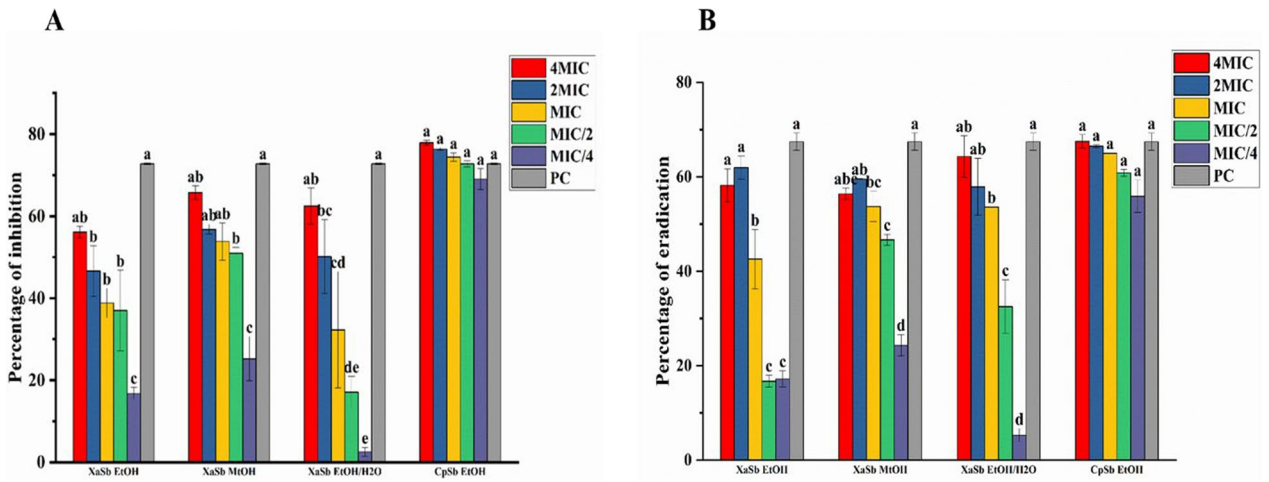


Fig. 4 Percentage of inhibition (**A**) and eradication (**B**) of *E. coli* ATCC 25922 biofilm by the ethanolic, methanolic, and hydroethanolic extract of *X. acutifolia* stem bark (XaSb EtOH, XaSb MtOH, and XaSb EtOH/H_2O, respectively) and the ethanolic extract of *C. procera* stem bark (CpSb EtOH); NC: negative control PC: positive control (Ciprofloxacin) Along the group, the histogrammes carrying the same letter are not significantly different ($P > 0.05$), Tukey

