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Antibacterial activity and antibiotic-potentiating effects of methanol extracts from *Ocimum basilicum* and *Sarcocephalus latifolius* against multidrug-resistant Gram-negative bacteria overexpressing efflux pumps

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Abstract

Background: The inappropriate use of antibiotics against bacterial infections leads to increased bacterial drug resistance. In the present study, the effectiveness of methanol extracts from the leaves and flowers of *Ocimum basilicum* and the roots of *Sarcocephalus latifolius* against multidrug-resistant (MDR) Gram-negative bacteria overexpressing efflux pumps was assessed.

Methods: The antibacterial properties of crude extracts (botanical, with and without phenylalanine arginine β-naphthylamide (PAβN), an inhibitor of the efflux pumps were performed using the liquid microdilution method. The effects of *O. basilicum* leaf extract on proton pumps H+/ATPases were analyzed using a spectrophotometric method. The study also involved screening the different extracts for phytochemicals using standard methods.

Results: The extracts of *O. basilicum* leaves and flowers and *S. latifolius* roots were active against at least 80% of the tested bacteria, with excellent activity against *E. coli* (AG100 and ATCC10536), *E. aerogenes* (EA3 and EA27), *P. stuartii* (PS2636 and ATCC29916) and *K. pneumoniae* KP55 (MICs ranging from 16 µg/mL to 64 µg/mL). At 32 µg/mL, the extract of *O. basilicum* leaves exhibited an inhibitory effect on the H+-proton pumps/ATPases of *E. coli* AG100. In the presence of PAβN, an improvement in the activity of the extracts against 100% of the tested bacteria was observed. All extracts enhanced the activity of tetracycline (TET), ciprofloxacin (CIP), cefixime (CFX), imipenem (IMI), levofloxacin (LEV), ceftriaxone (CTX), penicillin (PEN), and ampicillin (AMP) at MIC/2 and MIC/4. The activity improvement factors (AIF) ranged from 2 to 256. Terpenoids, saponins, and phenols were found in all the different extracts, meanwhile, flavonoids and alkaloids were specifically detected in the extract of the leaves of *O. basilicum* and the roots of *S. latifolius*.

Conclusion: The tested plants, *O. basilicum* and *S. latifolius* are important sources of antibacterial substances to combat bacterial infections involving MDR Gram-negative bacteria overexpressing efflux pumps.

Keywords: Antibacterial; modes of action; multidrug resistance; *Ocimum basilicum*; *Sarcocephalus latifolius*.

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Background

Since their discovery in 1928, antibiotics have significantly reduced morbidity and mortality related to bacterial infections [1]. As long as antibiotics are used, their surprising effectiveness has been accompanied by their inappropriate use in human and veterinary medicine. This has generated selection pressure on bacteria that have very quickly developed resistance mechanisms including enzymatic inactivation, target modification, reduced membrane permeability, and expression of efflux pumps limiting the use of antibiotics [1, 2]. Resistance through the expression of efflux pumps gives bacteria multidrug resistance phenotypes. They become able to expel a wide range of antibiotics from different families. Over the past decade, the prevalence of bacterial resistance to antibiotics has been increasingly reported in Gram-negative bacteria including Enterobacteriaceae (*Klebsiella pneumoniae, Escherichia coli, Enterobacter aerogenes, etc.*) and *Pseudomonas aeruginosa* [3]. The latter are top priority on the list established by the World Health Organization (WHO) in 2017 and overexpress respectively the AcrAB-TolC and MexAB-OprM efflux pumps of the Resistance Nodulation Cell Division (RND) family [4-6]. They significantly reduce the concentration of cytoplasmic antibiotics, thus leading to therapeutic failures linked to morbidity. In light of the global threat posed by antibiotic resistance, there is a growing focus on researching and developing new antibacterial substances [7, 8]. These substances should have the ability to combat infections on their own and also enhance the effectiveness of clinically used antibiotics. While there are various sources of antimicrobial substances, medicinal plants offer several advantages due to their diverse phytochemical structures and their historical use in traditional medicine [9]. It has been demonstrated that extracts from medicinal plants contain active substances that can be used alone or in combination with common antibiotics to combat multidrug-resistant (MDR) Gram-negative bacteria [10-14]. As part of this approach, we have selected *Ocimum basilicum* Linné (Lamiaceae), which has been traditionally used to treat diarrhea, relieve itching, and combat infectious diseases [15]. We also selected *Sarcocephalus latifolius* Smith (Rubiaceae), also known as *Nauclea latifolia* Sm., traditionally used to treat hepatitis, diarrhea, jaundice, rheumatism, constipation, and eye and muscle pain [16, 17].

