Investigational
Medicinal Chemistry & Pharmacology

Research Article Open Access

Antibacterial potential and modes of action of the methanol extracts of *Elephantopus mollis* Kunth (Asteraceae) against multidrug-resistant Gram-negative bacteria overexpressing efflux pumps

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Abstract

Background: Bacterial drug resistance still constitutes a major clinical issue. In the present study, the *in vitro* antibacterial potential, and modes of action of *Elephantopus mollis* were investigated.

Methods: The antibacterial activity of methanol extracts of the various parts of *E. mollis*, their association with an efflux pump inhibitor, phenylalanine-arginine *β*-naphthylamide (PAβN), and the potentiating effect of several standard antibiotics were determined using the broth microdilution method. The effects of *E. mollis* leaf extract on H+-proton pump/ATPase function and bacterial growth kinetics were determined using standard methods. Phytochemical screening of the extracts was carried out using standard qualitative methods.

Results: The crude extract (botanicals) from *E. mollis* leaf and flower had antibacterial activities with a 100% inhibition spectrum against bacterial strains and isolates, and the MIC values ranging from 16 to 256 µg/mL and 64 to 1024 µg/mL respectively. Botanical from the leaf showed excellent activity with a MIC of 16 µg/mL against *K. pneumoniae* KP55, a MIC of 32 µg/mL against *K. pneumoniae* (K2), and *P. stuartti* (NEA16). Botanicals from the leaf inhibited the exponential growth phase and H^+-proton pump/ATPases of *K. pneumoniae* ATCC11296. In the presence of PAβN, the activity of *E. mollis* extracts was increased on 90% (leaves and flowers) and 63% (roots) of the multidrug-resistant (MDR) bacteria tested. The various extracts of *E. mollis* potentiated the activities of the antibiotics: doxycycline, levofloxacin, vancomycin, imipenem, ceftriaxone, and ciprofloxacin against at least 70% of bacterial strains and isolates, with factors of increase in activity ranging from 2 to 128. Extracts from all parts of *E. mollis* contained alkaloids, flavonoids, tannins, and phenols.

Conclusion: The results show that *E. mollis* is a source of antibacterial phytomedicine that can be used to treat bacterial infections caused by Gram-negative bacteria expressing MDR phenotypes.

Keywords: Antibiotics; Asteraceae; bacteria; efflux pumps; *Elephantopus mollis*; multidrug resistance.

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Citation on this article: Mapie Tiwa S, Matieta VY, Ngakam R, Kengne Fonkou G, Megaptche JF, Nayim P, Mbaveng AT, Kuete V. Antibacterial potential and modes of action of methanol extracts of Elephantopus mollis Kunth (Asteraceae) against multidrug-resistant Gram-negative bacteria overexpressing efflux pumps. Investigational Medicinal Chemistry and Pharmacology (2024) 7(1):86; Doi: https://dx.doi.org/10.31183/imcp.2024.00086

Background

Bacterial multidrug resistance is the ability of a pathogenic bacterium to survive at least two antibiotics belonging to different families, thus leading to an increasing mortality rate and a considerable economic impact [1]. According to the World Health Organization (WHO), of the 2.7 million neonatal deaths recorded each year, 560,000 cases are caused by microbial infections. However, half of this mortality rate is in developing countries, particularly in South Asia and sub-Saharan Africa [2]. In 2019, the death rate due to antimicrobial resistance was estimated at approximately 4.19 million deaths worldwide while 1.27 million of these deaths were attributed to infectious diseases due to multidrug-resistant (MDR) pathogenic bacteria [3]. Several bacteria have been increasingly implicated in infectious diseases in humans, specifically, *Enterococcus spp, Enterobacter spp, Klebsiella pneumoniae, Staphylococcus aureus, Acinetobacter baumanii*, *Pseudomonas aeruginosa*, as well as *Escherichia coli* [4]. The inappropriate and abusive use of antibiotics in humans and animals is the main reason for the occurrence of antibiotic resistance. The most predominant resistance mechanisms in bacteria are, among others: enzymatic inactivation, modification of the target, modification of membrane permeability, formation of biofilm, and overexpression of efflux pumps. Indeed, efflux pump systems can identify and expel from the bacterial cell a wide range of chemically unrelated substances, including antibiotics. In Gram-negative bacteria, efflux pumps of the resistance nodulation cell division (RND) type are responsible for resistance to many families of antibiotics: these are AcrAB-TolC pumps in Enterobacteriaceae and MexAB-OprM in *P. aeruginosa* [5, 6].

Good strategies for effectively combating bacterial resistance and multidrug resistance are based on the search and development of novel antibacterial molecules from the plant kingdom [7-11]. Several African medicinal plants and their phytochemicals previously displayed good efficiency against MDR Gram-negative bacteria [12-19]. To improve our library of the antibacterial plant acting in MDR bacteria, the present study focused on *Elephantopus mollis* Kunth (Asteraceae), a plant native to South America [20]. The plant is commonly known as brown tobacco, false tobacco, and elephant foot. The plant is traditionally used for the treatment of pathologies such as cough, dysentery, hepatitis, cancer, and liver infection [21]; it is also used against fever, wounds, skin conditions, and intestinal disorders [22]. Herein, the antibacterial activity of botanicals from various parts of the plant was determined in a panel of MDR Gram-negative bacteria. The modes of action of the botanicals from the botanicals were also determined.

