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Unveiling the anti-Klebsiella activity of methanol extracts from *Hallea ciliata* leaves and barks against multidrug-resistant strains overexpressing AcrAB-TolC efflux pumps

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Abstract

Background: Infectious diseases are considered one of the most critical threats to public health. The present work aimed to determine the anti-Klebsiella activity of methanol extracts (botanicals) of *Hallea ciliata* leaves (HCL) and bark (HCB) against multidrug-resistant phenotypes over-expressing ACrAB-TolC efflux pumps.

Methods: The antibacterial activity of botanicals alone and in combination with PAβN and antibiotics, was determined using the broth microdilution method. The effects of HCL on H+/ATPases and the qualitative phytochemical screening were assessed using standard experimental protocols.

Results: The results indicate that HCL and HCB had antibacterial activity against 75% and 56.25% of the tested bacterial strains, with minimum inhibitory concentrations (MIC) ranging from 64 to 1024 µg/mL and from 64 to 512 µg/mL, respectively. They showed strong activity against *Klebsiella oxytoca* KO107 and *Klebsiella pneumoniae* K2 with MICs of 64 µg/mL for HCL and HCB, respectively. When the functioning of proton pump dysfunction was assessed in the presence of HCL, it was observed to affect *Klebsiella oxytoca* KO107. Furthermore, when combined with PAβN, the botanicals demonstrated improved activity against 100% of the tested strains and isolates, with the activity improvement factor (AIF) ranging from 2 to 256. Additionally, the study found that the botanicals enhanced the activity of certain antibiotics at MIC/2 and MIC/4, including ampicillin, penicillin, and ciprofloxacin. Phytochemical screening of the botanicals revealed the presence of alkaloids, triterpenes, phenols, flavonoids, saponins, and anthocyanins.

Conclusion: Overall, HCL and HCB are sources of antibacterial substances that could be valuable in combating multidrug-resistant (MDR) bacteria from the *Klebsiella* genus that over-express efflux pumps.

Keywords: Antibiotic-potentiating activity; *Hallea ciliata*; Klebsiella; multidrug resistance; Rubiaceae.

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Background

Infectious diseases pose a significant threat to public health [1]. Despite the effectiveness of antibiotic therapy, they caused approximately 4.95 million deaths worldwide in 2019 due to the emergence of antimicrobial resistance. In sub-Saharan African countries, this resistance led to 1.27 million deaths, primarily caused by multidrug-resistant (MDR) bacteria, including those of the genus *Klebsiella* [2]. MDR bacteria, such as *Klebsiella pneumoniae* and *Klebsiella oxytoca*, are responsible for a significant portion of nosocomial infections, ranging from 10% to 25% [3, 4]. Antibiotic therapy, once seen as a beacon of hope in combating bacterial infections, has lost its effectiveness due to inappropriate use. This has led to the development of resistance in bacteria through various mechanisms, such as reduced membrane permeability, enzymatic inactivation, modification of antibiotic targets, and overexpression of efflux pumps [5]. Efflux is a common resistance mechanism used by *Klebsiella* species. According to the World Health Organization (WHO), these bacteria pose the greatest threat to public health due to their high level of antibiotic resistance, which limits treatment options [2]. *Klebsiella* species overexpress efflux pumps of the Resistance-Nodulation-Division (RND) family such as AcrAB-TolC, allowing them to expel various compounds, including antibiotics, into the environment [2, 6]. This efflux significantly reduces the effectiveness of antibiotics, leading to an increase in therapeutic failures. In response to this threat, it is necessary to search for new natural substances to combat bacterial multidrug resistance. Medicinal plants from the African flora are considered promising sources of natural substances that are effective against MDR bacteria [7-15]. Recent investigations by various authors have demonstrated that the African flora contains potential natural substances against multi-resistant *Klebsiella* species [16-26]. Furthermore, certain classes of bioactive compounds found in medicinal plants, have been shown to inhibit bacterial growth and enhance the effectiveness of antibiotics against MDR bacteria [27-30]. *Hallea ciliata* (Aubrév. & Pellegr.) J.-F.Leroy (Synonym: *Mitragyna ciliata* Aubrév. & Pellegr) is a Cameroonian medicinal plant of the Rubiaceae family. It is traditionally used in Cameroon to treat chest pain, headaches, rheumatism, lung infections, malaria, and infected wounds [31]. In the present study, antibacterial activity of *H. ciliata* extracts, the mode of action on H+/ATPase proton pumps, and the synergistic effects in combination with common antibiotics on MDR *Klebsiella* species is reported.