Methods

Plant material and extraction

The leaves and flowers of *Ocimum basilicum* were harvested on October 2, 2023, in Koundoul, Chad in the Chari-Baguirmi Region, and the roots of *Sarcocephalus latifolius* were harvested on October 31, 2023, in Laï, Chad in the Tandjilé Region. Their identification was made by Mr. Tchatchouang Ngansop Eric, botanist expert at the National Herbarium of Cameroon (HNC) in Yaoundé, with the reference numbers 11955/SRFC (*Ocimum basilicum*) and 67005/HNC (*Sarcocephalus latifolius*). The leaves and flowers of *O. basilicum* and the roots of *S. latifolius* were cleaned, dried in the shade, and crushed. The powders obtained were macerated in methanol in a ratio of 1/3 (m/v) at room temperature for 48 hours. During this period, the mixtures were stirred three times a day to maximize the extraction yield of secondary metabolites. The macerates obtained were filtered using Whatman paper No. 1. The filtrates obtained were concentrated using a rotary evaporator at 65°C and dried in an oven at 45°C to remove the residual solvent. The crude extracts (botanicals) were then kept in the refrigerator at 4°C for further use.

Chemicals and culture media

Dimethyl sulfoxide (DMSO) served to solubilize plant extracts. Eight antibiotics, namely Ampicillin (AMP), levofloxacin (LEV), penicillin (PEN), cefixime (CFX), tetracycline (TET), ciprofloxacin (CIP), imipenem (IMI), and ceftriaxone (CTX) were used. 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) was used. All chemicals were purchased from Sigma-Aldrich (St. Quentin Fallavier, France).

Tested bacteria

The Gram-negative bacteria tested included both reference strains and clinical isolates of *Escherichia coli (*ATCC10536, AG102, and AG100), *Klebsiella pneumoniae (*ATCC11296, KP55, and KP63), *Pseudomonas aeruginosa* (PA01, PA124, and PA0100), *Enterobacter aerogenes* (EA3, EA298, and EA27), and *Providencia stuartii* (ATCC29916, PS2636, and NEA16). Their bacterial features were previously reported [5, 6, 12, 18-26]. *Escherichia coli (*AG102, and AG100), *Klebsiella pneumoniae (*KP55, and K2), *Enterobacter aerogenes* (EA3, EA298, and EA27), and *Providencia stuartii* (PS2636, and NEA16) are clinical bacterial strains overexpressing AcrAB-TolC efflux pumps while *Pseudomonas aeruginosa* PA124 overexpressed MexAB-OprM pumps [27-31].

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) [10, 11, 32-36]. 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 1024 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 [5, 6, 35, 37]. 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 [37-39] . 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 [40-42]. Each experiment was repeated three times in triplicate.

Evaluation of the effect of O. basilicum leaf extract *on the functioning of H+/ATPases proton dependent pumps of E. coli* AG100

The effects of *O. basilicum* leaf extract were assessed on the H^+-ATPase-mediated proton pumping of *E. coli* AG100 at 0.5×MIC, MIC, and 2×MIC as earlier described [36]. 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 [43, 44].

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 [6]. The ratio $MIC_{(sample\ alone)}/MIC_{(sample\ +PA\beta N)}$ 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 *E. coli* (ATCC10536, AG102), *P. aeruginosa* (PA0100 and PA124), *K. pneumoniae* (KP55 and ATCC11296), *P. stuartii* NEA16, and *E. aerogenes* EA282. 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 [21, 45]. The tested antibiotics included CTX, AMP, PEN, CFX, LEV, CIP, TET, and IMI. The tested bacteria were *E. coli* AG102, *P. aeruginosa* (PA0100 and PA124), *K. pneumoniae* (KP55 and ATCC11296), *P. stuartii* NEA16, and *E. aerogenes* EA282. 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 *P. aeruginosa* PA0100, 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 [46].

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) [7, 47].

Interpretation of antibacterial data

Updated and rationally defined cutoff points of the antibacterial botanicals have been defined, considering the various bacterial species [48-51]. For Enterobacteria: 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) [48]. For *P. aeruginosa:* outstanding activity (MIC ≤ 32 µg/mL), excellent activity (32 < MIC ≤ 128 µg/mL), very good activity (128 < MIC ≤ 256 µg/mL), good activity (256 < MIC ≤ 512 µg/mL), average activity (512 < MIC ≤ 1024 µg/mL), weak activity or not active (MIC values >1024 µg/mL) [49]. Bactericidal activities are considered when the ratios MBC/MIC are below or equal to 2 [41, 42, 52, 53].

The above appreciation criteria will be used to appreciate the antibacterial activities of the studied samples.

Results

Phytochemistry

Phytochemical screening of methanol extract from the botanicals from the flower and leaves of *O. basilicum* and the roots of *S. latifolius* revealed the presence of phenols, terpenoids, and saponins in the three plant extracts; The roots extract of *S. latifolius* also contained flavonoids, alkaloids, and anthocyanins, meanwhile flavonoids and alkaloids were also detected in the botanical from the leaves of *O. basilicum*.