Methods

Plant material and extraction

The leaves, flowers, and roots-stems of *Elephantopus mollis* Kunth were collected in the locality of Fokoué, Menoua Department, West Region of Cameroon in December 2022. The identification of the plant was made at the Herbarium Cameroon National (HNC) in Yaoundé under voucher number 35121/HNC. The different parts of *E. mollis* were air-dried and powdered. The powder resulting from the different parts was macerated in methanol at a ratio of 1/3 (m/v) for 48 hours. Subsequently, the macerate obtained was filtered using Whatman filter paper n°1. The filtrate obtained was concentrated under a vacuum at 65°C. The crude extract obtained was completely dried in an oven at 40°C to remove the residual solvent and kept at 4°C until further use.

Chemicals and culture media

para-Iodonitrotetrazolium chloride ≥ 97% (INT) was used as the bacterial growth indicator. Dimethyl sulfoxide (DMSO) served to solubilize plant extracts. Eight antibiotics from four families, namely ampicillin, ceftriaxone, imipenem, tetracycline, doxycycline, vancomycin, levofloxacin, and ciprofloxacin were used. Five culture media were used: Mueller Hinton Agar (MHA), for the activation of bacterial strains and isolates; Mueller Hinton Broth (MHB), used during microdilution as a nutrient medium for bacteria; Eosin methylene blue (EMB), specific and differential culture medium to confirm the purity of bacterial strains and isolates belonging to species of the genus *Escherichia coli* and *K. pneumoniae*; MacConkey, specific and differential culture medium to confirm the purity of bacterial strains and isolates belonging to species of the genus *E. coli*; and Cetrimide, specific and differential culture medium to confirm the purity of *P. aeruginosa*. All chemicals were purchased from Sigma-Aldrich (St. Quentin Fallavier, France).

Bacterial strains and isolates

Five Gram-negative bacterial species, each including three bacterial strains or isolates were used in this work. They were *Escherischia coli (*ATCC10536, AG102, and AG100), *Klebsiella pneumoniae (*ATCC11296, KP55, and K2), *Pseudomonas aeruginosa* (PA01 and PA124), *Enterobacter aerogenes* (EA3, EA298, and EA27), and *Providencia stuartii* (ATCC29916, PS2636, and NEA16). Their bacterial features are shown in Table 1.

Determination of minimal inhibitory (MIC) and bactericidal (MBC) concentrations

The bacterial inoculum was prepared as previously described [23-29] in comparison to the turbidity of a standard McFarland 0.5 ($1.5x10^8$ CFU/mL). The various plant extracts and the reference drug (imipenem) were dissolved in DMSO-MHB. Plant extracts were prepared at 8192 µg/mL, and antibiotics at 1024 µg/mL. PAβN was prepared at 100 µg/mL. Botanicals were tested alone, 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 botanicals [28, 30-32]. The minimal inhibitory (MIC) and bactericidal (MBC) concentrations of botanicals alone were determined using a 96-well broth microdilution method combined with the rapid INT colorimetric method [32-34]. The reference drug used was imipenem for positive 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 (the least concentration for which no color change is observed) 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 upon 48 h of additional incubation [35-37]. Each experiment was repeated three times in triplicate.

Evaluation of the effect of the methanol extract of *Elephantopus mollis* leaves on growth kinetics of *K. pneumoniae* ATCC11296.

To evaluate the effect of the crude extract from the leaf of *Elephantopus mollis* on the kinetics of bacterial growth, the optical densities (OD) were measured following the protocol previously

described [24]. The *P. stuartii* ATCC29916 strain was activated onto MHA at 37°C for 18 h. Subsequently, a few colonies of this bacterial culture were removed to prepare a suspension with turbidity corresponding to McFarland 0.5 (1.5×10^8 CFU/mL). With MHB, 20 mL of inoculum solution was prepared at a concentration of 10^6 CFU/mL. These inocula were treated with the botanicals at MIC/2, MIC, and 2×MIC, and the whole was incubated with stirring at a speed of 130 rpm using a magnetic stirrer to allow good dispersion of these. A positive control contained CIP at MIC while the negative control was MHB + the bacterial suspension. After incubation times of 0 min, 1 h, 2 h, 4 h, 6 h, 8 h, 10 h, 12 h, 14 h, 16 h, 18 h, and 20 h, 200 µL of each solution were introduced into the wells of flat-bottomed microplates and the OD were read at 600 nm. Each test was repeated 3 times.

Evaluation of the effect of E. mollis leaf extract on the H^+-ATPases pumps

The effects of leaf methanol extract were assessed on the kinetic growth and H^+-ATPase-mediated proton pumping of *K. pneumoniae* ATCC11296, at 0.5×MIC, MIC, and 2×MIC as earlier described [29]. The action on kinetic growth consisted of measuring the absorbance (600 nm) of the bacterial solution treated with extracts at various concentrations over 20 hours, whereas the action on H^+-ATPase-mediated proton pumping was done by controlling the acidification of the bacterial growth medium over 60 min. Elaborated procedures were previously described [38, 39].

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. 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* PA01, which then allowed the selection of appropriate sub-inhibitory concentrations of MIC/2 and MIC/4 for further combination testing (Data not shown). Antibiotic-resistance modulating 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 [40].

Phytochemical screening of E. mollis extracts

Phytochemical screening was done following the standard methods described for alkaloids, anthocyanins, flavonoids (Shinoda test), phenols, saponins, tannins, and triterpenes (Liebermann-Burchard test) [9, 41].