Methods

Plant material and extraction

The leaves and bark were gathered in Fondonera in the Santchou District (West Region of Cameroon) and later identified at the National Herbarium of Cameroon by Mr. TCHATCHOUANG NGANDOF Eric, comparing them with the reference samples preserved under code 27311/SRFCam. The plant parts were harvested and dried away from the sun. They were then crushed, and the obtained powders were soaked in methanol (in a 1:3 ratio of plant material to methanol) for 48 hours at room temperature, with shaking to enhance the extraction. The powder-solvent mixture was then filtered using Whatman No. 1 paper. The resulting filtrates were concentrated using a BÜCHI R-200 rotary evaporator at 65°C and then dried at 45°C until the residual solvent completely evaporated, resulting in crude extracts from the leaves (HCL) and barks (HCB). These extracts referred to as botanicals were stored in dark, sterile bottles at 4°C for future use.

Chemicals and culture media

Dimethyl sulfoxide (DMSO) served to solubilize plant extracts. Antibiotics used included β-lactams: ampicillin (AMP), penicillin (PEN), carbapenem imipenem (IMI), the cephalosporins cefixime (CFX), and ceftriaxone (CTX); fluoroquinolones: ciprofloxacin (CIP) and levofloxacin (LEV), and the tetracycline (TET). Mueller Hinton Agar (MHA) was used for the activation of bacteria; Mueller Hinton Broth (MHB) was used for microdilution as a nutrient medium for bacteria. para-Iodonitrotetrazolium chloride ≥ 97% (INT) was used as the bacterial growth indicator. The efflux pump inhibitor (EPI), phenylalanine-arginine *β*-naphthylamide (PAβN) at 0.2% was used. All chemicals were purchased from Sigma-Aldrich (St. Quentin Fallavier, France).

Tested bacteria

The *Klebsiella* species tested included both reference strains of *Klebsiella pneumoniae* ATCC11296 and clinical isolates *Klebsiella pneumoniae* K2, KP55, K24, KP203, KP175, KP77, KP93, KP126, and KP81, and *Klebsiella oxytoca* clinical isolates KO249, KO96, KO107, KO95, KO26, and KO55. Their bacterial features were previously reported by Kuete et al. [32, 33] for ATCC11296, K2, and KP55, Kengne et al. [34] and Kengne et al. [35] for K24, KP203, KP175, KP77, KP93, KP126, KP81, KO249, KO96, KO107, KO95, KO26, and KO55. KP55, KP24, KP126, ATCC11296, KO107, KO96 are clinical bacterial strains overexpressing AcrAB-TolC efflux pumps as reported earlier [35].

Determination of minimal inhibitory and bactericidal concentrations

The bacterial inoculum was prepared as previously described by comparing it to the turbidity of a standard McFarland 0.5 ($1.5x10^8$ CFU/mL) [16, 22, 36-40]. The various plant extracts and the reference drug (IMI) were dissolved in DMSO-MHB. Plant extracts were prepared at 8192 µg/mL, and antibiotics at 512 µg/mL. PAβN was prepared at a concentration of 100 µg/mL. The botanicals were tested alone and then in the presence of PAβN (EPI). The combination of plant extracts with EPI was intended to evaluate the function of efflux pumps in bacterial resistance to the test extracts [32, 33, 39, 41]. The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of test samples alone were determined using a 96-well broth microdilution method combined with the rapid INT colorimetric method [41-43] . IMI was used as a positive antibacterial control, whereas DMSO 2.5%+MHB and MHB alone were used as negative controls. MIC was considered the lowest concentration of plant extract which produced complete inhibition of bacterial growth after 18 to 24 hours of incubation at 37°C, whereas MBC was considered the lowest concentration of a sample that did not induce a color change with the addition of INT following additional 48 hours of incubation [23, 44, 45]. Each experiment was repeated three times in triplicate.

Evaluation of the effect of HCL on the functioning of H+/ATPases proton dependent pumps of K. oxytoca KO107

The effects of HCL were assessed on the H^+-ATPase-mediated proton pumping of *K. oxytoca* KO107 at 0.5×MIC, MIC, and 2×MIC as earlier described [40]. The action on H^+-ATPase-mediated proton pumping was done by controlling the acidification of the

bacterial growth medium over 60 min following the procedures previously described [46-49].

