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Multidrug-resistant profile of bacteria and synergistic effect between antibiotics and *Trichoscypha acuminata* and *Ongokea gore* extracts, medicinal plants.

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Abstract:-The aim of the present study was to establish the antimicrobial susceptibility profiles of bacteria from patients in treatment failure and to present the association of plant extracts and antibiotics in order to improve the effectiveness of antibiotics against multi-resistant bacteria. The identification of bacterial strains and the determination of their susceptibility to antibiotics were carried out using API 20E galleries. The plants extracts were tested alone and then in combination with the antibiotics by agar diffusion method. The predominant species isolated were *Escherichia coli* and *Staphylococcus aureus*. 25% of isolates were found resistant to antibiotics. The rate of resistance to antimicrobial agents was 89.95%. The extracts of *Trichoscypha acuminata* and *Ongokea gore* stem bark have intermediary growth inhibitory effects on the majority of tested pathogens, especially upon the MDR strains, with zone of inhibition diameters (ZIDs) ranging between 8 and 13 mm. The interactions between antimicrobial agents and plants extracts showed synergistic effects through significant improvement in ZID of the tested antibiotics against about 30% of bacteria. Infection with a single, multi-resistant bacterium can lead to treatment failure. The combinations show that extracts of *Trichoscypha acuminata* and *Ongokea gore* potentiate antibiotics against a few bacterial strains, comprising multi-resistant strains.

Keywords
Multidrug-resistant; drug interaction; synergy; antimicrobial; *Trichoscypha acuminata; Ongokea gore.*

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Introduction

Introduction actualisée

Antibiotics have long been the cornerstone for treating bacterial infections, effectively eliminating pathogenic bacteria with relatively few side effects and saving millions of lives in humans and animals over the past decades. However, the emergence and rapid spread of antibiotic-resistant strains soon after the introduction of new drugs into clinical use have drastically undermined their long-term effectiveness, contributing to the selection of multidrug-resistant bacteria and frequent treatment failures. Today, multidrug-resistant bacteria are a critical global public health threat, responsible for increasing mortality, prolonged illness, and substantial additional healthcare costs worldwide. Recent data from the World Health Organization (WHO) indicate that one in six bacterial infections globally was resistant to commonly used antibiotics in 2023, with resistance rising in more than 40 % of monitored pathogen-drug combinations between 2018 and 2023. Such resistance is particularly pronounced in Gram-negative organisms like *Escherichia coli* and *Klebsiella pneumoniae*, where resistance to third-generation cephalosporins and other essential antibiotics has severely limited treatment options, especially in low-resource regions (WHO, 2025[a]).

The WHO has developed and updated a Bacterial Priority Pathogens List (BPPL) to guide research and development (R&D) priorities for new antibacterials. The BPPL 2024 classifies drug-resistant bacteria into critical, high, and medium priority pathogens based on burden, resistance profiles, and need for novel therapies. Critically resistant pathogens include carbapenem-resistant *Acinetobacter baumannii* and third-generation cephalosporin- and carbapenem-resistant Enterobacterales, while high priority pathogens include methicillin-resistant *Staphylococcus aureus* (MRSA) and carbapenem-resistant *Pseudomonas aeruginosa* (WHO, 2024).

The scarcity of novel antibiotic classes entering clinical use remains a looming issue, with only a limited number of innovative agents progressing through clinical development and weak pipelines for addressing critical priority pathogens. Moreover, 90 products (~50 antibiotics and 40 non-traditional agents) were reported in clinical development as of early 2025, but many lack mechanisms that circumvent cross-resistance (WHO, 2025[b]).

In response to this escalating crisis, alternative strategies are urgently explored. One promising approach is the combination of existing antibiotics with non-antibiotic compounds to enhance efficacy, suppress resistance, and lower the Minimum Inhibitory Concentrations (MICs) required to inhibit pathogen growth. Studies demonstrate that plant extracts and phytochemicals can act synergistically with standard antibiotics to restore or boost their activity against resistant bacteria (Donkor et al., 2024).

Plant-derived antimicrobial compounds are particularly attractive due to their diversity, lower cost of production, and reduced side-effect profiles compared to many synthetic drugs. They may not exhibit strong antimicrobial activity alone, but when used in combination with conventional antibiotics, they can significantly enhance therapeutic outcomes (Angelini, 2024).

Gabon, with its exceptional and partially unexplored biodiversity, provides a rich reservoir of potential bioactive molecules. Given that individual plant species can produce hundreds of distinct secondary metabolites, investigating local flora for synergistic antimicrobial properties holds considerable promise.

Trichoscypha acuminata Engl (Anacardiaceae) is a shrub common in forest undergrowth, traditionally used in Gabonese medicine for its purported antimalarial, anti-dysenteric, and reproductive health benefits (Walker & Sillans, 1961).

Ongokea gore (Hua) Pierre (Olacaceae), a large rainforest tree found in central Africa, is traditionally used for treating abscesses, rheumatism, and as hemostatic and purgative remedies (Walker & Sillans, 1961).

The present study aims to isolate and characterize the antimicrobial susceptibility profiles of bacteria from patients with therapeutic failures and assess the potential of plant extracts in combination with antibiotics to improve or restore antibiotic efficacy against multidrug-resistant bacterial pathogens.