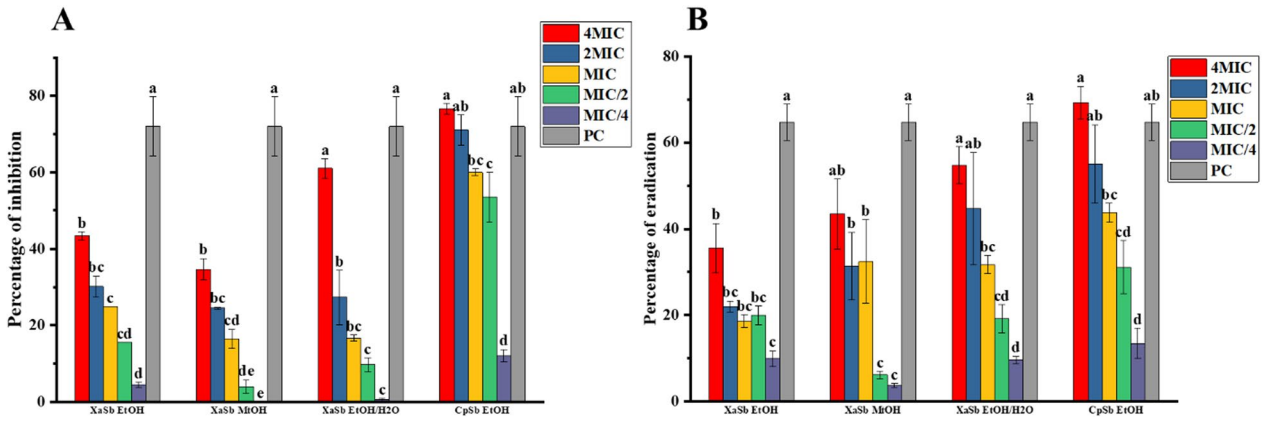


Fig. 5 Percentage of inhibition (**A**) and eradication (**B**) of *P. aeruginosa* NR 48982 biofilm by the ethanolic, methanolic, and hydroethanolic extract of *X. acutifolia* stem bark (XaSb EtOH, XaSb MtOH, and XaSb EtOH/H_2O, respectively) and the ethanolic extract of *C. procera* stem bark (CpSb EtOH); PC: positif control (ciprofloxacin), NC: negative control; along the group, the histograms carrying the same letter are not significantly different ($P > 0.05$), Tukey

Table 3 Phytochemical constitution of the selected extracts

Extracts	Phytochemicals					
	Alkaloids	Flavonoids	Phenolics	Triterpenes	Tannins	Saponins
XaSb EtOH	+	+ +	+ +	+	+ +	–
XaSb MtOH	+	+	+ +	+	+ +	+
XaSb EtOH/H_2O	–	+	+	+	+	+ +
CpSb EtOH	–	+	+	+	+	+

XaSb EtOH: ethanolic extract of *X. acutifolia* stem bark; XaSb MtOH: methanolic extract of *X. acutifolia* stem bark; XaSb EtOH/H_2O: hydroethanolic (70%) extract of *X. acutifolia* stem bark; CpSb EtOH: ethanolic extract of *C. procera* stem bark; (–) = absent; (+) = present in low concentration; (+ +) = present in high concentration

Table 4 Quantitative approximation of secondary metabolites

Extracts	Phenolic content		
	Total phenolics (mg GAE/g E)	Flavonoids (mg QUE/g E)	Tannin (mg CE/g E)
XaSb EtOH	1717.17 ± 70.78^{a}	541.28 ± 64.73^{a}	171.75 ± 36.52^{a}
XaSb MtOH	1270.54 ± 24.66^{b}	484.65 ± 26.97^{ab}	129.34 ± 13.30^{a}
XaSb EtOH/H2O	1715.40 ± 33.60^{a}	395 ± 7.07^{b}	121.37 ± 16.99^{a}
CpSb EtOH	1271.30 ± 45.08^{b}	240.15 ± 18.02^{c}	199.04 ± 38.5^{a}

XaSb EtOH: ethanolic extract of *X. acutifolia* stem bark; CpSb EtOH: ethanolic extract of *C. procera* stem bark; XaSb MtOH: methanolic extract of *X. acutifolia* stem bark; XaSb EtOH/H_2O: hydroethanolic (70%) extract of *X. acutifolia* stem bark; GAE: gram equivalent of gallic acid; QUE: gram equivalent of quercetin; CE: gram equivalent of catechin; E: extract; along the columns, values carrying the same letter superscripts are not significantly different ($p > 0.05$), Tukey

solvents in plant extraction. Non-toxic and manageable solvents are identified to be critical in the extraction and purification of bioactive compounds from plants [35]. Hence, extracts were obtained from the leaves, and stem bark of *A. floribunda, C. procera, H. acida, I. gaboneines, N.laevis,* and *X. acutiflora* with various solvent and extraction yields ranging from 2.233 to 47.81% were recorded. The disparity in the yields could be caused by the plants'different chemical compositions as well as the organic solvents'polarity-dependent affinity for the secondary metabolites [36]. The aqueous extract of *I. gabonensis* stem bark produced the highest extraction yield. Water has been noted to potentially improve extraction efficiency by facilitating the diffusion of the bioactive components across plant tissues [37, 38]. Furthermore, water is a low-viscosity solvent, and such solvents may speed up mass transfer during the preparation of plant extracts leading to quicker and more efficient extraction of desired compounds from

Table 5 DPPH, ABTS, and FRAP radical scavenger parameters of the selected plant extracts