Evaluation of the effect of efflux pumps on the antibacterial activity of the samples

Botanicals and IMI were also tested in the presence of PAβN (30 µg/mL) as previously described [32]. The ratio MIC $_{(\text{sample alone})}$/MIC $_{(\text{sample +PAβN})}$ referred to as the activity improvement factor (AIF) was used to determine the fold increase of the antibacterial activity of the samples in the presence of PAβN. The bacteria tested included *K. pneumoniae* (K2, KP55, K24, KP175, and KP93), and *Klebsiella oxytoca* (KO249, KO096, and KO095). IMI at concentrations ranging from 1 to 128 µg/ml to serve as a reference. Each assay was repeated thrice.

Determination of the antibiotic-potentiating effects of the botanicals

The effects of the association of the botanicals with antibiotics were determined against the MDR bacteria using the broth microdilution method as previously described [18, 26]. The tested antibiotics included CTX, AMP, PEN, CFX, LEV, CIP, TET, and IMI. The tested bacteria were *K. pneumoniae* K2, KP55, K24, KP175, KP93, and *Klebsiella oxytoca* KO249, KO96, and KO95. At first, extracts were used at the sub-inhibitory concentrations of MIC/2, MIC/4, MIC/8, and MIC/16 for a preliminary assay on KP175, which then allowed the selection of appropriate sub-inhibitory concentrations of MIC/2 and MIC/4 for further combination testing (Data not shown). Activity modulation factor (AMF) was calculated as the ratio of the MIC of the antibiotic alone versus MIC in combination with the plant extract. The potentiation effect was considered for AMF ≥ 2 [50].

Phytochemical screening of botanicals

Phytochemical screening was done following the standard methods described for alkaloids, anthocyanins, flavonoids (Shinoda test), phenols, saponins, and triterpenes (Liebermann-Burchard test) [10, 51].

Interpretation of antibacterial data

Updated and rationally defined cutoff points of the antibacterial botanicals have been defined for Enterobacteria as follows: outstanding activity (MIC ≤8 µg/mL), excellent activity (8 < MIC ≤64 µg/mL), very good activity (64 < MIC ≤128 µg/mL), good activity (128 < MIC ≤256 µg/mL), average activity (256 < MIC ≤512 µg/mL), weak activity (512 < MIC ≤1024 µg/mL), and not active (MIC values >1024 µg/mL) [52]. These appreciation criteria will be used to discuss the antibacterial activities of the studied samples.

Results

Antibacterial activity

The antibacterial activity of various extracts of *H. ciliata* was assessed by determining their MICs and MBCs against a range of *Klebsiella* strains and isolates. MIC/MBC ratio determined whether the extracts have bacteriostatic or bactericidal effects. Detailed MICs and MBCs values can be found in Table 1. It appears that HCL and HCB had various degrees of antibacterial activities against *Klebsiella* strains and isolates tested. HCL exhibited antibacterial activities with an inhibition spectrum of 75% and MICs ranging from 64 to 1024 µg/mL. It had an excellent activity (MIC of 64 µg/mL) against the *K. oxytoca* KO107. Very good activity was recorded against *K. pneumoniae* (K2, K24, KP77) with a MIC of 128 µg/mL, and good activity against *K. pneumoniae* (ATCC11296, KP126) with a MIC of 256 µg/mL. Moderate activity against *K. pneumoniae* (KP55, KP81), *K. oxytoca* (KO26, KO55) with a MIC of 512 µg/mL and low activity against K. oxytoca (KO95) with a MIC of 1024 µg/mL were also achieved. HCB displayed an inhibition spectrum of 56.25%, with excellent activity (MIC 64 µg/mL) observed against *K. pneumoniae* K2. Very good activities against *K. pneumoniae* (K24, KP126) and *K. oxytoca* (KO107) with a MIC of 128 µg/mL, good activity against *K. pneumoniae* (KP55, KP203) and *K. oxytoca* KO26 (MIC of 256 µg/mL), as well as an average activity against *K. pneumoniae* ATCC11296 and *K. oxytoca* KO55 (MIC of 512 µg/mL), were also achieved. A total of 9 out of 16 isolates (56.25%) showed a bactericidal effect (CMB/MIC ≤ 4) against all strains and isolates tested.