Interpretation of antibacterial data

Several cutoff points are available for the interpretation of the antibacterial activity of plant products including extracts from edible plants [7, 42]. According to Kuete [7], the following threshold values are applied to botanicals: significant activity (MIC <100 µg/mL), moderate (100 <MIC ≤ 625 µg/mL), and low or negligible (MIC> 625 µg/mL). According to Tamokou et al. [42], the cutoff point for the antibacterial activity of botanicals from edible plants are as follows: highly active (MIC below 100 µg/mL), significantly active (100 ≤ MIC ≤ 512 µg/mL), moderately active (512 < MIC ≤ 2048 µg/mL), low activity (MIC > 2048 µg/mL), and considered not active (MIC > 10 mg/mL). However, updated and rationally defined cutoff points of the antibacterial botanicals have been defined, considering the various bacterial species [43-46]. 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) [43]. 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) [44]. The above appreciation criteria have been used to discuss the antibacterial activities of samples reported in the present study.

Results

Antibacterial activity of the crude extracts

The antibacterial activity of the botanicals from leaves, flowers, and roots of *E. mollis* was evaluated by determining the MICs and MBCs on a panel of 15 strains and isolates belonging to 5 bacterial species: *P. aeruginosa*, *K. pneumoniae*, *E. coli*, *E. aerogenes,* and *P. stuartti*. To determine whether the extracts of *E. mollis* had bactericidal or bacteriostatic effects, the MMC/MIC ratio was calculated, and all the results are recorded in Table 2. The different botanicals displayed MICs varying from 16 to 2048 µg/mL. The botanical from the leaves had an inhibition spectrum of 100% against the bacteria tested, with MICs ranging from 16 to 256 µg/mL. It showed excellent activity with a MIC of 16 µg/mL against *K. pneumoniae* ATCC11295, a MIC of 32 µg/mL against *K. pneumoniae* K2 and *P. stuartti* NEA16, a MIC of 64 µg/mL against *K. pneumoniae* KP55, *P. stuartti* (ATCC29761 and PS2636), *E. aerogenes* (EA27 and EA298) and *E. coli* AG100. However, against the other tested enterobacteria, it had good activities. Against *P. aeruginosa* PA124, the botanical from the leaf had excellent activity with a MIC of 128 µg/mL and very good activity against *P. aeruginosa* (PA01 and PA121) with a MIC of 256 µg/mL. The extract from the leaves of *E. mollis* had a bactericidal effect against *K. pneumoniae* ATCC11295*, E. aerogenes* EA298, and *P. aeruginosa* (PA01, PA121, and PA124). The botanical from the flowers exhibited an inhibition spectrum of 100% against the tested bacteria with MIC values ranging from 64 to 1024 µg/mL. It showed excellent activity with a MIC of 64 µg/mL against *K. pneumoniae* ATCC11295 and very good activity with a MIC value of 128 µg/mL against *P. stuartti* ATCC29761 and *E. coli* (AG100 and AG102). However, it had good activities with other Enterobacteria. The extract of *E. mollis* flowers showed good activity (256 µg/mL) against *P. aeruginosa* PA124 and moderate activity against all other strains and isolates of *P. aeruginosa* tested. The extract of *E. mollis* flowers had a bacteriostatic effect against *P. stuartti* ATCC29761 and *E. coli* (AG100 and AG102); it was bactericidal against the other bacterial strains and isolates. The methanol extract of the roots of *E. mollis* showed an antibacterial inhibition spectrum of 86.66% with MIC values ranging from 128 to 2048 µg/mL. In general, the root extract had activities ranging from moderate to low. Nevertheless, this extract displayed very good activity against *K. pneumoniae* ATCC11295 with a MIC value of 128 µg/mL. The extract from the roots of *E. mollis* showed bactericidal effects against *K. pneumoniae* ATCC11295 and *P. stuartti* PS2636.

Effect of methanol extract of E. mollis leaves on the growth kinetics of K. pneumoniae ATCC11296

The kinetics of the growth of *K. pneumoniae* ATCC11296 in the presence of the leaf extract as well as the control drug, ciprofloxacin was evaluated, and the results are depicted in Figure 1. It was found that the growth curve of *K. pneumoniae* ATCC11296 in the absence of extract at MIC/2 presents all the phases of bacterial growth except the last phase: a latency phase (0 - 2 h), an exponential phase (2 - 10 h), and a stationary phase (10 -20 h). The curve in the presence of the extract from the leaves of *E. mollis* at the MIC shows a decrease in the exponential phase ranging from (2 -8 h) and an extension of the stationary phase from (8 -20 h). In the presence of the extract at 2MIC and ciprofloxacin at MIC, inhibition of growth in the exponential phase ranges from 2 - 6 h, and a prolongation of the stationary phase lasted from 6 - 20 h.

Effect of E. mollis leaf extract on H^+-ATPase pumps of K. pneumoniae ATCC11296

The ability of *E. mollis* leaf extract to interfere with the functioning of the H^+-ATPase proton pumps of *K. pneumoniae* ATCC11296 was assessed by measuring at different times the pH of the medium containing *K. pneumoniae* ATCC11296 in the presence of the leaf extract (Figure 2). At MIC/2 there was a decrease in pH values of the culture medium, indicating its acidification, from pH 6.4 to pH 4; i.e., a decrease of 2.4. At MIC and 2MIC, less pronounced acidification of the medium (pH 4.65 and 5.15, respectively) was observed. This is an indication that the extracts exert a dose-dependent inhibition of the H^+-ATPase proton pumps.

PAβN improves the activity of botanicals from E. mollis

The MICs botanicals alone and in the presence of PAβN are shown in Table 3. PAβN enhanced the activity of the extracts of *E. mollis* with an increase factor ranging from 2- to 64-fold. The increase was recorded in 90.90% (10/11) of the bacteria tested in the cases of leaves and flower extracts, and 63.63% (7/11) in the case of the root extract. The combination of the root extract with PAβN showed the highest increase in activity of up to 64-fold on *P. aeruginosa* (PA01 and PA124). This is an indication that the constituents of the botanicals are the substrates of bacterial efflux pumps.