Antibacterial activity

Table 1 summarizes the MICs and MBCs values resulting from the evaluation of the antibacterial activity of the different extracts and IMI. It appears that the botanicals from the leaves and flowers of *O. basilicum* and the roots of *S. latifolius* displayed antibacterial activities with MICs ranging from 16 µg/mL to 1024 µg/mL. The spectrum of inhibitory activity of the crude extract of the leaves of *O. basilicum* was 93.33% (14/15) with MICs ranging from 16 to 1024 µg/mL. However, this extract exhibited excellent activity against *E. coli* AG100 with a MIC of 16 µg/mL; *P. stuartii* with a MIC of 32 µg/mL; against *E. coli* ATCC10536, *P. stuartii* ATCC29916 and *E. aerogenes* EA3 with a MIC value of 64 µg/mL. We noted very good activity against *E. aerogenes* EA27 with a MIC of 128 µg/mL and good activity against the enterobacteria *E. coli* AG102, *E. aerogenes* EA282, and *K. pneumoniae* KP55 with a MIC of 256 µg/mL. This extract was found to be bactericidal against *P. aeruginosa* (PA01, PA124), *E. coli* AG102, *P. stuartii* NEA16, *E. aerogenes* (EA3 and EA282), and *K. pneumoniae* (KP63, ATCC11296) and bacteriostatic against *E. coli* (AG100 and ATCC10536), *P. stuartii* (PS2636 and ATCC29919) and *E. aerogenes* EA27. The botanical from *O. basilicum* flowers had an inhibitory activity spectrum of 80% (12/15) against the bacterial strains and isolates tested with MICs ranging from 16 µg/mL to 512 µg/mL. It showed excellent activity against *P. stuartii* PS2636 with a MIC of 16 µg/mL, against *P. stuartii* ATCC29916, and *E. aerogenes* EA3 with a MIC of 64 µg/mL. Against *E. coli* ATCC10536, *E. aerogenes* EA27, and *K. pneumoniae* KP55, very good activity with MICs of 128 µg/mL was observed. Good antibacterial activities with a MIC of 256 µg/mL were obtained against *E. aerogenes* EA27, *K. pneumoniae* KP55. Its bactericidal effect was extended against *E. coli* (AG102 and ATCC10536), *P. stuartii* (PS2636 and NEA16), against *E. aerogenes* (EA27 and EA282), and *K. pneumoniae* (KP55, KP63, and ATCC11296) and bacteriostatic against *P. stuartii* ATCC29916 and *E. aerogenes* EA3. The extract of the roots of *S. latifolius* had an inhibitory activity spectrum of 93.33% (14/15) with MICs ranging from 32 µg/mL to 1024 µg/mL. It showed excellent activity against *E. coli* AG100, *E. aerogenes* EA3 and *P. stuartii* PS2636 with a MIC of 32 µg/mL, against *E. coli* AG102, *E. aerogenes* EA 27 and *K. pneumoniae* KP55 with a MIC of 64 µg/mL. The activity of this extract against *P. stuartii* ATCC29916, *E. coli* ATCC10536, *K. pneumoniae* (KP63 and ATCC11296) was very good with a MIC of 128 µg/mL. Against enterobacteria *E. aerogenes* EA282 good activity with a MIC of 256 µg/mL was recorded. Moderate activity against *P. stuartii* NEA16 with a MIC and good activity against *P. aeruginosa* PA01 with a MIC of 512 µg/mL was observed. This

extract was bactericidal against *P. aeruginosa* (PA0100 and PA01), *E. coli* (AG102 and ATCC10536), *P. stuartii* (NEA16 and ATCC29916), *E. aerogenes* (EA3, EA27 and EA282), and *K. pneumoniae* (KP55 and ATCC11296). It was bacteriostatic against *E. coli* AG102 and *K. pneumoniae* KP63.

Effect of O. basilicum leaf extract on the functioning of H+ proton pumps/ATPases of E. coli AG100

The mode of action of the botanical from *O. basilicum* leaves was studied using its action on the functioning of the H+ proton pumps/ATPases of *E. coli* AG100. Hence, the variation of pH as a function of time in a reaction medium containing the bacterium and the crude extract was evaluated. The results are depicted in Figure 1. At MIC/2, the pH of the medium decreased over time, reaching the lowest value of 6.5 from the initial pH of 7.2 at time T0. At MIC, there was an increase in pH compared to the negative control, while at 2MIC, there was a significant increase compared to the negative control, with a pH difference of 0.58.

PAβN improves the activity of botanicals and IMI

The effect of EPI, PAβN, was performed on the eight most resistant bacteria. Table 2 summarizes the MICs of crude extracts of the leaves, flowers of *O. basilicum* and roots of *S. latifolius* in the presence of PAβN. It appears that the antibacterial effect of the botanicals from the leaves and flowers of *O. basilicum* and the roots of *S. latifolius* tested in the presence of PAβN was improved with activity AIF ranging from 2 to 256. The activity of the botanicals from *O. basilicum* and S. *latifolius* was improved against all (8/8) of the bacterial strains and isolates tested. The botanical from the roots of *S. latifolius* associated with PAβN revealed the highest AIF of 256 on *P. aeruginosa* PA124. The botanical from the leaves of *O. basilicum* also had a significant AIF value of 256 on *P. aeruginosa* PA100.