Effect of HCL on H+ proton pumps/ATPases

To verify the ability of HCL to alter the bacterial H+ proton pumps/ATPases of *K. oxytoca* KO107, the pH of the medium containing the bacteria was measured in the presence and absence of this extract. Figure 1 shows the different peaks of variation of the pH of HCL at MIC/2, MIC, and 2MIC. It can be noted that a considerable decrease in pH (7 to 6.6) is observed in the absence of HCL (negative control, C-) for *K. oxytoca* KO107 for 60 min. Also, a slight decrease in pH (7 to 6.83) is observed during the first 30 min in the presence of HCL at (MIC/2, MIC, and 2MIC) and the antibiotic, CIP (positive control, C+), then a significant increase in pH (6.83 to 7.05) during the next 30 min for HCL at 2MIC.

PAβN increased the activity of both HCL and HCB.

The botanicals HCL and HCB were tested with and without an efflux pump inhibitor (EPI), PAβN on eight different strains to determine the effects of the inhibitor on the efflux activity. The MIC values in the presence and absence of the inhibitor are summarized in Table 2. It appears that in the presence of PAβN, the activity of these extracts was potentiated against 100% of the bacteria tested, with AIF values ranging from 4 and 256. HCL combined with EPI showed an AIF of 256 against *K. pneumoniae* (KP175, KP93), and 128 against *K. oxytoca* (KO95, KO96) while the reference antibiotic with EPI showed AIF of 16 against *K. oxytoca* KO95. Similarly, HCB combined with EPI showed an AIF value of 256 against *K. pneumoniae* (KP175, KP93) and K. oxytoca KO96.

Antibiotic-activity modulation effects of HCL

To choose the different extracts to be used in association with antibiotics, a preliminary test was carried out at sub-inhibitory concentrations (MIC/2, MIC/4, MIC/8, MIC/16) of extracts on MDR *K. pneumoniae* KP175. It was found that HCL and HCB enhanced the effectiveness of the antibiotics at lower concentrations (MIC/2 and MIC/4) against 50 to 87.5% of the tested bacteria, with a fold increase ranging from 2 to 64 (Data not shown). HCL and HCB were also combined with eight (08) antibiotics at MIC/2 and MIC/4, and the findings are summarized in Tables 3 and 4. Table 3 shows that at MIC/2 and MIC/4, HCL potentiated the activity of antibiotics with AMF ranging from 2 to 64. PEN and AMP were potentiated against 100% of the bacteria tested, and CIP against 90%. At

MIC/4, AMP was potentiated against 100% of the tested bacteria, CIP against 90%, and PEN against 60%. Similarly, potentiating effects were observed for CFX, TET, and LEV against 60% of the bacteria at MIC/2 and against 50% at MIC/4. In addition, IMI and CTX showed synergy against 50% of strains at MIC/2 and against 25% for IMI at MIC/4 (Tables 3). Also, the AMF values in Table 4 show potentiation of the activity of antibiotics by HCB at MIC/2 and MIC/4. Synergistic effects were observed against 90% of the strains and isolates tested for AMP, 75% for PEN, and 60% for CIP. Similarly, 60% for CFX, and 50% for LEV, TET, and CTX at MIC/2. At MIC/4, synergistic effects were observed with percentages of potentiation against 60%, 50% of strains and isolates tested for LEV, 40% for TET and CTX, and 25% for CFX. IMI was potentiated against 10% of strains and isolates at MIC/2 and MIC/4.

Phytochemistry

Phytochemical screening of HCL and HCB revealed the presence of phenols, terpenoids, and alkaloids in the two plant extracts; HCL additionally contained flavonoids, meanwhile saponins and anthocyanins were also detected in HCB.