Materials and methods

Phytochemical study

Plant material and extract preparation

The plant materials used in this study consisted of *Trichoscypha acuminata* (stem bark) and *Ongokea gore* (stem bark), which were harvested in Oyem region, Gabon in August 2017. The plants were first identified locally by their local names, amvut and angèc, respectively, and then authenticated by a taxonomist at the Institute for Research in Tropical Ecology (IRET). A voucher specimen has been deposited in herbarium (*Ongokea gore*: Larebio.EMM005 and *Trichoscypha acuminata*: Larebio.EMM006) at Laboratory of Biochemistry Research (LAREBIO), Department of Chemistry, Faculty of Sciences, University of Sciences and Technologies of Masuku (USTM) in Franceville (Gabon). The plants were then air-dried in the shade for 10 days before the steam distillation at room temperature. The powered stem-bark of plants (100 g) was separately extracted with 500 mL (of each) of ethanol 50% (Ethanol – Aqueous 50/50; Et-Aq) and methanol 100% (MeOH) by maceration (Abubakar and Haque, 2020) for 48 h. The filtrate was collected after 48 hours. To make the dry extracts, the extracts were concentrated in vacuous at 40°C in a rotary evaporator.

Phytochemical screening

Each extract was subjected to preliminary phytochemical (qualitative) tests to detect the presence of flavonoids, coumarins, tannins, total phenolic, saponosides, triterpenoids, alkaloids, oses, holosides, cardiac glycosides, reducing sugars, and anthracenosids according to the standard technics (Sofowora 1993; Harborne 1998).

Quantification of phytoconstituents

The total polyphenol content of each extract was determined by Folin-Ciocalteu method (Singleton et al., 1999) with minor modifications (Ngoua-Meye-Misso et al., 2018). Gallic acid was used as the standard, and the absorbance was measured at 735 nm using a spectrophotometer (Evolution 60S, USA). The results were expressed as gallic acid equivalents.

The total flavonoid content was determined by aluminum trichloride ($AlCl_3$) method (Quettier-Deleu et al., 2000) with slight modifications (Ngoua-Meye-Misso et al., 2028). Quercetin was used as the standard, and the absorbance was measured at 415 nm using a spectrophotometer (Evolution 60S, USA). The results were expressed in quercetin equivalents.

The tannin content was determined according to the reference method of the European Community (European Communities, 1984). Absorbance was measured at 525 nm using a spectrophotometer (Evolution 60S, USA), and tannic acid was used as a standard. The results were expressed as tannic acid equivalents.

The proanthocyanidin content was determined according to the method described by Prigent (Prigent 2005) with minor modifications (Ngoua-Meye-Misso et al., 2017). Absorbances were read at 550 nm using a spectrophotometer (Evolution 60S, USA). Apple procyanidins were used as a standard, and the results were expressed in apple procyanidin equivalent (APE).

Antibacterial activity

Isolation, identification, and antibiotic sensitivity of bacterial strains

Bacteria were isolated at Omar Bongo Ondimba Army Instruction Hospital (OBO AIH) in Libreville from clinical specimens like urine, pus, urinary catheter, vaginal, nasal, and rectal swabs collected from adult patients, mainly those in therapeutic failure without distinction of sexes. For each patient in therapeutic defeat, nasal and rectal swabs were taken systematically. Nasal strains were searched for MDR strains, including: methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-resistant coagulase-negative *Staphylococcus* (MRSCN), enteric bacterium (*Klebsiella pneumonia*) resistant to 3rd generation cephalosporins (carrier of a cephalosporinase or broad-spectrum beta-lactamase), Ticarcillin-resistant *Pseudomonas aeruginosa* or

Imipenem-resistant *Acinetobacter baumanii*. Anal swabs were examined for the presence of an *Enterobacterium* (*Klebsiella pneumonia*) resistant to third generation cephalosporins and a Ticarcillin resistant *Pseudomonas aeruginosa*. Each sample was grown on a specific medium and incubated. All isolates were identified to species level using standard methods such as API 20E gallery or API 10S strips, various Slidex kits, and verified using the VITEK-2 system (BioMérieux, France) according to the manufacturer's instructions.

All isolates were tested for sensitivity using the reference BioMerieux ATB test strips (ATB UR EU (08), ATB G, ATB-Staph, ATB-Strep; (BioMérieux, France) and Vitek 2 compact automaton. The resistance to both Penicillin G and Oxacillin were indicated a methicillin-resistant-like profile (Krucke et al., 2009). All tests were done following the manufacturer's instructions.

Bacterial strains

The antibacterial screening of some antibiotics and plant extracts alone and their combinations were tested against a panel of multidrug-resistant bacteria, including eight strains of clinical isolates obtained from patients in therapeutic failure in Omar Bongo Ondimba Army Instruction Hospital (OBO AIH) in Libreville, Gabon. The micro-organisms used were:

Hospital bacterial strains: *Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia, Klebsiella pneumoniae* spp. *pneumonia, Klebsiella oxytocae, Streptococcus agalactiae, Enterobacter cloacae,* and *Acinetobacter baumannii*. Reference bacterial strains: *Escherichia coli* CIP 105182, *Staphylococcus aureus* ATCC 25293 BHI, *Pseudomonas aeruginosa* CRBIP19249, and *Enterococcus faecalis* CIP10397.

These strains were resistant to different antibiotics, such as Amikacin (AKN), Ampicillin (AMP), Amoxicillin (AMX), Cefalotin (CFT), Cefuroxim (CXM), Cefixim (CFM), Cefotaxime (TCX), Cefoxitin (CXT), Cefoxitin 32 (CXT 32), Ceftazidim (CAZ), Cefepime (CPM), Ciprofloxacin (CIP), Co-Trimoxazole (COT), Fosfomycin (FOS), Gentamicin (GEN), Imipenem (IMI), Meropenem (MERO), NALI, Nalidixic acid (NALF), Levofloxacin (LVX), Ofloxacin (OFL), Piperacillin (PIC), Piperacillin + Tazobactame (TZP), Ticarcillin (TIC), Tetracycline (TET), Tobramycin (TOB).