Antibiotic-potentiating effects of the botanicals

Botanicals at MIC/2 and MIC/4 were tested in combination with antibiotics, and the results are shown in Tables 4 to 6. The activities of the antibiotics were improved by the extracts on at least one tested bacterium, with activity increase factors ranging from 2- to 128-fold. Botanical from the roots of *E. mollis* potentiated (at MIC/2 and MIC/4) the activity of doxycycline, vancomycin, ciprofloxacin, imipenem, and levofloxacin on at least 80% of the bacteria tested. it potentiated the effects of ceftriaxone on at least 70% of bacteria tested. The root extract potentiated the effects of tetracycline and ampicillin on at least 60% and 40% of the bacteria tested, respectively (Table 4). It was found that the methanol extract of the leaf (at MIC/2 and MIC/4) enhanced the activity of doxycycline, vancomycin, ciprofloxacin, imipenem, ceftriaxone, and levofloxacin vis-a-vis at least 80% of bacteria tested. This extract potentiated tetracycline and ampicillin against at least 60% and 40% of bacteria, respectively (Table 5). The botanical from the flowers (at MIC/2 and MIC/4) potentiated the activity of ciprofloxacin, imipenem, ceftriaxone doxycycline, vancomycin, and levofloxacin vis-a-vis at least 80% of bacteria tested. It potentiated the effects of tetracycline and ampicillin against at least 70% and 30% of bacteria, respectively (Table 6).

Phytochemical composition of the botanicals

The crude extract from the leaf of *E. mollis* contained all the investigated classes of secondary metabolites, namely alkaloids, anthocyanins, flavonoids, phenols, saponins, tannins, and triterpenes (Table 7). They were selectively present in roots and flower extracts.

Discussion

Medicinal plants are an undeniable source of effective and low-toxic natural substances that can help fight against recalcitrant human pathologies such as microbial, parasitic, viral infections, and MDR cancer phenotypes [47-70]. This work constitutes a good model for the discovery of substances to counteract bacterial resistance, given the MDR features of many bacteria tested. According to the established classification scales, the extract from the leaves of *E. mollis* showed excellent activity [43] against *K. pneumoniae* ATCC11295, *K. pneumoniae* K2 and KP55, *P. stuartti* (NEA16, ATCC29761 and PS2636), *E. aerogenes* (EA27 and EA298) and *E. coli* AG100. It also displayed excellent and very good activities against aeruginosa PA124 and P. aeruginosa (PA121 and PA01), respectively. These results are in agreement with those obtained by Nguyen et al. [71] who highlighted the significant antibacterial activity of the water decoction of the leaves of *E. mollis* against the Enterobacteriaceae *E. coli, S. Typhi,* and *S. flexneri*. Ohana et al. [22] also demonstrated that the hydro-ethanolic extract of *E. mollis* leaves has exceptional [43, 44] antibacterial activity against susceptible strains of *E. coli*, *P. aeruginosa* and *K. pneumoniae* with a MIC value of 5 µg/mL, thus confirming the interesting antibacterial activity of the leaves of *E. mollis*. A significant shortening of the exponential growth phase of this bacterium in the presence of the extract of the leaves of *E. mollis* was observed. A decrease in the bacterial population at this phase of bacterial growth could be because the extract from the leaves of *E. mollis* denatures the enzymes and proteins, and inhibits the transport systems of the bacteria, leading to the death of certain bacteria.

H^+-ATPase proton pumps are involved in the regulation of bacterial cytoplasmic pH and the supply of energy in the form of ATP to the bacterium. These two elements are necessary for the growth of bacteria [72]. An increase in the environmental pH in the presence of an antibacterial substance can lead to the inhibition by this substance of the H+-ATPase-dependent proton pumps leading to the death of the bacterium [73]. *K. pneumoniae* has an optimal growth pH between 6-8 [74]. According to the results obtained, there was a considerable decrease in pH at the level in the negative control and the extract of the leaves of *E. mollis* at MIC/2; in the presence of the extract of the leaves of *E. mollis* at MIC and 2MIC, there was a slowing down of the acidification of the medium marked, indicating that at these concentrations, the botanical inhibits the functioning of the proton pumps of *K. pneumoniae* ATCC11296. The H+-ATPase proton pumps would be the target of the action of the botanical from the leaves of *E. mollis*. MDR Gram-negative bacteria including Enterobacteriaceae and *P. aeruginosa* actively over-express efflux pumps, and consequently are resistant to several antibiotics. The use of EPI in combination with the

botanicals tested in this study could be helpful for the antimicrobial fight against MDR bacteria. The effect of the association of extracts from the leaves, flowers, and roots of *E. mollis* and imipenem could also be useful to fight bacterial drug resistance. These results are similar to those of Kuete et al. [30] and Youmbi et al. [75] who showed that the combination of plant extracts with PAβN improved their activities.

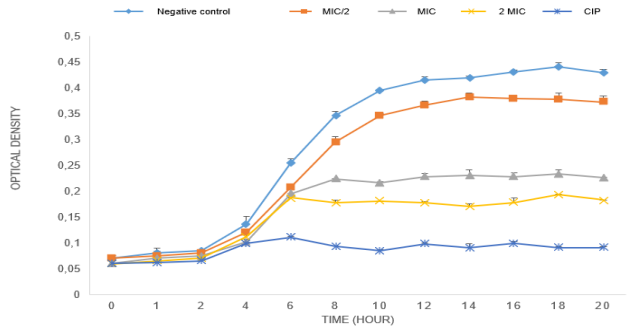

Figure 1. Effect of the methanol extract of *Elephantopus mollis* leaves on growth kinetics of *K. pneumoniae* ATCC11296.