Discussion

Bacterial infections continue to be a significant public health issue, leading to an increasing number of deaths globally, particularly in sub-Saharan African countries [1, 2]. Numerous research studies have highlighted the crucial role that medicinal plants can play in combating human ailments [20, 26, 53-70]. In this context, the antibacterial activity of *H. ciliata*, a medicinal plant traditionally used to treat various diseases, was assessed against MDR strains and isolates of the *Klebsiella* genus. The antibacterial activity of different extracts of *H. ciliata* against Enterobacteria was discussed according to the classification made by Kuete in 2023 [52]. HCL showed excellent activity against *K. oxytoca* KO107 and very good activity against *K. pneumoniae* (K2, K24, Kp77). Similarly, HCB showed excellent activities against *K. pneumoniae* K2, and very good activities against *K. pneumoniae* (K24) and *K. oxytoca* KO107. These results contradict those obtained by Adesegun et al. [71] who found poor antibacterial activity of the methanol extract of *H. ciliata* leaves in Nigeria. The discrepancy could be due to variation in pedoclimatic conditions between the two geographical areas [72]. Additionally, there are few previous studies regarding the antibacterial activity of plants of the genus *Hallea*. However, some authors, including Demgne et al. [47] and Praptiwi et al. [73], have shown that plants of the Rubiaceae family, to which H. ciliata belongs, exhibit antibacterial activities comparable to those obtained in the present work. Qualitative phytochemical screening of HCL and HCB revealed the presence of alkaloids, phenols, triterpenes, flavonoids, anthocyanins, and saponins. These results, for HCL, do not corroborate those obtained by Adesegun et al. [71] who demonstrated through their work the presence of saponins in the *H. ciliata* leaf extract. This difference could also be explained by the different pedoclimatic conditions between the geographical areas [72]. On the other hand, these results corroborate those obtained by Koffi et al. [74] who highlighted the presence of triterpenes and saponins in the *H. ciliata* bark extract. The richness in secondary metabolites of the different extracts of *H. ciliata* would explain the antibacterial activities observed. The antibacterial activity of a plant depends on its qualitative and quantitative composition in secondary metabolites [75]. The anti-Klebsiella activity presented by these extracts is due to all the secondary metabolites including alkaloids, phenols, triterpenes, and flavonoids for HCL and alkaloids, phenols, triterpenes, saponins, and anthocyanins for the HCB.

Proton pumps are the essential element in the control of cellular pH; they regulate the amount of proton H+ that leaves the cytoplasm to the extracellular medium depending on the concentration gradient across the plasma membrane by supplying the cell with energy in the form of ATP [76, 77]. The dysfunction of these pumps leads to a cessation of energy supply and a weak acidification of the extracellular medium. Not only do these pumps supply the bacterial cell with energy but also provide maintenance in cellular homeostasis by controlling cytoplasmic acidity for cell survival and growth. A significant increase in the pH of the culture medium at 2MIC in the presence of HCL and *K. oxytoca* KO107 was recorded. Bavishi & DuPont [76] demonstrated that an antibacterial substance that induces an increase in environmental pH is an inhibitor of proton pumps. Thus, H+/ATPases proton pumps are one of the targets involved in the anti-Klebsiella activity of HCL because the latter induces their inhibition, probably leading to a loss of cellular homeostasis.

The insensitivity of MDR bacteria that overexpress efflux pumps is due to the extrusion of antibacterial agents from the cell into the surrounding environment. These antibacterial agents are substrates of the RND pumps used by MDR bacteria of the *Klebsiella* genus [6]. One effective method to combat the multidrug resistance of these bacteria is to increase their sensitivity by combining them with an efflux pump inhibitor (EPI). PAβN is known as an efflux pump inhibitor of the RND AcrAB-TolC family. Combining *H. ciliata* extracts with PAβN has demonstrated a significant enhancement of HCL and HCB activity, with AIF values ranging from 4 to 256. These results support findings by Fonkou et al. [35] and Matieta et al. [16], which indicated the overexpression of efflux in strains and isolates of *Klebsiella* bacteria. The substantial improvement in the activity of the extracts not only justifies the overexpression of efflux in our strains and isolates but also suggests that these extracts are substrates of the RND family pumps (AcrAB-TolC). In fact, it was reported that antibacterial products demonstrating significant activity improvement in combination with PAβN are substrates of the AcrAB-TolC pumps [32, 33]. Botanicals can improve the activity of antibiotics against MDR bacteria [20, 25, 28, 38, 40, 68, 78]. In the present study, HCL and HCB improved the activity of antibiotics with AMF of 2 to 64. Indeed, the HCL improved the activity of antibiotics with potentiating powers against 100% of strains/isolates for PEN, AMP, and 90% for CIP. This could be explained by the fact that the latter would act as EPIs.