Preparation of extracts and antibiotics used for antibacterial activity

Antibiotic powders of Ampicillin (AMP), Amoxicillin (AMX), Doxycycline (DOX), methanolic and hydro-ethanolic (Ethanol – Aqueous 50/50; Et-Aq) extracts of plants were used. Each antibiotic was individually combined with the plant extracts according to te table below:

Antibiotics \ plant extracts	100 mg/mL of hydro-ethanolic extract (Et-Aq)	100 mg/mL of Methanolic extract (MeOH)

100 mg/mL Ampicillin (AMP)	100 mg/mL of AMP + Et-Aq	100 mg/mL of AMP + MeOH
100 mg/mL Amoxicillin (AMX)	100 mg/mL of AMX + Et-Aq	100 mg/mL of AMX + MeOH
100 mg/mL Doxycycline (DOX)	100 mg/mL of DOX + Et-Aq	100 mg/mL of DOX + MeOH

For antibacterial screening, stock antibiotic/extract solutions were prepared (100 mg/mL) for each and all combinations.

Antibacterial screening

The antibacterial screening of antibiotics and extracts alone and in combination was carried out using agar well diffusion method (Wikler 2006). The bacteria grown in nutrient broth at 37°C for 18 h were standardized by using normal saline to turbidity of 0.5 MacFarland standards (10^8 cfu/mL). The Petri dishes (90 mm in diameter) were prepared with 15 mL of a base layer of Müeller-Hinton gelose medium, and the test bacteria were inoculated on nutrient agar plates and spread uniformly using a sterile glass spreader. Six millimeters of sterile paper discs (Whatman N° 3) soaked with 20 µL of the antibiotics/extracts dilution or combinations (100 mg/mL) were placed on agar in 15 mm of the Petri dishes' periphery. The Petri dishes were incubated aerobically at 37°C for 18 to 24 h. The effect of antibiotics, extracts, and combinations was reflected by the appearance of a transparent circular zone around the disc, corresponding to the absence of growth. The inhibition zone diameter was measured in mm. All tests were performed in triplicate and antibacterial activity was expressed as the mean of the zone of inhibition diameters (ZIDs) produced. The percentage improvement in the activity of antibiotics was calculated on the basis of the combinations having a synergetic effect according to Veras et *al.,* (2012).

For antibacterial screening, stock antibiotic/extract solutions were prepared (100 mg/mL) for each combination.

Statistical analysis

All tests were carried out in triplicate to ensure reproducibility of the results. Data was expressed as the mean ± standard deviation (SD) of three independent experiments. Data was analyzed using Staplus Build 8.03/Corev7.811(x86_64) software. Values of $p \leq 0.05$ were considered to be statistically significant.

Results

Bacteria Isolated and antibiotic resistance

The species distribution of bacterial strains from clinical specimens of 57 inpatients in therapeutic failure at Omar Bongo Ondimba Army Instruction Hospital (OBO AIH) in Libreville is presented in table 1. The present survey shows that 56 bacterial strains were isolated. The predominant species isolated were *Escherichia coli* (38.60%), *Staphylococcus aureus* (12.28%), *Klebsiella pneumonia* (8.77%), *Streptococcus agalactiae*, *Enterobacter cloacae* (7.02% each), *Pseudomonas aeruginosa*, *Acinetobacter baumanii*, *Proteus mirabilis* (5.26% each) and *Proteus vulgaris* (3.51%). The percentages of other bacterial strains were less than 2% (Table 1).

The reference BioMerieux ATB test strips were used to assess the sensitivity of all isolates. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2008 and the Clinical Laboratory Standards Institute (CLSI) 2008 were used to develop these tests. For each bacterium, categories of antibiotic with epidemiological significance were created. Using documentation and breakpoints from the CLSI, the EUCAST, the United States Food and Drug Administration (FDA), lists of antibiotic categories proposed for antimicrobial susceptibility testing were constructed. Thus, multidrug resistance (MDR) was defined as acquired non-susceptibility to at least one antimicrobial agent in three or more antibiotic categories (Magiorakos et al., 2012).

Only 14 out of 56 isolates (25%) were found to be resistant to antibiotics (Table 1). All of them were multidrug resistant. The resistant bacterial species and their resistance to different antibiotics are summarized in table 2. Overall, the resistance rate to antimicrobial agents was at 89.95%. Individually, with a percentage of resistance of 71.43%, the resistance rate was lowest for Ceftazidime (CAZ), Cefepime (CPM), and Imipenem (IMI), respectively. Meropenem (MERO), Tobramycin (TOB), Ciprofloxacin (CIP), Co-Trimoxazole (COT) and Fosfomycin (FOS) exhibited 85.71% of resistance, while the highest antibiotic resistance rates were recorded for all the rest of the antibiotics (100% of resistance). The analysis in table 2 reveals that the multidrug-resistant clinical isolates are all highly resistant to at least 84.62% of the antibiotics tested.

Table 1: Prevalence of bacterial species isolated and frequencies of multidrug resistant bacteria in each strain.

Bacteria	Frequency	Proportions of multi-resistant bacteria / non-resistant bacteria				
		% of bacteria / Number of isolates	MDR	% MDR / Bacterial species	No MDR	% No MDR
Escherichia coli	22	38.60	3	13.63	19	86.36
Staphylococcus aureus	7	12.28	2	28.57	5	71.43
Pseudomonas aeruginosa	3	5.26	0	0	3	100
Klebsiella pneumoniae	5	8.77	4	80.0	1	20.0
Acinetobacter baumanii	3	5.26	2	66.66	1	33.33
Streptococcus agalactiae	4	7.02	1	25.0	3	75.0
Enterobacter cloacae	4	7.02	2	50.0	2	50.0
Proteus mirabilis	3	5.26	0	0	3	100
Proteus vulgaris	2	3.51	0	0	2	100
Pantoae spp	1	1.75	0	0	1	100
Ornithinolytica spp	1	1.75	0	0	1	100
Klebsiella oxytoca	1	1.75	1	100	0	0
Totale and quota (%)	56	100	14	25.0	42	75.0

MDR = Multidrug resistant bacteria; No MDR = No multidrug resistant bacteria

Antimicrobial activity

Tables 3 and 4 present the results of experiments conducted using the well-diffusion method concerning the antimicrobial activities (zones of inhibition) of antibiotics and plant extracts alone and their combination effect (mm) against eight multidrug-resistant clinical isolates and four reference strains. The antibacterial activity as a function of the zone of inhibition diameter (ZID) is interpreted as follows: ZID < 6 mm: resistant; 6 mm ≤ ZID ≤ 13 mm: intermediary; ZID ≥ 13 mm: sensitive (Billerbeck 2007).