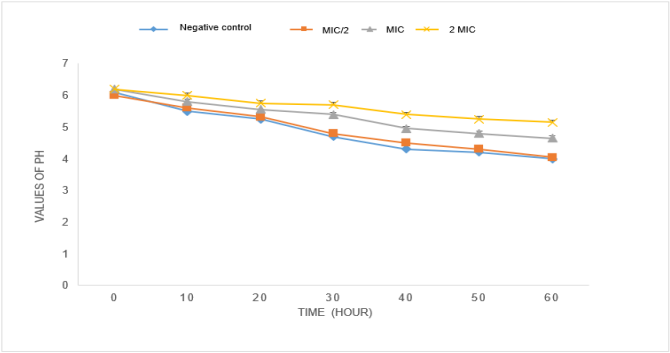

Figure 2. Effect of the methanol extract of *Elephantopus mollis* leaves on H^+-proton pumps/ATPases of *K. pneumoniae* ATCC11296.

Table 1. Features of bacterial strains and isolates used.

Bacterial strains/isolates	Features	References
Escherichia coli		
ATCC10536	Reference ATCC strains	[30, 31]
AG102	Wild-type strain of *E. coli* K-12 overexpressing AcrAB and Mar A pumps	[76, 77]
AG100	Wild-type E. coli K-12 expressing AcrAB efflux pumps	[78, 79]
Klebsiella pneumoniae		
ATCC11296	Reference ATCC strains	[30, 31]
KP55	Clinical MDR isolate, Tet^r, Amp^r, Atm^r, Cef^r	[80, 81]
K2	Clinical over-expressing MDR AcrA-TolC pumps	Laboratory collection of UNR-MD1, University of Marseille,France
Pseudomonas aeruginosa		
PA01	Reference ATCC strains	[30, 31]
PA124	Clinical over-expressing MDR MexAB-OprM pumps	[78, 82]
P121	Clinical over-expressing MDR MexAB-OprM pumps	Laboratory collection of URMSA, University of Dschang, Cameroon
Enterobacter aerogenes		
EA3	Clinical MDR isolate Chl^r, Nor^r,	[31, 83]
EA298	Clinical MDR isolate Mox^r, Cft^r, Atm^r, Fep^r	[5, 6]
EA27	Clinical MDR isolate, Kan^r, Amp^r, Nal^r, Str^r, Tet^r; expressing the energy-dependent efflux of norfloxacin and chloramphenicol	[6, 84]
Providencia stuartii		
ATCC29916	Reference ATCC strains	[30, 31]
PS2636	Clinical MDR isolate of *Providencia stuartii* expressing AcrAB-TolC pumps	[85]
NEA16	Clinical MDR isolate of *Providencia stuartii* expressing AcrAB-TolC pumps	[86, 87]

ATCC: American Type Culture Collection; MDR: multidrug-resistant; $Ofxa^r$, Kan^r, Tet^r, Erm^r, Amp^r, Nal^r, Str^r, Atm^r, Cef^r, Cip^r, Im/Cs^r, Chl^r, Gen^r, Nis^r, Flx^r, Dox^r, Cro^r, TOB^r resistance respectively to: Ofloxacin, kanamycin, tetracycline , erythromycin, ampicillin, nalidixic acid, streptomycin, aztreoname, cefepime, ciprofloxacin, imipenem/cilastatin sodium, chloramphenicol, gentamicin, nisin, flomoxef, doxycycline, ceftriaxone and Tobramycin AcrAB-TolC, AcrAB and Mar A: efflux pumps.

Table 2. MICs and MBCs (µg/mL) of extracts from different parts of *Elephantopus mollis.*

Bacteria	Botanicals and ATB											
	Roots			Leaves			Flowers			ATB (imipenem)		
	MIC	MBC	R	MIC	MBC	R	MIC	MBC	R	MIC	MBC	R
K. pneumoniae												
K2	512	-	nd	32	256	8	512	-	nd	<4	64	>16
KP55	2048	-	nd	64	2048	32	512	-	nd	<4	64	>16
ATCC11296	128	1024	4	16	64	4	64	256	4	<4	-	nd
P. stuartti												
ATCC29761	512	-	nd	64	512	8	128	1024	8	<4	16	>4
NEA16	2048	-	nd	32	512	16	256	1024	4	8	64	8
PS2636	1024	2048	2	64	512	8	256	1024	4	8	64	8
E. aerogenes												
EA3	1024	-	nd	128	-	nd	256	-	nd	8	64	8
EA27	1024	-	nd	64	512	8	256	1024	4	8	16	2
EA298	1024	-	nd	64	1024	4	512	-	nd	8	8	1
P. aeruginosa												
PAO1	>2048	-	nd	256	1024	4	1024	2048	2	16	64	4
PA121	2048	-	nd	256	512	2	1024	-	nd	16	128	8
PA124	>2048	-	nd	128	256	2	512	1024	2	4	32	8
E. coli												
AG100	512	-	nd	64	512	8	128	1024	8	8	64	8
AG102	1024	-	nd	128	1024	8	128	2048	8	16	64	4
ATCC10536	512	-	nd	128	1024	8	512	2048	4	8	64	8

R: MBC/MIC ratio; >2048: or inactive; nd: not determined; MIC: minimal inhibitory concentration; MBC: minimum bactericidal concentration, ATB: Antibiotic.

Table 3. Effects of the combination of *Elephantopus mollis* extracts with PAβN.