Antibiotic-potentiating effects of botanicals

Following the initial assay to determine the appropriate sub-inhibitory concentrations (MIC/2 and MIC/4), we conducted combinations of *O. basilicum* and *S. latifolius* extracts with antibiotics. The results can be found in Tables 3, 4, and 5. It can be observed that botanicals from *O. basilicum* and *S. latifolius* potentiated the activity of antibiotics against the tested MDR bacteria, with AMF varying from 2 to 256 (Tables 3 and 4). The crude extract of the flowers of *O. basilicum* potentiated at MIC/2 the activity of TET, CIP, CTX, IMI, and LEV against at least 75% of the bacterial isolates and strains tested; it potentiated that of CFY, PEN, and AMP against at least 50% of the bacteria tested. At MIC/4, we noted the potentiation of the activity of TET, CIP, CFX, IMI, and AMP against at least 62.5% of the bacteria tested (Table 3). At MIC/4, the activity of LEV was potentiated at 37.5%, and CFX and PEN at 50% (Table 3). Extract of the leaves of *O. basilicum* potentiated (at MIC/2) the activity of TET, CIP, CFX, IMI, and LEV against 100% of the bacteria tested. The activities of CFX and PEN were potentiated against 85.5% and AMP against 75% of the bacteria tested. At MIC/4, this extract potentiated the effect of CFX against 100% of the bacteria tested; LEV, IMI, and CFX against 85.5% of the bacteria tested. This extract also potentiated (at MIC/4) the effects of AMP and TET against 75% and 62.5% respectively, and CIP against 37.5% of the bacteria tested (Table 4). The extract of the roots of *S. latifolius* potentiated (at MIC/2) the effects of CFX and LEV against 100% of the bacteria tested. We noted that at MIC/2 and MIC/4, this extract potentiated the following antibiotics against at least 75% of the bacteria: TET, PEN, IMI, and AMP (Table 5). The effects of CIP and CTX were potentiated at MIC/2 against 75.5% and 87.5% of the bacteria tested, respectively. At MIC/4, we noted a potentiation effect of the botanical for LEV, CTX, and CIP against at least 50%, 50%, and 62.5%, respectively, while that of CFX was potentiated against 100% of the bacteria tested (Table 5).

Discussion

The rising challenge of treating infectious diseases caused by MDR bacteria, along with the high death rate due to bacterial resistance to antibiotics, poses a significant public health concern. Consequently, there is an urgent need to develop new active substances to tackle this major health issue. African flora has the potential to alleviate various human ailments, as previously documented [21-23, 54-70].These motivated the assessment of the antibacterial activity and potentiating effect of raw extracts from *O. basilicum* and *S. latifolius* against Gram-negative bacteria that overexpress efflux pumps. Based on the updated classification of the antibacterial activity of plant products against Enterobacteria [48], a botanical extract has excellent activity when 8 < MIC ≤ 64 µg/mL, and very good activity when 64 < MIC ≤ 128 µg/mL. Consequently, the extract of the leaves of *O. basilicum* was active against 93.33% of the tested bacteria and showed excellent activity against *E. coli* ATCC10536 and AG100, and *P. stuartii* ATCC29916 and PS2636. We also noted a very good antibacterial activity against *E. aerogenes* EA27. The extract from the flowers of *O. basilicum* showed excellent activity against *P. stuartii* PS2636 and ATCC29916, and *E. aerogenes* EA3; a very good activity against *E. coli* ATCC10536. The crude extract of the roots of *S. latifolius* showed excellent activity against *E. coli* AG100 and AG102, *E. aerogenes* EA3 and EA27, *P. stuartii* PS2636, and *P. pneumoniae* KP55. Against *P. stuartii* ATCC29916, *E. coli* ATCC10536, and *K. pneumoniae* (KP63 and ATCC11296), we noted very good activity and good activity against *P. aeruginosa* PA01. These data reveal the potential of the different crude methanol extracts of *O. basilicum* and *S. latifolius* to inhibit the growth of MDR Gram-negative bacteria tested.

The lack of data from an *in vivo* antibacterial activity study is a limitation of this study. The results obtained are in agreement with previous reports. In effect, Tankeo and his collaborators have documented the antibacterial activity of *O. basilicum* leaves (harvested in Cameroon) against a panel of bacteria including those used in the present work [15]. Their work revealed activities with MIC values ranging from 128 µg/mL to 1024 µg/mL. Similarly, Adigüzel et al. [71] reported that crude ethanol, hexane, and methanol extracts of *O. basilicum* flowers had antimicrobial activity against 146 microbial agents including 55 bacteria (including the genus *Pseudomonas* and *Escherichia*) with MICs ranging from 62.50 µg/mL to 500 µg/mL. Ahoyo et al., [72] reported the antibacterial activity of *S. latifolius* leaves and roots against five Gram-positive and four Gram-negative bacteria.

To better understand the excellent antibacterial activity of the crude extract of *O. basilicum* leaves, its mode of action on the functioning of the H+/ATPase proton pumps of E. coli AG100 was investigated. It was found that inhibits the functioning of the H+/ATPase proton pumps. In effect, Ngakam et al. [13] have demonstrated that botanicals can target H+ proton pumps/ATPases to inhibit bacterial growth. Enterobacteria (*E. coli, E. aerogenes, P. stuartii, K. pneumoniae*) and bacteria of the genus *Pseudomonas* used in this study were documented as MDR

[5, 6, 31]. Among the resistance mechanisms developed by bacteria, the one developed by these bacteria of interest is active efflux including the AcrAB-TolC pumps expressed in Enterobacteriaceae and the MexAB-OprM pumps in *P. aeruginosa* [4]. PAβN, known for its inhibitory effect on efflux pumps of the RND family such as AcrAB-TolC and MexAB-OprM [73], improved the activity of the tested botanicals. This indicates that the bacteria tested overexpress efflux pumps and that the constituents of the tested crude extracts are their potential substrates. This also suggests that a possible combination of the botanicals from *O. basilicum* and *S. latifolius* with an EPI may be possible to combat MDR Gram-negative bacteria.