Antibiotics activity

Our results show that the reference strains and clinical isolates (MDR) were commonly susceptible (ZID > 13 mm) to all antibiotics tested except for 5 MDR, which showed an intermediary antibacterial activity (6 < ZID < 13 mm) (Tables 3 and 4). Indeed, *Enterobacter cloacae* (AMP and AMX); *Klebsiella pneumonia*, *Klebsiella pneumoniae spp*., *Streptococcus agalactiae* (AMP, respectively) and *Staphylococcus aureus* (AMX), *Klebsiella oxytocae* and *Klebsiella pneumoniae* spp (AMX) were resistant to the antibiotics AMX and AMP.

Trichoscypha acuminata and *Ongokea gore* extracts

The yields of different extracts of *T. acuminata* and *O. gore* were respectively 2.21% (Et-Aq), 5.44% (MeOH); 1.44% (Et-Aq) and 2.99% (MeOH) (Table 6). The results obtained using disc-diffusion method demonstrate that ethanol 50% (Et-Aq) and methanol 100% (MeOH) extracts of *Trichoscypha acuminata* and *Ongokea gore* stem bark contain bioactive compounds against reference strains and clinical isolates (Tables 3 and 4). Overall, these extracts showed intermediate growth inhibitory effects on the majority of tested pathogens, especially upon the MDR strains with ZID ranging between 8 and 13 mm. Some extracts exhibited sensitive effects through substantial ZID (> 13 mm), particularly on the reference strains. Only *Klebsiella oxytocae* (Et-Aq, MeoH) and *Staphylococcus aureus* (MeOH) were resistant through *Trichoscypha acuminata* extracts and *Klebsiella oxytocae* on Et-Aq extract of *Ongokea gore*.

Table 2: Susceptibility profile of isolated bacteria

Multidrug-resistant strains	Antibiotics (abbreviations) tested																										Susceptibility / Antibiotics Total (%)	
	β-Lactamines															Aminoglycosides				Quinolones					Others			
	AMP	AMX	TIC	PIC	TZP	CFT	CXM	CFM	TCX	CXT	CXT32	CAZ	CPM	IMI	MERO	GEN	TOB	AKN	TET	NALF	NALI	OFL	LVX	CIP	COT	FOS	S / I	R
Acinetobacter baumanii	R	R	R	R	R	R	R	R	R	R	R	I	R	R	R	R	R	R	R	R	R	R	R	R	R	R	3.85	96.15
Enterobacter cloacae	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S	R	R	R	R	R	R	R	R	R	R	S	11.54	88.46
Escherichia coli	R	R	R	R	R	R	R	R	R	R	R	R	I	I	R	R	I	R	R	R	R	R	R	R	R	R	11.54	88.46
Klebsiella oxytoca	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	0	100
Klebsiella pneumoniae	R	R	R	R	R	R	I	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	3.85	96.15
Staphylococcus aureus	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	0	100
Streptococcus agalactiae	R	R	R	R	R	R	R	R	R	R	R	I	I	R	R	R	R	R	R	R	R	R	R	S	S	R	15.38	84.62
Total resistance by antibiotic (%)	100	100	100	100	100	100	85.71	100	100	100	100	71.43	71.43	71.43	85.71	100	85.71	100	100	100	100	100	100	85.71	85.71	85.71	10.05	89.95

R: antibiotic resistant bacterial strain; S: A bacterial strain sensitive to the antibiotic; I: The bacterial strain with intermediate activity (the antibiotic was tested at two different concentrations, the highest concentration inhibiting bacterial growth and the lowest concentration allowing the bacterium to grow)

Amikacin (AKN), Ampicillin (AMP), Amoxicillin (AMX), Cefalotin (CFT), Cefuroxim (CXM), Cefixim (CFM), Cefotaxime (TCX), Cefoxitin (CXT), Cefoxitin 32 (CXT 32), Ceftazidim (CAZ), Cefepime (CPM), Ciprofloxacin (CIP), Co-Trimoxazole (COT), Fosfomycin (FOS), Gentamicin (GEN), Imipenem (IMI), Meropenem (MERO), NALI, Nalidixic acid (NALF), Levofloxacin (LVX), Ofloxacin (OFL), Piperacillin (PIC), Piperacillin + Tazobactame (TZP), Ticarcillin (TIC), Tetracycline (TET), Tobramycin (TOB).