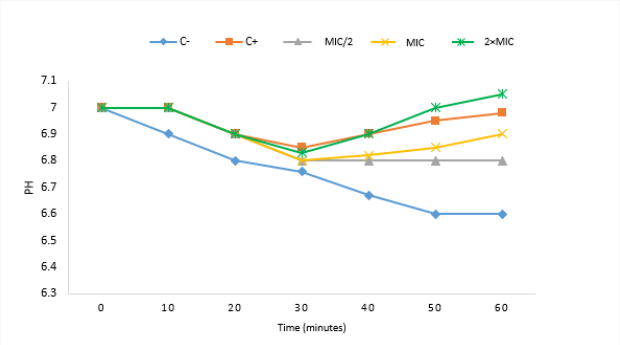

Figure 1. Effects of *Hallea ciliata* leaf extract (HCL) on H+ proton pumps/ATPases on *Klebsiella oxytoca* KO107

Table 1. Minimal inhibitory and bactericidal concentrations of the leave (HCL) and barks (HCB) extracts of *Hallea ciliata*, and IMI against the tested *Klebsiella* strains and isolates.

Bacteria	**Samples, MIC and MBC (in µg/mL), and MBC/MIC ratios**								
	Botanicals						**Antibiotic**		
	HCL			**HCB**			**Imipenem**		
	MIC	**MBC**	**R**	**MBC**	**MBC**	**R**	**MIC**	**MBC**	**R**
Klebsiella pneumoniae									
ATCC11296	256	512	2	512	1024	2	32	<32	nd
K2	128	2048	16	64	2048	32	32	128	4
KP55	512	2048	4	256	512	2	128	128	1
K24	128	1024	8	128	512	4	64	128	4
KP203	2048	2048	nd	256	>2048	nd	<1	16	nd
KP175	>2048		nd	>2048	nd	nd	16	128	8
KP77	128	1024	8	2048	>2048	nd	>128	nd	nd
KP93	>2048		nd	>2048	nd	nd	16	64	4
KP126	256	2048	8	128	2048	16	8	64	8
KP81	512	1024	2	>2048	nd	nd	32	>128	nd
Klebsiella oxytoca									
KO249	2048		nd	>2048	nd	nd	>128	nd	nd
KO96	1024		nd	2048	<2048	nd	32	128	4
KO107	64	128	2	128	2048	16	<1	128	nd
KO95	1024	2048	2	2048	<2048	nd	>128	nd	nd
KO26	512	2048	4	256	>2048	nd	32	128	4
KO55	512	2048	4	512	>2048	nd	16	>128	nd

MIC: Minimum Inhibitory Concentrations; MBC: Minimum Bactericidal Concentration; R: MBC/MIC ratio; nd: not determined; HCL: Hallea ciliata leaves extract; HCN: *Hallea ciliata* bark extract.

Table 2. Minimum inhibitory concentrations of the different extracts alone and in the presence of PAβN.

Bacteria	**HCL**			**HCB**			**Imipenem**		
	MIC *alone*	**+PA*β*N**	**R (AIF)**	**MIC *alone***	**+PA*β*N**	**R (AIF)**	**MIC *alone***	**+PA*β*N**	**R (AIF)**
Klebsiella pneumoniae									
K2	128	16	8	64	<8	*Nd*	<1	<1	1
KP55	512	16	**32**	256	64	**4**	8	<1	2
K24	128	<8	*16*	128	<8	16	32	8	4
KP175	>2048	<8	**256**	>2048	<8	**256**	64	8	8
KP93	>2048	<8	**256**	>2048	<8	**256**	<1	<1	1
Klebsiella oxytoca									
KO249	2048	32	**64**	>2048	64	*32*	32	16	2
KO96	1024	<8	*128*	2048	<8	*256*	8	<1	8
KO95	1024	<8	*128*	2048	64	**32**	128	8	**16**

MIC alone: Minimum inhibitory concentration in the absence of the inhibitor, +PAßN: Minimum inhibitory concentration in the presence of the inhibitor, R or AIF (activity improvement factor): MIC/+PAβN ratio, nd: not determined, HCL: *Hallea ciliata* leaves extract, HCB: *Hallea ciliata* bark extract.

Table 3. Effects of the combination of antibiotics and HCL against MDR bacteria.