To address the challenge of MDR Gram-negative bacteria, various research approaches could be pursued. Evidence from several researchers shows that using a combination of crude extracts from medicinal plants along with conventional antibiotics offers a potential alternative for combating antibiotic multi-resistance observed in Gram-negative bacteria [13]. In this study, the antibiotic-potentiating effect of botanicals demonstrated that the crude extract of the leaves of *O. basilicum* potentiated the activities of AMP, LEV, IMI, CFX, and CTX ON at least 75% bacteria tested, while the extract of the flowers potentiated the activities of IMI, LEV, TET, CIP, and CFX at least 75% of the studied bacteria. The crude extract of the roots of *S. latifolius* potentiated the activities of AMP, IMI, CIP, CEF, PEN, and TET by at least 75% against at least 75% of the bacteria studied. It has been suggested that when an extract potentiates the activity of at least 70% of antibiotics against at least 70% of the bacteria tested, it can be considered an efflux pump inhibitor (Braga et al., 2005, Fankam et al., 2011), therefore, the different extracts of *O. basilicum* and *S. latifolius* would be considered as EPI capable of interring with efflux pumps belonging to RND family.

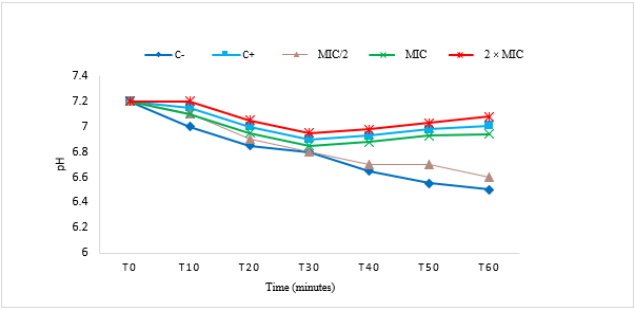

Figure 1. Effect of crude extract of *O. basilicum* leaves on H+ proton pumps/ATPases of *E. coli* AG100.

Table 1. Minimal inhibitory and bactericidal concentrations of the different extracts of *O. basilicum*, *S. latifolius*, and IMI against the tested bacteria.

Bacteria	Strains or isolates	Samples, MIC and MBC (in µg/mL), and MBC/MIC ratios											
		Botanicals and ATB											
		O. basilicum leaves			*O. basilicum flowers*			*S. latifolius rots*			ATB (imipenem)		
		MIC	MBC	R	MIC	MBC	R	MIC	MBC	R	MIC	MBC	R
P. aeruginosa	PAO1	1024	1024	**1**	>2048	-	nd	512	512	1	32	64	2
	PA124	1024	2048	**2**	>2048	-	nd	>2048	-	nd	>128	-	nd
	PA0100	>2048	-	nd	2048	>2048	nd	1024	2048	**2**	16	128	8
E. coli	AG100	**16**	512	32	512	>2048	nd	**32**	256	8	32	64	2
	AG102	256	512	**2**	512	512	**1**	**64**	64	**1**	32	64	2
	ATCC10536	**64**	512	8	128	512	**4**	128	512	**4**	32	32	1
P. stuartti	PS2636	**32**	1024	32	**16**	32	**2**	**32**	256	8	16	64	4
	NEA16	512	1024	**2**	512	2048	**4**	512	1024	**2**	64	128	2
	ATCC29916	**64**	512	8	**64**	1024	16	128	256	2	16	64	4
E.aerogenes	EA3	**64**	128	**2**	**64**	1024	16	**32**	128	**4**	8	16	2
	EA27	128	1024	8	256	256	**1**	**64**	64	1	<1	<1	<1
	EA282	256	256	**1**	512	512	**1**	256	256	**1**	32	32	1
K.pneumoniae	KP55	256	>2048	nd	256	512	**2**	**64**	512	8	64	128	2
	KP63	512	512	**1**	512	1024	**2**	128	512	**4**	16	32	2
	ATCC11296	512	512	**1**	512	512	**1**	128	512	**4**	64	128	2

R: MBC/MIC ratio; nd: not determined; MIC: minimum concentration; MBC: minimum bactericidal concentration; ATB: Antibiotic.