Extracts	DPPH			ABTS			FRAP
	RSA_{50} ± SD (µg/mL)	$EC_{50} \times 10^4$ (µg/mol)	AP $\times 10^{-6}$ (mol/µg)	RSA_{50} ± SD (µg/mL)	$EC_{50} \times 10^4$ (µg/mol)	AP $\times 10^{-6}$ (mol/µg)	IC_{50} ± SD (µg/mL)
XaSb EtOH	159.50 ± 2.20^{a}	3.19 ± 0.00^{a}	0.31 ± 0.00^{a}	144.20 ± 2.16^{b}	2.88 ± 0.08^{b}	0.35 ± 0.01^{b}	22.89 ± 1.36^{f}
XaSb MtOH	83.79 ± 1.92^{b}	1.68 ± 0.15^{b}	0.6 ± 0.05^{b}	76.90 ± 1.89^{c}	1.54 ± 0.08^{c}	0.65 ± 0.04^{c}	87.93 ± 1.94^{de}
XaSb EtOH/H_2O	104.30 ± 2.02^{ab}	2.09 ± 0.02^{ab}	0.479 ± 0.01^{ab}	77.91 ± 1.89^{c}	1.56 ± 0.04^{c}	0.64 ± 0.02^{c}	123.60 ± 2.09^{cd}
CpSb EtOH	≥ 500	/	/	67.95 ± 1.83^{c}	1.36 ± 0.11^{c}	0.74 ± 0.06^{c}	130.50 ± 2.12^{c}
Gallic acid	2.51 ± 0.40^{c}	0.05 ± 0.00^{c}	20.04 ± 1.61^{c}	4.21 ± 0.62^{d}	0.11 ± 0.00^{d}	8.85 ± 0.14^{d}	7.12 ± 0.86^{f}

XaSb EtOH: ethanolic extract of *X. acutiflora* stem bark; XaSb MtOH: methanolic extract of *X. acutiflora* stem bark; XaSb EtOH/H_2O: hydroethanolic (70%) extract of *X. acutiflora* stem bark; CpSb EtOH: ethanolic extract of *C. procera* stem bark; RSA_{50}, 50% radical scavenging activity; EC_{50} = 50% efficient concentration; ARP = antiradical power, IC_{50}: median inhibition concentration. Along the columns, values carrying the same letter superscripts are not significantly different ($p > 0.05$), Tukey

plant material. This can result in higher yields of active ingredients, making the process more effective [39].

The in vitro screening of different extracts against the ESKAPE bacteria showed MIC values ranging from 31.5 to 1000 µg/mL. XaSb EtOH, XaSb MtOH, XaSb EtOH/H_2O, and CpSb EtOH were observed to have the broadest spectra of activities with effects on five of the six tested bacterial strains (83.33%). These observations thus justify the use of *A. floribunda, C. procera, H. acida, I. gaboneines, N.laevis,* and *X. acutiflora* in the traditional treatment of various diseases among which diarrhea, dysentery, cutaneous diseases, fever, respiratory diseases, and headaches [22, 40]. According to Tamokou et al. [41], extracts with minimum inhibitory concentrations MICs of 100 ≤MIC ≤512 µg/mL; 512 ≤MIC ≤2048 µg/mL; MIC > 2048 µg/mL and MIC > 10 mg/mL can be categorized to be active, moderately active, poorly active, and inactive, respectively. Based on these aforestated MIC threshold values, the extracts from the stem bark of *X. acutiflora* and *C. procera* can be stated to show the highest inhibitory activity against ESKAPE pathogens in this study. The methanol and aqueous extract of *C. procera* leaf showed significant antibacterial activity against some bacterial strains among which are *P.areuginosa* and *S. aureus* with a diameter of inhibition of 6—22 mm [42]. The methanolic extract of *C. procera* leaf and latex have been shown potent activities against *E. coli*, *S. epidermides*, and *Bacillus spp*. with inhibition zones ranging from 11.0 to 23.5 mm [43]. More recently, the aqueous extract of *C. procera* extract was active against *S. aureus* and *S. epidermidis* with a MIC of 16 mg/mL and 128 mg/mL, respectively [44].