Bacteria	Botanicals and ATB											
	Roots			Leaves			Flowers			ATB (imipenem)		
	MIC alone	MIC with PAβN	R	MIC alone	MIC with PAβN	R	MIC alone	MIC with PAβN	R	MIC alone	MIC with PAβN	R
E. coli												
ATCC10536	512	512	1	128	16	8	512	16	32	8	2	4
AG100	512	512	1	64	32	2	64	16	4	8	<1/2	16
P. aeruginosa												
PA01	>2048	32	64	256	256	1	256	128	2	16	8	2
PA121	2048	32	64	256	16	16	256	16	16	16	<8	2
PA124	>2048	1024	2	128	<16	8	512	<16	32	4	<1	4
K. pneumoniae												
K2	512	64	8	32	16	2	512	128	2	<4	2	2
KP55	2048	1024	2	64	<16	4	512	256	2	< 4	1/4	16
E. aerogenes												
EA3	1024	32	32	128	64	2	256	128	2	8	8	1
E298	1024	1024	1	64	32	2	512	16	32	8	<1	8
P. stuartti												
NEA16	2048	2048	1	32	16	2	256	128	2	8	8	1
PS2636	1024	64	16	64	32	2	256	256	1	8	2	4

R: MIC alone *vs* MIC with PAβN ratio; MIC alone: Minimal inhibitory Concentration; MIC with PAβN: Minimal inhibitory Concentration in the presence of PAβN; ATB: Antibiotic

Table 4. Activity of antibiotics combined with the root extract of *Elephantopus mollis* against bacterial strains and isolates.

ATB	Extract concen-tration	MIC of antibiotics in the presence of extract and Antibiotic-resistance modulating factor (AMF)										PSP (%)
		E. coli		*E. aerogenes*		*K. pneumoniae*		*P. aeruginosa*		*P. stuartii*		
		AG100	ATC10536	EA298	EA3	KP55	K2	PA124	PA121	PS2636	NEA16	
TET	0	2	8	4	1/8	1	1/2	1	8	8	1/8	
	MIC/2	1/2	8	<1/16(64)	1/16(2)	<1/16(16)	<1/16(8)	<1/16(16)	8(1)	8(1)	<1/16(2)	60%
	MIC/4	2	8	<1/16(64)	1/8(1)	<1/16(16)	<1/16(8)	<1/16(16)	8(1)	8(1)	<1/16(2)	50%
CIP	0	1/4	1/4	1/4	1/4	1/2	1/4	1	1	1/4	1/2	
	MIC/2	<1/16(4)	<1/16(4)	<1/16(4)	1/16(4)	<1/16(8)	<1/16(4)	<1/16(16)	1/16(16)	1/8(2)	<1/16(8)	100%
	MIC/4	1/8(2)	1/8(2)	<1/16(4)	1/8(2)	<1/16(8)	<1/16(4)	<1/16(16)	1/2(2)	1/8(2)	<1/16(8)	100%
IMI	0	8	8	8	8	<4	<4	4	16	8	8	
	MIC/2	4(2)	8(1)	2(4)	<1/4(32)	<1/8(32)	1(4)	<1/2(8)	<1/2(32)	4(2)	1(8)	90%
	MIC/4	4(2)	8(1)	8(1)	2(4)	<1/8(32)	1(4)	<1/2(8)	<1/2(32)	4(2)	8(1)	70%
CEF	0	16	16	8	128	16	256	64	32	8	8	
	MIC/2	16(1)	8(2)	<2(4)	<2(64)	<2(8)	256(1)	2(32)	2(16)	4(2)	8(1)	70%
	MIC/4	32(0,5)	8(2)	4(2)	4(32)	<2(8)	256(1)	4(16)	<2(16)	8(1)	8(1)	60%
DOX	0	1	2	2	8	2	4	1/4	2	1	2	
	MIC/2	1/2(2)	1(2)	1(2)	8(1)	1/16(32)	1/16(64)	<1/16(4)	<1/16(32)	1(1)	1(2)	80%
	MIC/4	1/2(2)	1/2(4)	1(2)	8(1)	1/16(32)	1/16(64)	<1/16(4)	<1/16(32)	1(1)	1(2)	80%
LEV	0	1/4	1/4	1/4	1/2	1/2	1/2	1/2	4	1	1/2	
	MIC/2	1/4(1)	1/16(4)	<1/16(4)	1/16(8)	<1/16(8)	1/2(1)	1/16(8)	1(4)	<1/16(16)	<1/16(8)	80%
	MIC/4	1/4(1)	1/4(1)	<1/16(4)	1/16(8)	<1/16(8)	< 1/16(8)	1/16(8)	1(4)	<1/16(16)	<1/16(8)	80%
VAN	0	8	64	256	128	256	64	256	64	2	2	
	MIC/2	4(2)	1(64)	8(32)	64(2)	<2(128)	8(8)	128(2)	4(16)	< 1/2(4)	< 1/2(4)	100%
	MIC/4	4(2)	1(64)	8(32)	64(2)	<2(128)	8(8)	128(2)	4(16)	1(2)	< 1/2(4)	100%
AMP	0	256	256	256	256	256	256	256	256	256	256	
	MIC/2	256(1)	256(1)	256(1)	8(32)	<2(128)	256(1)	256(1)	<2(128)	256(1)	<2(128)	40%
	MIC/4	256(1)	256(1)	256(1)	8(32)	<2(128)	256(1)	256(1)	<2(128)	256(1)	<2(128)	40%

MIC: minimal inhibitory concentration; (): Antibiotic-resistance modulating factor (AMF); PSP (%): percentage of strain where potentiation effect was observed; ATB: Antibiotics; DOX: Doxycycline, LEV: Levofloxacin; VAN: Vancomycin; AMP: Ampicillin; TET: Tetracycline; CIP: Ciprofloxacin; IMI: Imipenem; CEF: Ceftriaxone.