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

Bacteria	Strains or isolates	Samples, MIC alone and with PA*β*N (in µg/mL), and their ratios											
		Botanicals and ATB											
		***O. basilicum* leaves**			***O. basilicum* flowers**			***S. latifolius* roots**			**ATB (imipenem)**		
		MIC	MIC (+PA*β*N)	AIF	MIC	MIC (+PA*β*N)	AIF	MIC	MIC (+PA*β*N)	AIF	MIC	MIC (+PA*β*N)	AIF
E. coli	ATCC10536	64	< 8	8	128	< 8	16	128	32	4	32	< 1	**32**
	AG102	256	< 8	32	512	< 8	64	64	< 8	8	32	16	**2**
P. aeruginosa	PA0100	>2048	< 8	**256**	2048	128	16	1024	< 8	128	16	< 1	**16**
	PA124	1024	< 8	**128**	>2048	32	**64**	>2048	< 8	**256**	>128	>128	>1
K. pneumoniae	KP55	256	32	8	256	< 8	32	64	< 8	8	64	< 1	**64**
	ATCC11296	512	<8	64	512	< 8	**64**	128	64	2	64	< 1	**64**
P. stuartii	NEA16	512	< 8	64	512	< 8	**64**	512	128	4	64	< 1	64
E. aerogenes	EA282	256	16	16	512	16	32	256	16	16	32	16	**2**

AIF or activity improvement factors: $MIC_{alone}/MIC_{with\ PA\beta N}$, ratio; MIC: Minimum Inhibitory Concentration; $MIC_{with\ PA\beta N}$: Minimum Inhibitory Concentration in the presence of PAβN; ATB: Antibiotic.

Table 3. Effects of the combination of antibiotics and crude extract of *O. basilicum* flowers against MDR bacteria.

ATB	Crude extract	Bacteria, MIC in µg/mL, and AMF								PBS
		P. aeruginosa		*E. coli*		*K. pneumoniae*		*P. stuartii*	*E. aerogenes*	
		PA0100	PA124	AG102	ATCC10536	ATCC11296	KP55	NEA16	EA282	
TET	0	64	64	>128	16	32	32	>128	16	
	MIC/2	<1 **(64)**	64 (1)	<1 **(128)**	<1 **(16)**	64 (0.5)	8 **(4)**	64 **(2)**	<1 (**16**)	**75%**
	MIC/4	4 **(16)**	64 (1)	2 **(64)**	16 (1)	64 (0.5)	8 (**4**)	64 (**2**)	<1 (**16**)	**62.5%**
CTX	0	1024	512	256	64	512	256	64	32	
	MIC/2	512 (**2**)	<8 **(64)**	1024	32 (**2**)	<8 **(64)**	16 (**16**)	<8 (**8**)	< 8 (**4**)	**87.5%**
	MIC/4	512 (**2**)	<8 **(64**)	1024	32 (**2**)	<8 (**64**)	256 (1)	<8 **(8)**	16 **(2)**	**75%**
CFX	0	64	512	128	512	512	256	64	512	
	MIC/2	512	32 (**16**)	1024	512 (**1**)	<8 **(64)**	256 (1)	<8 **(8)**	16 **(32)**	**50%**
	MIC/4	512	64 **(8)**	1024	512 (**1**)	64 **(64)**	512	<8 (**8**)	16 **(32)**	**50%**
PEN	0	32	512	1024	256	128	256	64	16	
	MIC/2	<8 **(4)**	<8 **(64)**	<8 **(128)**	128**(16)**	64 (0.5)	8 **(32)**	>1024	32 (0.5)	**62.5%**
	MIC/4	<8 **(4)**	512 (1)	<8 **(128)**	128 **(4)**	256 (0.5)	128 (**2**)	>1024	32 (0.5)	**50%**
IMI	0	16	>128	32	32	64	64	64	32	
	MIC/2	<1 **(16)**	8 (**16**)	<1 (**32**)	16 (**2**)	8 (**8**)	64 (1)	<1 (**64**)	32 (1)	**75%**
	MIC/4	<1 (**16**)	32 (**4**)	<1 (**32**)	32 (1)	32 (**2**)	64 (1)	<1 (**64**)	64 (0.5)	**62.5%**
AMP	0	1024	1024	>1024	>1024	32	32	>1024	>1024	
	MIC/2	16 **(64)**	<8**(128)**	1024 (1)	>1024(1)	<8 (**4**)	<8 **(4)**	>1024 (1)	512 **(2)**	**62.5%**
	MIC/4	16 **(64)**	<8 **(128)**	1024 (1)	>1024(1)	<8 **(4)**	<8 **(4)**	>1024 (1)	512 **(2)**	**62.5%**
CIP	0	8	8	8	4	8	8	32	4	
	MIC/2	4 (**2**)	<1 (**8**)	32(0.25)	4 (1)	<1 (**8**)	<1 (**8**)	<1 (**32**)	<1 (**4**)	**75%**
	MIC/4	8 (1)	2 (**4**)	32(0.25)	4 (1)	2 (**2**)	2 (**4**)	<1 (**32**)	2 (**2**)	**62.5%**
LEV	0	16	32	8	64	32	64	2	4	
	MIC/2	<8 (**2**)	16 **(2)**	32 (1)	1 (**64**)	16 **(2)**	4 (**16**)	<1 (**2**)	<1 (**4**)	**87.5%**
	MIC/4	<8 (**2**)	64 (0.5)	32 (0.25)	<1 (**64**)	64 (0.5)	4 (**16**)	2 (1)	4 (1)	**37.5%**

MIC: minimum inhibitory concentration; (): activity modulation factor (AMF); LEV: Levofloxacin; AMP: Ampicillin; TET: Tetracycline; CIP: Ciprofloxacin; IPM: Imipenem; CTX: Ceftriaxone; CFX: cefixime; PEN: penicillin; PBS: Percentage of bacteria where synergy is observed.