The time-kill kinetics test was used to evaluate the time dependant activity of the most potent and broadly active extracts on the most sensitive bacteria (*E. coli, S. pneumoniae, and P. aeruginosa*) at different concentrations (1 MIC, 2 MIC, 4 MIC) over 24 h. The extracts at 4 MIC presented a continuous reduction in bacterial load of *E. coli, S. pneumoniae, and P. aeruginosa,* indicating a bactericidal effect. Similar results were observed with ciprofloxacin, the positive control used. A hybrid composite based on *C. procera,* and polyaniline demonstrated bactericidal activity against *E. coli* and *S. aureus*, after 4 h and 3 h, respectively [45]. Biofilm, a significant virulence factor of ESKAPE bacteria like *E. coli* and *P. aeruginosa,* poses a challenge for the medical community in addressing bacterial resistance. Plant extracts containing significant amounts of polyphenolic compounds often inhibit cell adherence and efficiently reduce microbial colonization, epithelial mucosa secretion, and adhesion of the EPS matrix to surfaces [46, 47]. In this study, extracts from *X. acutiflora* and *C. procera* showed significant inhibition and eradication of *E. coli* and *P. aeruginosa* biofilm, with percentages of 77.88% and 67.49% for *E. coli*, and 76.6% and 69.29% for *P. aeruginosa*, respectively. The antibiofilm activity was dose dependent and comparable to ciprofloxacin used as a positive control. Inhibition of initial cell attachment is critical to prevent colonization and the development of infection, thus being proposed as one of the strategies against bacterial biofilm infections [48].

Table 6 Cytotoxicity effect of the selected plant extract on the Raw and Vero cell lines

Extracts	CC_{50} ± SD (µg/mL)	
	Raw	Vero
XaSb EtOH	274.40 ± 1.13	550.50 ± 1.13
XaSb MtOH	315.10 ± 1.56	141.35 ± 1.34
XaSb EtOH/H_2O	353.75 ± 2.33	188.00 ± 7.64
CpSb EtOH	14.52 ± 2.01	138.20 ± 1.41
Podophyllotoxin	1.36 ± 0.43	0.18 ± 0.01

XaSb EtOH: ethanolic extract of *X. acutifolia* stem bark; XaSb MtOH: methanolic extract of *X. acutifolia* stem bark; XaSb EtOH/H_2O: hydroethanolic (70%) extract of *X. acutifolia* stem bark; CpSb EtOH: ethanolic extract of *C. procera* stem bark