Antibiotics	**HCL**	**Bacteria, MIC in µg/mL, and AMF 8(in bracket)**								**PBS**
		Klebsiella pneumoniae					***Klebsiella oxytoca***			
		K2	**KP55**	**K24**	**KP175**	**KP93**	**KO249**	**KO96**	**KO95**	
CIP	0	64	64	8	64	64	16	1	32	
	MIC/2	16**(4)**	4**(16)**	<1**(8)**	16**(4)**	16**(4)**	2**(8)**	<1**(1)**	4**(8)**	90
	MIC/4	16**(4)**	16**(4)**	<1**(8)**	64**(4)**	32**(2)**	2**(8)**	<1**(1)**	16**(2)**	90
LEV	0	8	64	16	16	16	16	64	8	
	MIC/2	<1**(8)**	16**(4)**	32**(0.5)**	32**(0.5)**	8**(2)**	4**(4)**	<1**(64)**	16**(0.5)**	60
	MIC/4	4**(2)**	16**(1)**	64**(0.25)**	64**(0.25)**	8**(2)**	4**(4)**	<1**(64)**	32**(0.25)**	50
TET	0	16	>128	128	64	64	>128	4	32	
	MIC/2	<1**(16)**	32**(4)**	128**(1)**	<1**(64)**	128**(0.5)**	<1**(128)**	4**(1)**	<1**(32)**	60
	MIC/4	<1**(16)**	64**(2)**	128**(1)**	64**(1)**	128**(0.5)**	<1**(128)**	4**(1)**	<1**(32)**	50
CFX	0	16	128	64	>1024	256	128	32	64	
	MIC/2	16**(1)**	32**(4)**	1024**(0.0625)**	16**(64)**	1024**(0.25)**	128**(1)**	<8**(4)**	<8**(8)**	50
	MIC/4	16**(1)**	32**(4)**	1024**(0.062)**	16**(64)**	1024**(0.25)**	128**(1)**	<8**(4)**	<8**(8)**	50
AMP	0	>1024	1024	>1024	>1024	1024	>1024	>1024	>1024	
	MIC/2	512**(2)**	256**(4)**	256**(4)**	256**(4)**	256**(4)**	256**(4)**	256**(4)**	256**(4)**	100
	MIC/4	512**(2)**	256**(4)**	256**(4)**	256**(4)**	512**(2)**	256**(4)**	512**(2)**	256**(4)**	100
PEN	0	>1024	128	>1024	16	1024	>1024	>1024	>1024	
	MIC/2	256**(4)**	64**(2)**	256**(4)**	<8**(2)**	128**(8)**	256**(4)**	256**(4)**	256**(4)**	100
	MIC/4	512**(2)**	1024**(0.125)**	256**(4)**	256**(0.062)**	1024**(1)**	256**(4)**	512**(2)**	256**(4)**	60
IMI	0	32	128	64	16	16	>128	32	>128	
	MIC/2	2**(16)**	2**(64)**	128**(0.5)**	16**(1)**	<1**(16)**	>128**(1)**	16**(2)**	>128**(1)**	50
	MIC/4	32**(1)**	2**(64)**	128**(0.5)**	64**(0.25)**	<1**(16)**	>128**(1)**	32**(1)**	>128**(1)**	25
CFX	0	32	1024	512	256	128	256	32	256	
	MIC/2	<8**(4)**	32**(32)**	1024**(0.5)**	128**(2)**	128**(1)**	256**(1)**	16**(2)**	128**(2)**	60
	MIC/4	16**(2)**	128**(8)**	1024**(0.5)**	128**(2)**	1024**(0.125)**	256**(1)**	16**(2)**	256**(1)**	50

MIC: Minimum Inhibitory Concentration; (): AMF: Activity modulation factor; PBS: Percentage of bacteria where synergy is observed.

Table 4. Effects of the combination of antibiotics and HCB against MDR bacteria.