Antibiotic combinations

Although the majority of bacteria are resistant to many antibiotics, only AMP, AMX and DOX were used in the combination trials with the plant extracts. The interactions between the antimicrobial agents and the plant extracts showed synergistic effects through a substantial improvement in the ZID of the tested antibiotics (9.09% to 2167% with *Trichoscypha acuminata* and 8.7% to 29026.88% with *Ongokea gore*) against 31.94% of the strains for *Ongokea gore* extracts and 29.17% of the strains for *Trichoscypha acuminata* extracts (Tables 3 and 4). In agreement with the results obtained, the combinations show that several bacterial strains have an antagonistic effect of 45.83% and 58.33% on the combinations with *Ongokea gore* and *Trichoscypha acuminata* extracts, respectively. These combinations decreased the antibacterial activity of the antibiotics and/or extracts, with a significant reduction in the ZID of the tested antibiotics and/or extracts against the tested bacteria strains. Nevertheless, only combinations of the antibiotics with *Trichoscypha acuminata* extracts on *E. coli* CIP 105182; *S. aureus* ATCC 25293 BHI; *P. aeruginosa* CRBIP 19249; *Acinetobacter baumanii*; *Enterobacter cloacae*; *Klebsiella oxytocae*; *Klebsiella pneumoniae*; *Klebsiella pneumoniae spp*; *Streptococcus agalactiae* gave statistically significant results with p-values <0.05; <0.001; and <0.0001. The combinations with *Ongokea gore* extracts have a statistically significant result on all bacteria tested with p-values <0.05; <0.001; and <0.0001 (Table 4). Combinations of antibiotics with *Ongokea gore* extracts had more indifferent effects (22.23%) than *Trichoscypha acuminata* (12.50%). These combinations do not alter the individual activities of the antibiotics or plant extracts.

Phytochemical screening and phenolic compounds

The phytochemical screening yielded the various types of natural substances from *Trichoscypha acuminata* and *Ongokea gore* (Table 5). The phytochemical screening attests to the presence of several secondary metabolites in the extracts, mainly phenolic content (catechin, flavonols, coumarins, and proanthocyans), oses, holosides, sterols, triterpenes, and digitoxigenin.

Since phenolic components contribute significantly to the overall antibacterial activity (Mandal et al., 2017), it was reasonable to determine their total amount in the tested extracts. The result of total phenols, total flavonoids, total tannins, and total proanthocyanidin content is shown in table 6. The contents of total phenolic ranged from 1184.78 to 3111.22 mg GAE/g of extract and were highest in the methanol extract of *Trichoscypha acuminata*. Total flavonoids ranged from 88.88 to 405.72 mg EQ/g of extract and were abundant in the methanol extract of *Ongokea gore*. Total tannins ranged from 8.37 to 163.93 mg EAT/g of extract and were abundant in methanol extract of *Trichoscypha acuminata*. Among extracts, proanthocyanidin content ranged between 12.11 and 654.89 mg APE/g of extract. The content of proanthocyanidins is very high in the methanol extract of *Trichoscypha acuminata*. Overall, it appears that extracts of *Trichoscypha acuminata* have the highest content of phenolic compounds.

Table 3: Modification of the antibiotic activity of antibiotics by *Trichoscypha acuminata* extracts against eight multidrug-resistant clinical isolates and four reference strains.

Bacteria	Standard antibiotic			Extracts		Combinations					
	AMP	AMX	DOX	Et-Aq	MeOH	AMP+MeOH	AMP+Et-Aq	AMX+MeOH	AMX+Et-Aq	DOX+MeOH	DOX+Et-Aq
Reference strains											
E. coli CIP 105182	16.33 ± 0.58	14.67 ± 0.58	31 ± 0.0	15 ± 0.0	12.33 ± 0.58	15.67 ± 0.57, I	20.67 ± 0.58, S*	24 ± 1.0, S**	21.67 ± 0.58, S**	26 ± 0.0, A*	17 ± 0.0, A**
Increase (%)			-			-	26.58	63.60	47.72	-	-
S. aureus ATCC25293 BHI	17 ± 0.0	15 ± 0.0	41 ± 1.0	10 ± 0.0	15 ± 0.0	29 ± 0.0, S*	33 ± 0.0, S**	38.68 ± 0.58, S**	20 ± 1.0, S**	45 ± 0.0, S***	22.33 ± 0.58, A**
Increase (%)			-			70.59	94.12	157.87	33.33	9.76	-
P. aeruginosa CRBIP19249	30.33 ± 0.58	28.67 ± 0.58	35 ± 0.0	25.33 ± 0.58	23.33 ± 0.58	19 ± 0.58, A**	19 ± 1.0, A**	23 ± 0.58, A**	17 ± 2.0, A***	26 ± 0.0, A*	25.67 ± 0.58, A*
Increase (%)			-			-	-	-	-	-	-
E. faecalis CIP10397	24 ± 1.0	21.67 ± 0.58	40 ± 0.0	20.33 ± 0.58	21.33 ± 0.58	21.67 ± 0.58, A	32 ± 2.0, S	30 ± 0.0, S	27 ± 0.0, S	36 ± 0.0, A	30 ± 0.0, A
Increase (%)			-			-	33.33	38.44	24.60	-	-
Multidrug-resistant clinical isolates											
Acinetobacter baumanii	35 ± 0.0	18 ± 1.0	32.33 ± 2,08	12.33 ± 0.58	11.67 ± 0.58	11.67 ± 0.58, A***	12 ± 0.0, A	19.33 ± 0.58, I***	17 ± 2.0, I	32.33 ± 0.58, I	21.67 ± 0.58, A*
Increase (%)			-			-	-	-	-	-	-
Escherichia coli	24 ± 1.0	40 ± 0.0	36 ± 0.0	14.33 ± 0.58	12.67 ± 0.0	30 ± 0.0, S	29 ± 0.0, S	35.33 ± 0.58, A	35 ± 0.0, A	31.33 ± 0.58, A	31.67 ± 0.58, A
Increase (%)			-			25	20.83	-	-	-	-
Enterobacter cloacae	12 ± 0.0	9 ± 0.0	30 ± 1.0	10.33 ± 0.58	10.67 ± 0.58	R, A	R, A	20.33 ± 0.58, S***	20 ± 1.0, S***	28.67 0.58, A	17.33 ± 0.58, A**
Increase (%)			-			-	-	125.89	122.22	-	-
Klebsiella oxytocae	15 ± 0.0	R	23 ± 1.0	R	R	R, A	R, A	21.67 ± 1.53, S***	20.67 ± 0.58, S***	24.67 ± 0.58, I	12 ± 0.0, A***
Increase (%)			-			-	-	> 2167	> 2067	-	-
Klebsiella pneumoniae	10 ± 0.0	21 ± 0.0	26 ± 1.0	12 ± 0.0	13.33 ± 0.58	21.33 ± 0.58, S	15.67 ± 0.58, I*	21.67 ± 0.58, I	21.67 ± 0.58, I	30 ± 0.0, S*	23 ± 0.0, A
Increase (%)						113.30	-	-	-	15.38	-
Klebsiella pneumoniae spp	9.33 ± 0.58	R	25 ± 0.0	11.7 ± 0.5	13 ± 1.0	23 ± 1.0, S***	8 ± 0.0, A	R, A	R, A	20 ± 0.0, A*	12 ± 0.0, A***
Increase (%)			-			146.52	-	-	-	-	-
Streptococcus agalactiae	7.33 ± 0.58	25 ± 0.0	40 ± 0.0	11 ± 0.0	12.67 ± 0.58	10.67 ± 0.58, A	11 ± 0.0, I	20.67 ± 0.58, A	21 ± 0.0, A	35.33 ± 0.58, A*	21.33 ± 0.58, A***
Increase (%)			-			-	-	-	-	-	-
Staphylococcus aureus	20 ± 0.0	9 ± 0.0	22 ± 1.7	10.33 ± 0.58	R	R, A	R, A	R, A	R, A	20 ± 0.0, A	24 ± 0.0, S
Increase (%)			-			-	-	-	-	-	9.09