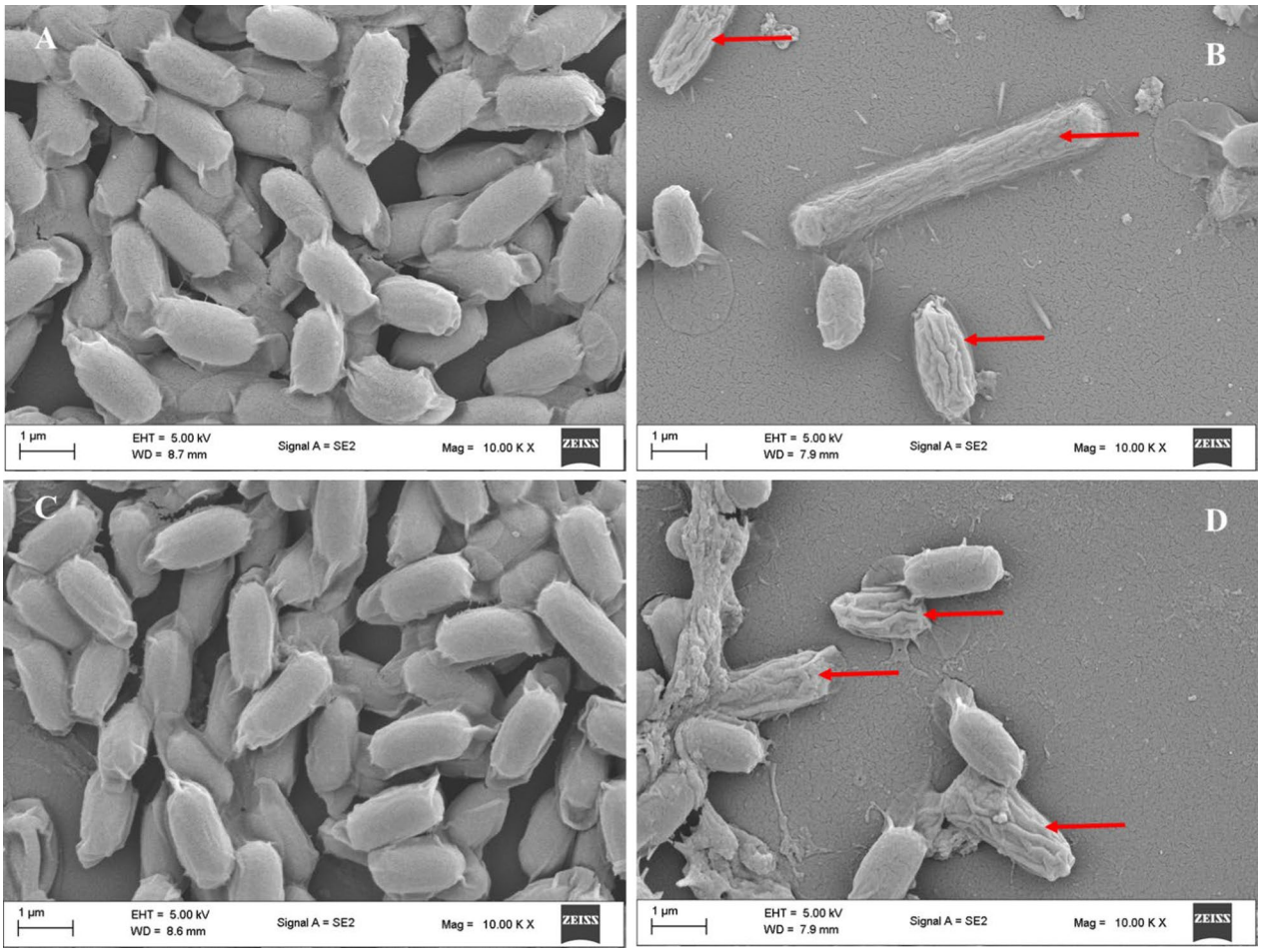


Fig. 6 Effect of the ethanolic extract of *X. acutifolia* stem bark (XaSb EtOH) on the morphological change of *E. coli* and *P. areuginosa*. **A**: untreated *E. coli*; **B**: *E. coli* treated with XaSb EtOH at 10 × MICs; **C**: untreated *P. areuginosa*; **D**: *P. areuginosa* treated with XaSb EtOH at 10 × MICs

Phytochemical screening showed that all the selected plant extracts had flavonoids, phenolics, triterpenes, and tannins. However, saponins were recorded to be absent in the XaSb EtOH. These secondary metabolites have been demonstrated to contribute to the antibacterial activities of plants [49–51]. The methanolic extract of the flower of *C. procera* was reported to be rich in tannin, phenol, terpenoids, glycoside, quinone, anthraquinone, anthocyanin, coumarin, and steroids [52]. The polyphenol quantification revealed that XaSb EtOH possessed the highest amount of phenolic compound (1717.17 ± 70.78 mg GAE/g E) and flavonoid (541.28 ± 64.73 mg QUE/g E). In contrast, no significant difference was observed between the condensed tannin content of all the tested plant extracts. This result indicates that ethanol is the most effective solvent for extracting phenolic compounds and flavonoids from *X. acutiflora*. Several antioxidants, including flavonoids and polyphenols, have been used recently for their advantageous effects against oxidative stress induced by pathogenic microbes [53, 54]. All the selected plant extracts except CpSb EtOH scavenged the DPPH radical with the RSA_{50} ranging from 370.7 ± 2.57 to 83.79 ± 1.92 µg/mL. The best anti-DPPH activity was obtained with XaSb MtOH (83.79 ± 1.92 µg/mL). The ABTS results showed that the lowest scavenging concentration with CpSb ETOH being 67.95 ± 1.83 µg/mL, with a 50% scavenging concentration of 83.79 ± 1.92 µg/mL for the XaSb MtOH. XaSb EtOH displayed the lowest Fe^{3+}-reducing concentration (22.89 ± 1.36 ug/mL) indicating that it possesses the highest total antioxidant potential. Given that XaSb EtOH also showed the highest antibacterial activity, there may exist a notable relationship between the antibacterial activity and the antioxidant capability of plant compounds. XaSb MtOH presented significant antiradical activities with an IC_{50} of 19.90 µg/ml, 16.27 µg/ml, 211.82 mg AAE/g extract, and 116.38 mg AAE/g extract, respectively, against DPPH, ABTS, FRAP, and PAP [55]. Leaf, fruit, flower, and latex

Table 7 Identified compounds in the ethanolic extract of *X. acutifolia* stem bark