Table 5. Activity of antibiotics combined with antibiotics and the leaves extract of *Elephantopus mollis* against bacterial strains and isolates.

ATB	Extract concen-tration	MIC of antibiotics in the presence extract and Antibiotic-resistance modulating factor (AMF)										PSP (%)
		E. coli		*E. aerogenes*		*K. pneumoniae*		*P. aeruginosa*		*P. stuartii*		
		AG100	ATCC10536	EA298	EA3	KP55	K2	PA124	PA121	PS2636	NEA16	
TET	0	2	8	4	1/8	1	1/2	1	8	8	1/8	
	MIC/2	2(1)	8(1)	1/8(32)	1/16(2)	<1/16(16)	<1/16(8)	<1/16(16)	8(1)	8(1)	<1/16(2)	60%
	MIC/4	2(1)	8(1)	1/2(8)	1/16(2)	<1/16(16)	<1/16(8)	<1/16(16)	8(1)	8(1)	1/8(1)	50%
CIP	0	1/4	1/4	1/4	1/4	1/2	1/4	1	1	1/4	1/2	
	MIC/2	1/8(2)	<1/16(4)	1/4(1)	1/16(4)	<1/16(8)	<1/16(4)	<1/16(16)	1/16(16)	1/8(2)	1/4(2)	90%
	MIC/4	1/4(1)	<1/16(4)	1/8(2)	1/16(4)	<1/16(8)	<1/16(4)	<1/16(16)	1/2(2)	1/8(2)	1/4(2)	90%
IMI	0	8	8	8	8	<4	<4	4	16	8	8	
	MIC/2	8(1)	2(4)	<1/2(16)	2(8)	2(2)	1(4)	<1/2(8)	4(4)	1(8)	8(1)	80%
	MIC/4	8(1)	2(4)	1(8)	2(4)	2(2)	1(4)	2(2)	4(4)	1(8)	8(1)	80%
CEF	0	16	16	8	128	16	256	64	32	8	8	
	MIC/2	8(2)	2(8)	4(2)	2(64)	<2(8)	256(1)	2(32)	<2(16)	4(2)	8(1)	80%
	MIC/4	8(2)	8(2)	8(1)	16(8)	<2(8)	256(1)	2(32)	8(4)	4(2)	16(0,5)	70%
DOX	0	1	2	2	8	2	4	1/4	2	1	2	
	MIC/2	1/2(2)	1/8(16)	1(2)	8(1)	1/16(32)	<1/16(64)	<1/16(4)	<1/16(32)	<1/16(16)	1/8(16)	90%
	MIC/4	1(1)	1/4(8)	1(2)	8(1)	1/16(32)	<1/16(64)	<1/16(4)	<1/16(32)	<1/16(16)	1/8(16)	80%
LEV	0	1/4	1/4	1/4	1/2	1/2	1/2	1/2	4	1	1/2	
	MIC/2	1/4(1)	1/16(4)	1/8(2)	1/16(8)	<1/16(8)	<1/16(8)	1/16(8)	1/16(64)	1/2(2)	1/4(2)	90%
	MIC/4	1/4(1)	1/8(2)	1/8(2)	1/4(2)	<1/16(8)	<1/16(8)	1/8(4)	1/16(64)	1/2(2)	1/2(1)	80%
VAN	0	8	64	256	128	256	64	256	64	2	2	
	MIC/2	4(2)	1/2(128)	8(32)	2(64)	<2(128)	8(8)	128(2)	4(16)	1(2)	< 1/2(4)	100%
	MIC/4	4(2)	1/2(128)	8(32)	32(4)	<2(128)	8(8)	256(1)	4(16)	1(2)	1(2)	90%
AMP	0	256	256	256	256	256	256	256	256	256	256	
	MIC/2	256(1)	256(1)	256(1)	2(128)	<2(128)	256(1)	256(1)	<2(128)	256(1)	<2(128)	40%
	MIC/4	256(1)	256(1)	256(1)	256(1)	<2(128)	256(1)	256(1)	<2(128)	256(1)	128(2)	30%

MIC: minimal inhibitory concentration; (): Antibiotic-resistance modulating factor (AMF); PSP (%): percentage of strain where potentiation effect was observed; ATB: Antibiotics; DOX: Doxycycline, LEV: Levofloxacin; VAN: Vancomycin; AMP: Ampicillin; TET: Tetracycline; CIP: Ciprofloxacin; IMI: Imipenem; CEF: Ceftriaxone.

Table 6. Activity of antibiotics combined with antibiotics and the flower's extract of *Elephantopus mollis* against bacterial strains and isolates.