Antibiotics	HCL	Bacteria, MIC in µg/mL, and AMF 8(in bracket)								PBS
		Klebsiella pneumoniae					*Klebsiella oxytoca*			
		K2	KP55	K24	KP175	KP93	KO249	KO96	KO95	
CIP	0	64	64	8	64	64	16	1	32	
	MIC/2	8**(8)**	8**(8)**	<1**(8)**	128**(0.5)**	4**(16)**	32**(0.5)**	128**(0.007)**	4**(8)**	60
	MIC/4	16**(4)**	8**(8)**	<1**(8)**	128**(0.5)**	4**(16)**	32**(0.5)**	128**(0.007)**	8**(4)**	60
LEV	0	8	64	16	16	16	16	64	8	
	MIC/2	2**(4)**	8**(4)**	32**(0.5)**	<1**(16)**	16**(1)**	16**(1)**	<1**(64)**	16**(0.5)**	50
	MIC/4	2**(4)**	8**(4)**	32**(0.5)**	<1**(16)**	16**(1)**	16**(1)**	<1**(64)**	16**(0.5)**	50
TET	0	16	>128	128	64	64	>128	4	32	
	MIC/2	16**(1)**	32**(4)**	64**(2)**	16**(4)**	128**(0.5)**	32**(4)**	4**(1)**	32**(1)**	50
	MIC/4	16**(1)**	64**(2)**	64**(2)**	16**(4)**	128**(0.5)**	>128**(1)**	4**(1)**	32**(1)**	40
CFX	0	16	128	64	>1024	256	128	32	64	
	MIC/2	16**(1)**	<8**(16)**	256**(0.25)**	256**(4)**	<8**(32)**	>1024**(0.125)**	16**(2)**	256**(0.25)**	50
	MIC/4	16**(1)**	32**(4)**	256**(0.25)**	>1024**(1)**	<8**(32)**	>1024**(1)**	16**(2)**	256**(0.25)**	40
AMP	0	>1024	1024	>1024	>1024	1024	>1024	>1024	>1024	
	MIC/2	256**(4)**	256**(4)**	256**(4)**	256**(4)**	1024**(1)**	256**(4)**	512**(2)**	256**(4)**	90
	MIC/4	512**(2)**	256**(4)**	256**(4)**	256**(4)**	1024**(1)**	256**(4)**	512**(2)**	256**(4)**	90
PEN	0	>1024	128	>1024	>1024	1024	>1024	>1024	>1024	
	MIC/2	256**(4)**	256**(0.5)**	256**(4)**	256**(4)**	1024**(1)**	256**(4)**	512**(2)**	256**(4)**	75
	MIC/4	512**(2)**	256**(0.5)**	256**(4)**	256**(4)**	1024**(1)**	256**(4)**	512**(2)**	256**(4)**	75
IMI	0	32	128	64	16	16	>128	32	>128	
	MIC/2	32**(1)**	>128**(1)**	64**(1)**	>128**(0.125)**	<1**(16)**	>128**(1)**	>128**(0.25)**	>128**(1)**	10
	MIC/4	32**(1)**	>128**(1)**	128**(0.5)**	>128**(0.125)**	<1**(16)**	>128**(1)**	>128**(0.25)**	>128**(1)**	10
CFX	0	32	1024	512	256	128	256	32	256	
	MIC/2	16**(2)**	>1024**(1)**	128**(4)**	1024**(0.25)**	<8**(16)**	128**(2)**	64**(0.5)**	<8**(32)**	60
	MIC/4	32**(1)**	>1024**(1)**	256**(2)**	>1024**(0.25)**	<8**(16)**	1024**(0.25)**	64**(0.5)**	<8**(32)**	25

MIC: Minimum Inhibitory Concentration; (): AMF: Activity modulation factor; PBS: Percentage of bacteria where synergy is observed.

Conclusion

The methanol extracts of *H. ciliata* have been shown to have anti-*Klebsiella* properties against MDR phenotypes. The leaf extract acts by inhibiting H+ proton pumps/ATPases. Both leaf and bark extracts were found to be substrates of bacterial efflux pumps and enhanced the activity of commonly used antibiotics. In conclusion, botanicals from *H. ciliata* have the potential to be used as antibacterial agents either alone or in combination with efflux pump inhibitors or antibiotics to combat *Klebsiella* infections.

Abbreviations

AIF : activity improvement factors
AMF : Activity modulation factor
AMP: ampicillin
ATCC: American-type culture collection
CFU: Colony Forming Unit
CFX: cefixime
CIP: ciprofloxacin
CTX: ceftriaxone
DMSO: Dimethylsulfoxide
EPI : efflux pump inhibitor
HNC: National Herbarium of Cameroon
IMI: imipenem
INT: Iodonitrotetrazolium chloride
LEV: levofloxacin
MBC: Minimum Bactericidal Concentration
MDR: Multidrug resistant
MHA: Mueller Hinton agar
MHB: Mueller Hinton broth
MIC: Minimal inhibitory Concentration
PAβN : phenylalanine arginine β-naphthylamide
PEN: penicillin
TET: tetracycline
WHO: World Health Organization

Authors' Contribution

DJA, LM, EC, AWBY, VYM, JRNK, INB, MFK, and JFM carried out the study; ATM and VK supervised the study; All authors read and approved the final version of the manuscript.

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Conflict of interest

The authors declare no conflict of interest.

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