I: Indifference; S: Synergy; A: Antagonism; R: resistant strain (no inhibition; no inhibition diameter observed); *= $p < 0.05$; **= $p < 0.001$; ***= $p < 0.0001$

Table 4: Modification of the antibiotic activity of antibiotics by *Ongokea gore* extracts against eight multidrug-resistant clinical isolates and four reference strains.

Bacteria	Standard antibiotic			Extracts		Combinations					
	AMP	AMX	DOX	Et-Aq	MeOH	AMP+MeOH	AMP+Et-Aq	AMX+MeOH	AMX+Et-Aq	DOX+MeOH	DOX+Et-Aq
Reference strains											
E. coli CIP 105182	16.33 ± 0.58	14.67 ± 0.58	31 ± 0.0	16.33 ± 0.58	17.33 ± 0.58	19 ± 0.0, S	39.33 ± 0.5, S***	15 ± 0.58, A	24.33 ± 0.58, S***	29.67 ± 0.58, A	26.67 ± 0.58, A*
Increase (%)			-			16.35	140.85	-	65.85	-	-
S. aureus ATCC25293 BHI	17 ± 0.0	15 ± 0.0	41 ± 1.0	9.33 ± 0.58	10 ± 0.0	25 ± 0.0, S**	21 ± 1.0, S***	17 ± 0.0, S	21 ± 1.0, S***	40 ± 0.0, I	39 ± 0.58, A
Increase (%)			-			47.06	23.53	13.33	40	-	-
P. aeruginosa CRBIP19249	30.33 ± 0.58	28.67 ± 0.58	35 ± 0.0	16.67 ± 0.58	20.33 ± 0.58	35 ± 0.58, S*	24 ± 1.0, A*	30 ± 0.58, I	20 ± 0.0, A*	30 ± 0.0, A	26.67 ± 0.58, A
Increase (%)			-			15.40	-	-	-	-	-
E. faecalis CIP10397	24 ± 1.0	21.67 ± 0.58	40 ± 0.0	12.67 ± 1.0	16.33 ± 0.58	27 ± 0.58, S	39 ± 0.0, S***	22 ± 0.58, I	19.33 ± 0.58, A	45 ± 0.0, S*	41 ± 0.0, I
Increase (%)			-			12.50	62.50	-	-	12.50	-
Multidrug-resistant clinical isolates											
Acinetobacter baumanii	35 ± 0.0	18 ± 1.0	32.33 ± 2,08	14.33 ± 0.58	12.67 ± 1.15	19.33 ± 0.58, A***	16 ± 1.0, A***	19.33 ± 0.58, I	21 ± 1.0, S	32.67 ± 0.58, I	29.67 ± 0.58, A
Increase (%)			-			-	-	-	16.67	-	-
Escherichia coli	24 ± 1.0	40 ± 0.0	36 ± 0.0	17 ± 0.0	15 ± 1.0	24 ± 1.0, I	20.33 ± 0.58, A*	38.67 ± 0.58, A	39.33 ± 0.58, I	40 ± 0.0, S*	35 ± 0.0, I
Increase (%)			-			-	-	-	-	11.11	-
Enterobacter cloacae	12 ± 0.0	9 ± 0.0	30 ± 1.0	10.67 ± 0.58	11.67 ± 0.68	R, A***	9 ± 1.0, A	R, A***	R, A***	28 ± 0.0, A	20.33 ± 0.58, A***
Increase (%)			-			-	-	-	-	-	-
Klebsiella oxytocae	15 ± 0.0	R	23 ± 1.0	R	11.33 ± 0.58	R, A***	8 ± 0.0, A*	R, A	R, I	25 ± 0.0, S	23 ± 0.0, I
Increase (%)			-			-	-	-	-	8.70	-
Klebsiella pneumoniae	10 ± 0.0	21 ± 0.0	26 ± 1.0	13 ± 0.0	14.33 ± 1.53	21 ± 1.0, S***	15 ± 1.0, S*	21 ± 1.0, I	20 ± 0.0, A	35.33 ± 0.58, S*	35 ± 0.0, S*
Increase (%)			-			110	50	-	-	35.89	34.62
Klebsiella pneumoniae spp	9.33 ± 0.58	R	25 ± 0.0	10.33 ± 0.58	18.33 ± 1.53	21 ± 1.0, S***	21 ± 1.0, S***	R, A	R, A	21.67 ± 1.15, A	22.67 ± 0.58, A
Increase (%)			-			125.08	125.08	-	-	-	-
Streptococcus agalactiae	7.33 ± 0.58	25 ± 0.0	40 ± 0.0	12.67 ± 0.57	14.67 ± 1.15	9 ± 0.0, A	35 ± 1.0, S**	24 ± 1.0, I	24.33 ± 0.58, I	40 ± 0.0, I	40 ± 0.0, I
Increase (%)			-			-	29026.88	-	-	-	-
Staphylococcus aureus	20 ± 0.0	9 ± 0.0	22 ± 1.7	11 ± 1.0	11 ± 0.0	R, A***	8.33 ± 0.58, A**	R, A**	R, A**	24 ± 0.0, S	25 ± 0.0, S
Increase (%)			-			-	-	-	-	9.09	13.64