Table 4. Effects of the combination of antibiotics and crude extract of *O. basilicum* leaves against MDR bacteria.

ATB	Crude extract	Bacteria, MIC in µg/mL, and AMF								PBS
		P. aeruginosa		*E. coli*		*K. pneumoniae*		*P. stuartii*	*E. aerogenes*	
		PA0100	PA124	AG102	ATCC10536	ATCC11296	KP55	NEA16	EA282	
TET	0	64	64	>128	16	32	32	>128	16	
	MIC/2	<1 **(64)**	<4 **(16)**	<1 **(128)**	<1 **(16)**	<1 **(32)**	<1 **(32)**	< 1 **(128)**	<1 (**16**)	**100%**
	MIC/4	2 **(32)**	16 **(4)**	2 **(64)**	16 (1)	32 (1)	8 (**4**)	>128 (1)	<1 (**16**)	**62.5%**
CTX	0	1024	512	256	64	512	256	64	32	
	MIC/2	16 (**64**)	16 **(32)**	16 (**16**)	<8 (**8**)	16 **(32)**	512	<8 (**8**)	<8 (**4**)	**87.5%**
	MIC/4	16 (**64**)	32 (**16**)	16 **(16)**	16 (**4**)	32 (**16**)	512	<8 **(8)**	16 **(2)**	**87.5%**
CFX	0	64	512	128	512	512	256	64	512	
	MIC/2	32 **(2)**	<8 (**64**)	16 (**8**)	256 **(2)**	<8 **(64)**	<8 **(32)**	< 8 **(8)**	<8 **(64)**	**100%**
	MIC/4	32 **(2)**	64 **(8)**	16 (**8**)	256 **(2)**	64 **(8)**	<8 **(32)**	<8 (**8**)	32 **(16)**	**100%**
PEN	0	32	512	124	256	128	256	64	16	
	MIC/2	<8 **(4)**	<8 **(64)**	<8 **(16)**	128 **(2)**	<8 **(16)**	<8 **(32)**	<4 **(16)**	16 (1)	**87.5%**
	MIC/4	64 (0.5)	64 **(8)**	64 **(2)**	256 (1)	32 (**4**)	<8 (**32**)	<4 **(16)**	16 (1)	**62.5%**
IMI	0	16	>128	32	32	64	64	64	32	
	MIC/2	<1 **(16)**	<1(**128**)	<1 (**32**)	<1 (**32**)	<1 (**64**)	<1 (**64**)	16 (**4**)	<1 (**32**)	**100%**
	MIC/4	<1 (**16**)	<1(**128**)	<1 (**32**)	<1 (**32**)	<1 (**64**)	64 (1)	32 (**2**)	16 (2)	**87.5%**
AMP	0	1024	1024	>1024	>1024	32	32	>1024	>1024	
	MIC/2	16 **(64)**	<8**(128)**	<8 (**128**)	>1024	<4 (**8**)	<4 **(8)**	>1024	<4 **(256)**	**75%**
	MIC/4	16 **(64)**	16 **(64)**	<8 (**128**)	>1024	8 **(8)**	16 **(2)**	>1024	32 **(32)**	**75%**
CIP	0	8	8	8	4	8	8	32	4	
	MIC/2	<1 **(8)**	<1 **(8)**	<1 **(8)**	<1 **(4)**	<1 **(8)**	<1 **(8)**	<1 (**32**)	<1 **(4)**	**100%**
	MIC/4	4 **(2)**	8 (1)	16 (0.5)	8 (0.5)	8 (1)	2 **(4)**	<1 (**32**)	4 (1)	**37.5%**
LEV	0	16	32	8	64	32	64	2	4	
	MIC/2	4 (**4**)	<1 **(32)**	<1 (**8**)	32 (**2**)	<1 **(32)**	<1 (**64**)	<1 (**2**)	<1 (**4**)	**100%**
	MIC/4	4 (**4**)	<1 **(32)**	16 (0.5)	32 (**2**)	<1 (**32**)	<1 (**64**)	<1 (**2**)	<1 **(4)**	**87.5%**

MIC: minimum inhibitory concentration; (): activity modulation factor (AMF); LEV: Levofloxacin; AMP: Ampicillin; TET: Tetracycline; CIP: Ciprofloxacin; IPM: Imipenem; CTX: Ceftriaxone; CFX: cefixime; PEN: penicillin; PBS: Percentage of bacteria where synergy is observed.

Table 5. Effects of the combination of antibiotics and crude extract of *S. latifolius* roots against MDR bacteria.