No	Tentative identification	R_t (min)	*m/z*	Total score	Formula	Pick intensity
1	Kelampayoside A	12.41	523.17	5.53	$C_{20}H_{30}O_{13}$	32,620
2	Coniferyl alcohol	18.21	179.07	6.88	$C_{10}H_{12}O_3$	22,706
3	8-iso- 15-keto-PGE2	23.61	349.20	5.65	$C_{20}H_{30}O_5$	11,870
4	Eschweilenol C	19.66	447.06	6.38	$C_{20}H_{16}O_{12}$	7196
5	(Z)– 3-Hexenyl beta-D-glucopyranoside	18.78	261.13	4.49	$C_{12}H_{22}O_6$	6263
6	Gentiobiosyl 2-methyl- 6-oxo- 2E,4E-heptadienoate	13.45	523.17	5.48	$C_{20}H_{30}O_{13}$	6233
7	Jasminoside I	21.81	491.21	4.57	$C_{22}H_{36}O_{12}$	5004
8	Seguinoside F	15.70	583.17	4.64	$C_{26}H_{32}O_{15}$	4901
9	[3,4,5-Trihydroxy- 6-(hydroxymethyl)oxan- 2-yl] 2,6-dimethyl- 8-[3,4,5-trihydroxy- 6-(hydroxymethyl)oxan- 2-yl]oxyoct- 2-enoate (Terpene glycosides)	14.78	509.22	4.22	$C_{22}H_{38}O_{13}$	4750
10	Luteolin- 7-beta-D-glucuronide	20.65	461.07	6.31	$C_{21}H_{18}O_{12}$	4509
11	Gibberellin A97	23.10	363.18	5.06	$C_{20}H_{28}O_6$	3940
12	Suberic acid	15.62	173.08	5.13	$C_8H_{14}O_4$	3552
13	Vanillic acid	15.06	167.03	5.62	$C_8H_8O_4$	3183
14	Shanzhiside	12.04	391.12	5.23	$C_{16}H_{24}O_{11}$	3018
15	Caloxanthone G	23.02	311.09	6.70	$C_{18}H_{16}O_5$	1896
16	Luteone	23.421	353.10	7.33	$C_{20}H_{18}O_6$	1841
17	Gentisic acid	8.06	153.02	8.20	$C_7H_6O_4$	1635
18	Hibiscitrin	10.77	495.08	6.30	$C_{21}H_{20}O_{14}$	181
19	Putranjivain A	12.91	541.06	4.76	$C_{46}H_{36}O_{31}$	1409
20	(+)-(7S,8S)-guaiacylglycerol-beta-vanillic acid ether	14.92	363.11	6.75	$C_{18}H_{20}O_8$	435
21	Corilagin	13.17	633.07	6.51	$C_{27}H_{22}O_{18}$	410
22	(+)-(7S,8S)-guaiacylglycerol-beta-vanillic acid ether	14.58	363.11	5.70	$C_{18}H_{20}O_8$	410
23	6-({5,7-dihydroxy- 2-[4-hydroxy- 3-(sulfooxy)phenyl]– 4-oxo- 4H-chromen- 3-yl} oxy)– 3,4,5-trihydroxyoxane- 2-carboxylic acid	18.67	557.02	5.64	$C_{21}H_{18}O_{16}S$	410
24	Spicatin	15.11	515.19	7.29	$C_{27}H_{32}O_{10}$	320

C. procera extract demonstrated an anti-DPPH activity (IC_{50}) of 1.7, 0.21, 0.27, and 0.43 mg/mL, respectively [56]. Yesmin et al. [42] demonstrated a significant anti-DPPH radical activity of the methanol extract of *C. procera* with an IC_{50} of 121.25 µg/ml. Various factors, including the plant part used, the plant's geographical location, the time of harvesting, the method of extraction, and the type of solvent used, can impact the composition of plant extracts, consequently influencing their pharmacological properties such as antimicrobial and antioxidant effects [42, 56–58].