I: Indifference; S: Synergy; A: Antagonism; R: resistant strain (no inhibition; no inhibition diameter observed); *= p <0.05; **= p <0.001; ***= p <0.0001

Table 5: Results of phytochemical screening of extracts from *Trichoscypha acuminata* and *Ongokea gore*.

Chemical constituents		*Trichoscypha acuminata* extracts		*Ongokea gore* extracts	
		Ethanol-water (Et-Aq)	Methanol (MeOH)	Ethanol-water (Et-Aq)	Methanol (MeOH)
Saponins		-	-	--	-
Tannins	Gallic	-	-	-	-
	Catechin	+++	+++	+++	+++
Alkaloïds		++	++	++	++
Polyphenols		+++	+++	+++	+++
Sterols and triterpenes		+++	+++	+++	+++
Oses and Holosides		+++	+++	+++	+++
Total Flavonoids		+++	+++	-	++
Cyanidins	Flavonols	+++	+++	-	-
	Flavones	-	-	+	+
	Flavanones	-	-	-	-
Anthocyanes		-	-	-	-
Anthracenics		+++	+++	+	+
Coumarines		+++	+++	+++	+++
Proanthocyanes		+++	+++	+	+
Cardiac glycosides	Digitoxine	-	-	-	-
	Digitoxigenine	+++	+++	+++	+++
	Gitoxine	-	-	-	-
	Gitoxigénine	-	-	-	-
Reducing sugars		+++	+++	++	+

Legend: -: Not detected, +: Rare, ++: Abundant, +++: Very abundant.

Table 6: Result of the determination of phenolic compounds of extracts from *Trichoscypha acuminata* and *Ongokea gore*.

Plants / Extracts		Yields (%)	Total phenolic content (TPC) (mg GAE/gof crude extract)	Total flavonoid content (TFC) (mg QE/g of crude extract)	Total tannins content (TTC) (mg TAE/g of crude extract)	Total Proanthocyanidins content (TPC) (mg APE/g of crude extract)
Trichoscypha acuminata	Ethanolic-water extract	2.21	2109.22 ± 0.27	118.74 ± 0.04	8.37 ± 0.03	176.0 ± 0.02
	Methanolic extract	5.44	3111.22 ± 0.63	88.88 ± 0.05	163.93 ± 0.03	654.89 ± 0.07
Ongokea gore	Ethanolic-water extract	1.44	1184.78 ± 0.02	272.91 ± 0.15	Nd	28.22 ± 0.01
	Methanolic extract	2.99	2516.44 ± 0.09	405.72 ± 0.22	141.70 ± 0.02	12.11 ± 0.01

GAE: Gallic acid equivalent; EQ: Quercetin equivalent; TAE: Tannic Acid Equivalent; APE: Apigenin acid equivalent; Nd: Not determined

Discussion

Bacteria isolated and antibiotic resistance

Antimicrobial resistance (AMR) is widely acknowledged as a global problem, yet in many parts of the world, its magnitude is still not well understood. Thus, knowledge of local and regional AMR is important for clinical decision making. However, AMR surveillance capacity is lacking throughout Africa, particularly in

Gabon, and current data on AMR are scarce. This study, using inpatients in a therapeutic failure focused approach, aimed to understand and describe the current status of AMR in these patients.

Thus, high levels of AMR to commonly-used antibiotics were registered in this study, including 71.43% to 100% multidrug-resistance (MDR) to β-Lactamines, emerging MDR to Quinolones and Aminoglycosides (100%, generally) except Ciprofloxacin and Tobramycin (85.71%, each one). In addition, relatively high levels of MDR to Co-Trimoxazole and Fosfomycin (85.71%, each one) among gram-negative and gram-positive infections were recorded. The highest levels of resistance were reported to be in *Klebsiella pneumoniae* (80%), *Acinetobacter baumanii* (66.66%) and *Enterobacter cloacae* (50%) despite the fact that the isolation frequency was higher in *Escherichia coli* (38% of isolated bacteria) and *Staphylococcus aureus* (12% of isolated bacteria). Relatively high levels of MDR were demonstrated for *Staphylococcus aureus* (28%), *Streptococcus agalactiae* (25%), and *Escherichia coli* (13%). Overall, out of the 26 antibiotics tested the multidrug-resistance ranges from 84 to 96%. With the except for *Klebsiella oxytocae* and *Staphylococcus aureus,* which showed 100% resistance to 26 antibiotics, including 2nd and 3rd generation cephalosporins. Among the bacteria isolated from patients in therapeutic failure at Army Instruction Hospital in Libreville, extensive multidrug resistance has been reported to AMP, AMX, TIC, PIC, CFT, CFM, TCX, CXT, CXT32, GEN, AKN, TET, NALF, NALI, OFL, LVX (100%), CXM, MERO, TOB, CIP, COT, FOS (85.71%), CAZ, CPM, and IMI (71.43%). The findings of this study reveal exceptionally high levels of antibiotic resistance, confirming that antimicrobial resistance (AMR) remains a critical public health concern, particularly in resource-limited hospital settings. The World Health Organization (WHO) emphasizes that resistance to essential antibiotics has increased globally, notably affecting pathogens such as *Escherichia coli*, *Klebsiella pneumoniae*, and *Acinetobacter* spp., with resistance rates to third-generation cephalosporins exceeding 55% in certain African regions (WHO, 2025[a]).