ATB	Extract concen-tration	MIC of antibiotics in the presence extract and Antibiotic-resistance modulating factor (AMF)										PSP (%)
		E. coli		*E. aerogenes*		*K. pneumoniae*		*P. aeruginosa*		*P. stuartii*		
		AG100	ATCC10536	EA298	EA3	KP55	K2	PA124	PA121	PS2636	NEA16	
TET	0	2	8	4	1/8	1	1/2	1	8	8	1/8	
	MIC/2	1/2(4)	8(1)	<1/16(64)	1/8(1)	1/2(2)	<1/16(8)	<1/16(16)	8(1)	8(1)	<1/16(2)	60%
	MIC/4	1(2)	8(1)	<1/16(64)	1/16(2)	1/2(2)	<1/16(8)	<1/16(16)	8(1)	8(1)	1/16(2)	70%
CIP	0	1/4	1/4	1/4	1/4	1/2	1/4	1	1	1/4	1/2	
	MIC/2	<1/16(4)	<1/16(4)	<1/16(4)	1/8(2)	<1/16(8)	<1/16(4)	<1/16(16)	1/16(16)	<1/16(4)	1/4(2)	100%
	MIC/4	1/8(2)	<1/16(4)	<1/16(4)	1/4(1)	<1/16(8)	1/8(2)	<1/16(16)	1/16(16)	1/8(2)	1/4(2)	90%
IMI	0	8	8	8	8	<4	<4	4	16	8	8	
	MIC/2	4(2)	< 1(8)	1/2(16)	2(4)	<1/8(32)	< 1(4)	<1/2(8)	16(1)	2(4)	8(1)	80%
	MIC/4	8(1)	1(8)	4(2)	4(2)	2(2)	1/2(8)	2(2)	16(1)	2(4)	8(1)	70%
CEF	0	16	16	8	128	16	256	64	32	8	8	
	MIC/2	<2(8)	2(8)	<2(4)	8(16)	<2(8)	256(1)	8(8)	8(4)	2(4)	8(1)	80%
	MIC/4	16(1)	4(4)	<2(4)	8(16)	<2(8)	256(1)	4(16)	8(4)	4(2)	8(1)	70%
DOX	0	1	2	2	8	2	4	1/4	2	1	2	
	MIC/2	<1/16(16)	1/16(32)	1(2)	1/4(32)	<1/16(32)	<1/16(64)	<1/16(4)	1/16(32)	<1/16(16)	1/2(4)	100%
	MIC/4	1/8(8)	1/16(32)	1(2)	8(1)	<1/16(32)	<1/16(64)	<1/16(4)	1/16(32)	<1/16(16)	1/2(4)	90%
LEV	0	1/4	1/4	1/4	1/2	1/2	1/2	1/2	4	1	1/2	
	MIC/2	1/8(2)	1/16(4)	<1/16(4)	1/8(4)	<1/16(8)	1/16(8)	1/16(8)	1/2(8)	<1/16(16)	1/4(2)	100%
	MIC/4	1/4(1)	1/16(4)	<1/16(4)	1/2(1)	<1/16(8)	1/16(8)	1/16(8)	1/2(8)	1/8(8)	1/2(1)	70%
VAN	0	8	64	256	128	256	64	256	64	2	2	
	MIC/2	4(2)	4(16)	8(32)	128(1)	<2(128)	8(8)	64(4)	4(16)	< 1/2(4)	1/2(4)	90%
	MIC/4	4(2)	8(8)	8(32)	128(1)	<2(128)	8(8)	64(4)	4(16)	1(2)	1(2)	90%
AMP	0	256	256	256	256	256	256	256	256	256	256	
	MIC/2	256(1)	256(1)	256(1)	256(1)	<2(128)	256(1)	256(1)	<2(128)	256(1)	<2(128)	30%
	MIC/4	256(1	256(1)	256(1)	256(1)	<2(128)	256(1	256(1	<2(128)	256(1)	64(4)	30%

MIC: minimal inhibitory concentration; (): Antibiotic-resistance modulating factor (AMF); PSP (%): percentage of strain where potentiation effect was observed; ATB: Antibiotics; DOX: Doxycycline, LEV: Levofloxacin; VAN: Vancomycin; AMP: Ampicillin; TET: Tetracycline; CIP: Ciprofloxacin; IMI: Imipenem; CEF: Ceftriaxone.

Table 7. Phytochemical composition of extracts from different parts of *Elephantopus mollis.*

Phytochemical classes	Botanicals		
	Roots	Leaves	Flowers
Alkaloids	+	+	+
Polyphenols	+	+	+
Flavonoids	+	+	+
Tannins	+	+	+
Triterpenes	-	+	+
Saponins	-	+	-
Anthocyanins	-	+	-

(+): present; (-): absent

Conclusion

In the present study, the antibacterial potential, and modes of action of botanicals from *Elephantopus mollis* against MDR Gram-negative bacteria were evaluated. It was shown that botanicals from *E. mollis* leaf and flower are potent sources of antibacterial agents against MDR bacteria. The botanical from the leaf of *E. mollis* exerts its antibacterial activity at the exponential phase of bacterial growth, probably through the inhibition of the H+-ATPase proton pumps. The constituents from the methanol extracts are potential substrates for bacterial efflux pumps. Botanicals from this plant have potentiating effects with doxycycline, ciprofloxacin, levofloxacin, imipenem, ceftriaxone, and vancomycin. Finally, the methanol extracts of the leaf, flower, and root of *E. mollis* are potential sources of effective antibacterial molecules that could be used alone and in combination with antibiotics or efflux pump inhibitors to overcome MDR pathogenic bacteria.

Abbreviations

AMF, antibiotic-resistance modulating factor; DMSO, dimethylsulfoxide, HNC, Cameroon national herbarium; INT, para-Iodonitrotetrazolium chloride; MDR, multidrug-resistant; MBC, minimal bactericidal concentrations; MHA, Mueller Hinton Agar; MHB, Mueller Hinton Broth; MIC, minimal inhibitory concentrations.

Authors' Contribution

SMT, VYM, RN, GKF, JFM, and PN carried out the study; ATM and VK supervised the study; All authors read and approved the final version of the manuscript.

Acknowledgments

The authors are grateful to the Cameroon National Herbarium for identifying the plant.

Conflict of interest

The authors declare no conflict of interest.

Article history:

Received: 5 June 2023
Received in revised form: 26 July 2023
Accepted: 01 August 2023
Available online: 01 August 2023

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