In this study, XaSb EtOH (10 × MICs) was selected to illustrate its destabilizing effect on *E. coli* and *P. aeruginosa* at the subcellular level using electron microscopy. *E. coli* and *P. aeruginosa* treated with XaSb EtOH presented a significant decrease in the number of cells and pronounced destruction of fundamental cellular structures, resulting in the rupture and release of cytoplasm into the surrounding environment compared to untreated controls. These results indicate that the bactericidal effect of XaSb EtOH may be attributed to cell membrane rupture and the subsequent cytoplasm leakage. The antimicrobial compounds found in plant extracts, such as polyphenols, tannins, and carotenoids, disrupted the normal structure of bacteria, causing dysfunction and eventual cell death [44]. Due to the significant biological activities recorded for XaSb EtOH in this study, it was considered expedient to identify the key phytochemicals present therein thus opening up future investigations into *X. acutiflora* as a source of lead drug compounds. Furthermore, there are currently no data on the specific phytocompounds contained in the bark of the plant; hence, the chemical components were tentatively identified using LC–MS/MS analysis. It is noteworthy that many of the compounds identified in the analysis have been previously acknowledged as remarkable antimicrobial and antioxidant agents, e.g., kelampayoside A, coniferyl alcohol, 8-iso- 15-keto-PGE2, eschweilenol C, vanillic acid, luteone, gentisic acid, hibiscitrin, and corilagin. Some of these compounds have also been found in the extracts of *X. aethiopica* fruit, especially vanillic acid and gentisic acid. Furthermore, the antibacterial activities of XaSb EtOH may be credited to the synergistic interaction of the identified compounds. Corilagin, for example, has

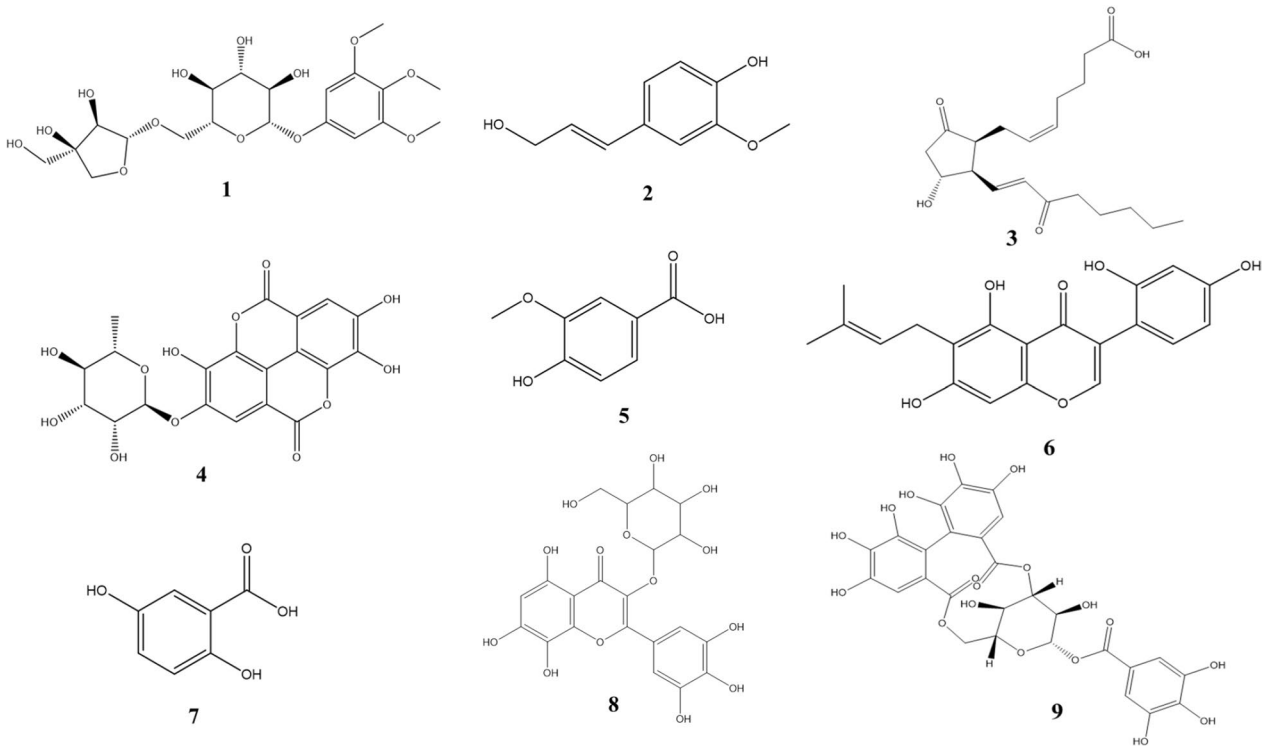


Fig. 7 Structure of the major compounds in the ethanolic extract of *X. acutifolia* stem bark known for their antimicrobial and antioxidant activities. 1: kelampayoside A, 2: coniferyl alcohol, 3: 8-iso- 15-keto-PGE2, 4: eschweilenol C, 5: vanillic acid, 6: luteone, 7: gentisic acid, 8: hibiscitrin, 9: corilagin

been shown to increase the susceptibility of *P. aeruginosa* by inhibiting the branch migration activity of PaRuvAB with an IC_{50} value of 0.40 µM [59]. Corilagin also exhibits its significant activity against *E. coli,* and *C. albicans* by affecting membrane permeability with the MIC value of 62.5 µg/mL [60]. Other *X. aethiopica* compounds, such as vanillic acid and gentisic acid, have also been demonstrated to be active against *Mycobacterium tuberculosis* (MIC; 83.3 µg/mL) [61] and *Schizosaccharomyces octosporus* (MIC; 1000 µg/mL), respectively [62].