ATB	Crude extract	Bacteria, MIC in µg/mL, and AMF								PBS
		P. aeruginosa		*E. coli*		*K. pneumoniae*		*P. stuartii*	*E. aerogenes*	
		PA0100	PA124	AG102	ATCC10536	ATCC11296	KP55	NEA16	EA282	
TET	0	64	64	>128	16	32	32	>128	16	
	MIC/2	<1 **(64)**	<1 **(64)**	64 **(2)**	<1 **(16)**	32 (1**)**	8 **(4)**	< 1 **(128)**	<1 (**16**)	**87.75%**
	MIC/4	8 **(8)**	4 **(16)**	64 **(2)**	16 (1)	32 (1)	8 **(4)**	< 1 **(128**	<1 (**16**)	**75%**
CTX	0	1024	512	256	64	512	256	64	32	
	MIC/2	32 (**32**)	16 **(32)**	512 (0.5)	32 (**2**)	32 (**16**)	64(**4**)	<8 (**8**)	< 8 (**4**)	**87.5%**
	MIC/4	32 (**32**)	16 **(32)**	512 (0.5)	32 (**2**)	32 (**16**)	1025	128	128)	**50%**
CFX	0	64	512	128	512	512	256	64	512	
	MIC/2	16 **(4)**	16 (**32**)	<8 (**16**)	8 **(64)**	16 **(32)**	32 **(8)**	<8 (**8**)	16 **(32)**	**100%**
	MIC/4	16 **(4)**	16 **(32)**	16 (**8**)	64 **(8)**	32 **(16)**	32 **(8)**	<8 (**8**)	16 **(32)**	**100%**
PEN	0	32	512	1024	256	128	256	64	16	
	MIC/2	<8 **(4)**	<8 **(64)**	512 **(2)**	< 8 **(32)**	<8 **(16)**	<8 **(32)**	<8 **(8)**	16 (1)	**87.5%**
	MIC/4	16 (**2**)	<8 **(64)**	1024 **(1)**	128 **(2)**	<8 **(16)**	<8 **(32)**	16 **(4)**	16 (1)	**75%**
IMI	0	16	>128	32	32	64	64	64	32	
	MIC/2	<1 **(16)**	<1(**128**)	2 (**16**)	<1 (**32**)	<1 (**64**)	64 (**1**)	<1 (6**4**)	16 (**2**)	**87.5%**
	MIC/4	<1 (**16**)	<1 (**128**)	2 (**16**	16 (**2**)	<1 (**64**)	64 (1)	<1 (6**4**)	32 (1)	**75%**
AMP	0	1024	1024	>1024	>1024	32	32	>1024	>1024	
	MIC/2	<8 **(128)**	512 **(2)**	<8 (**128**)	>1024	< 8 (**4**)	<8 (**8**)**)**	<8 (**128)**	<8 (**128)**	**87.5%**
	MIC/4	16 **(64)**	1024 **(1)**	<8 (**128**)	>1024	< 8 (**4**)	<8 (**8**)**)**	<8 (**128)**	32 **(32)**	**75%**
CIP	0	8	8	8	4	8	8	32	4	
	MIC/2	4 **(2)**	16 **(**0.5)	8 (1)	<1 **(4)**	<1 **(8)**	2 **(4)**	<1 (**32**)	<1 **(4)**	**75%**
	MIC/4	4 **(2)**	16 **(**0.5)	8 (1)	<1 **(4)**	4 **(2)**	4 **(2)**	<1 (**32**)	4 (1)	**62.5%**
LEV	0	16	8	8	64	32	64	2	4	
	MIC/2	4 (**4**)	<1 (**8)**	<1 (**8**)	< 1 (**64**)	<1 **(32)**	8 (**8**)	<1 (**2**)	<1 (**4**)	**100%**
	MIC/4	16 (**1**)	8 **(1)**	4 (**2**)	< 1 (**64**)	<1 (**32**)	8 (**8**)	<1 (**2**)	4 **(1)**	**50%**

MIC: minimum inhibitory concentration; (): activity modulation factor (AMF); LEV: Levofloxacin; AMP: Ampicillin; TET: Tetracycline; CIP: Ciprofloxacin; IPM: Imipenem; CTX: Ceftriaxone; CFX: cefixime; PEN: penicillin; PBS: Percentage of bacteria where synergy is observed.

Conclusion

In this study, the antibacterial properties of methanol extracts from *Ocimum basilicum* and *Sarcocephalus latifolius* were evaluated. It has been found that the methanol extracts from the leaves and flowers of O*. basilicum*, as well as the roots of *S. latifolius*, exhibited antibacterial activity against MDR Gram-negative bacteria. The extracts had excellent activity against many strains and clinical isolates. Specifically, the extract from *O. basilicum* leaves inhibited the functioning of the H+/ATPase proton pumps of *E. coli* AG100. The botanicals from *O. basilicum* and *S. latifolius* are substrates of bacterial efflux pumps, and these extracts can enhance the effectiveness of various antibiotics, including β-lactams, cyclins, and quinolones. All the tested extracts contain phytochemicals with documented antibacterial activities. The findings suggest that these plants could be potential sources of antibacterial drugs, either alone or in combination with antibiotics, to combat resistant bacterial strains.

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

EC, DJA, AWBY, LM, VYM, JRNK, INB, 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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