Conclusion

This study explores six Cameroonian plant extracts'antioxidant and antibacterial potential against ESKAPE pathogens. The methanol extract of *C. procera* stem bark and the extract of *X. acutiflora* stem bark were identified as the most active. These extracts demonstrated significant in vitro antibacterial and antibiofilm properties as well as antioxidant activities and low cytotoxicity. They also contain various secondary metabolites such as flavonoids, phenolics, alkaloids, triterpenes, and tannins which may contribute to their observed effects. Compared to the positive control, the methanol extract of *C. procera* stem bark and the extract of *X. acutiflora* stem bark showed excellent ferric-reducing and ABTS activity and moderate DPPH activity. A strong correlation was found between the phytochemical constituents of the extracts and their antioxidant and antibacterial activities. The ethanolic extract of *X. acutiflora* stem bark demonstrated the ability to interact with and disrupt the morphological structure of the bacterial cells. Further research is needed to isolate, purify, and characterize the specific bioactive compounds present in these plants for potential use in complementary and alternative medicine for treating various diseases. In addition, the results of this study have also highlighted the potential of these indigenous plants as sources of small drug compounds to address the increasing concerns of antibiotic resistance.

Abbreviations

SEM	Scanning electron microscope
LC–MS	Liquid chromatography–mass spectrometry
MICs	Minimum inhibitory concentrations
MH	Mueller–Hinton
$MBIC_{50}$	50% Minimum biofilm inhibitory concentration
$MBEC_{50}$	50% Minimum biofilm eradicative concentration
BHI	Brain heart infusion
RSA_{50}	Radical scavenging activities 50
EC_{50}	50% Effective concentration
AP	Antiradical power
IC_{50}	50% Inhibitory concentration
CC_{50}	50% Cytotoxic concentration
PDA	Acquity photodiode array
MS	Mass spectrometry
ANOVA	One-way analysis of variance
GAE	Gallic acid equivalence
QUE	Quercetine acid equivalence

AAE Ascorbic acid equivalence

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s43094-025-00799-2.

Additional file 1.

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Author contributions
Branly-natalien Nguena-dongue involved in conceptualization, investigation, methodology, formal analysis, data curation, software, writing—original draft, writing—review & editing; Elisabeth Zeuko'o Menkem took part in writing—review & editing, validation, supervision, resources; Paul Keilah Lunga involved in writing—review & editing, visualization, resources, supervision; Stella Tofac Asong took part in investigation, methodology; Ayodeji Amobonye involved in writing—review & editing, visualization, validation, supervision; Santhosh Pillai involved in writing—review & editing, supervision, resources.

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Availability of data and materials
The data and materials that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Statement on studies involving plants
The study adhered to WHO guidelines for good agricultural and collection practices (GACP) for medicinal plants. All the plant in this study were collected from Bandounga forest, Tonga, Cameroon, and they were authenticated by Mr. Victor Nana, Senior Botanist, Cameroon National Herbarium, Yaounde, Cameroon. The identification of plant parts was also assisted by Mr. Victor Nana, Senior Botanist, Cameroon National Herbarium; the voucher specimens were all deposited at the Cameroon National Herbarium—*Allanblackia floribunda* Oliv. (Clusiaceae) (1380/HNC), *Calotropis procera* (Aiton) Dryand (Apocynaceae) (7808/SRF/Cam), *Irvingia gabonensis Baill.* (Irvingiaceae) (28054/HNC), *Newbouldia laevis* (P.Beauv.) Seem. (Bignoniaceae) (29469/HNC), *Hymenocardia acida* Tul. (Euphorbiaceae) (50114/HNC) and *Xylopia acutiflora* (Dunal) A.Rich (Annonaceae) (28,718 SRF/Cam).

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