Resistance to β-lactams and ESBL/Carbapenemase Production

The high resistance observed to nearly all β-lactam antibiotics is consistent with recent African data indicating that extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae are widely disseminated and frequently associated with multidrug-resistant (MDR) phenotypes. For instance, a systematic review conducted in Central Africa reported high prevalence rates of resistance to several antibiotic classes, including cotrimoxazole and amoxicillin/clavulanic acid, among clinical Enterobacteriaceae isolates (Garé et al., 2025). Furthermore, studies involving African pediatric populations document substantial resistance to third-generation cephalosporins and confirm the emergence and spread of such resistance mechanisms in *K. pneumoniae* and *E. coli*.

Multidrug Resistance to Quinolones and Aminoglycosides

The emergence of high resistance rates to quinolones and aminoglycosides observed in this study aligns with trends described in several regional reports. A review from East Africa, for example, demonstrated significant resistance to quinolones, although certain studies still report relative susceptibility of *E. coli* to ciprofloxacin in specific hospital populations, highlighting marked geographical variability (Ampaire et al., 2016). A meta-analysis published in Antimicrobial Resistance & Infection Control further indicates that resistance to carbapenems and fluoroquinolones is becoming increasingly prevalent in some African countries, partly driven by the dissemination of plasmid-mediated resistance genes such as *bla_OXA-48* and *bla_NDM-1* (Venne et al., 2023).

Resistance to Cotrimoxazole and Fosfomycin

The elevated levels of resistance to cotrimoxazole observed in this study are consistent with continental data demonstrating that this antibiotic class is among the least effective against clinical Enterobacteriaceae in many sub-Saharan African settings (Garé et al., 2025). In contrast, some analyses suggest that certain last-resort antibiotics, such as fosfomycin, may retain activity in specific contexts. However, African data remain limited and indicate considerable regional variability in susceptibility patterns (Venne et al., 2023).

Distribution of Resistant Species

Although *E. coli* and *Staphylococcus aureus* are frequently the most commonly isolated species, *Klebsiella* spp., *Acinetobacter* spp., and *Enterobacter* spp. are consistently associated with more severe resistance profiles in African hospital-based surveillance studies. Systematic reviews corroborate this trend, reporting high proportions of MDR strains among these pathogens across various African countries, particularly within intensive care units and surgical wards (Venne et al., 2023).

Overall, we found that all resistant bacteria isolated from patients in therapeutic failure were multidrug-resistance to the 26 antibiotics tested, which are commonly-used first-line empirical antibiotics in this region in general and in Gabon in particular (Ampaire et al., 2016; Tadesse et al., 2017). It is noted that, generally, the isolation frequency of resistant strains is three times lower than that of non-resistant strains (25% compared to 75%). Thus, the antibiotherapy failure mediated by a resistant subpopulation among gram-negative and gram-positive infections should alert us to the urgency of finding a solution to this problem of the emergence and spread of multidrug-resistance (Cantón and Morosini, 2011; Tadesse et al., 2017).

Antimicrobial activity

Higher plants produce hundreds to thousands of diverse chemical compounds with different biological activities. The antimicrobial compounds produced by plants are active against microorganisms that are pathogenic to plants and humans.Plant extracts with target sites other than those used by antibiotics are expected to be active against drug-resistant microbial pathogens (Bhatia et al., 2021). From this study, we can see that all antibiotics showed antibacterial activity against the four reference bacterial strains tested, but at different levels. All the multidrug-resistant bacteria tested were more or less sensitive to the three antibiotics at the concentration tested by the diffusion method, except for *Enterobacter cloacae* (AMP, AMX), *Klebsiella pneumoniae*, *Klebsiella pneumoniae* spp, *Streptococcus agalactiae* (AMP) and *Staphylococcus aureus* (AMX). *Klebsiella oxytocae* and *Klebsiella pneumoniae* ssp are resistant to amoxicillin. Overall, according to De Billerbeck's interpretation, the results obtained in this study show that the water-ethanol and methanol extracts of both plants have a low inhibitory effect (mostly intermediate activities) on the growth of the majority of bacteria tested. However, the reference bacteria have better sensitivity to plant extracts. The extracts of *Trichoscypha acuminata* show the highest antibacterial activity on reference strains, in particular on *Enterococcus faecalis* CIP 10397 and *Pseudomonas aeruginosa* CRBIP 19249 inhibition halos of 20 to 25 mm. The extracts of the two plants, among themselves, showed comparable antibacterial activity, while the bacteria tested showed a different sensitivity. Our results are in agreement with the research work of Sima Obiang et *al*., and according to De Billerbeck, plant extracts are moderately effective against reference bacteria tested (ZID < 13 mm) in general (Sima Obiang et al., 2016; Sima-Obiang et al., 2018; Sima-Obiang et al., 2019). Sima Obiang et *al*., also showed that *Staphylococcus aureus* ATCC 25293 BHI and *Enterococcus faecalis* CIP 103907 are significantly sensitive to extracts of *Coula edulis* and *Solanum torvum* with an inhibition zone diameter of 13 to 18 mm (Sima Obiang et al., 2016; Sima-Obiang et al., 2019).

Microbiological resistance is growing and there is an urgent need to look for new uses of antimicrobial drugs is urgently required. One approach could be the development of new substances or the combined use of antibiotics with plant extracts or other substances (Freitas et al., 2013). Indeed, in rational drug therapy, the simultaneous administration of two or more agents is often essential and sometimes mandatory in order to achieve the desired therapeutic goal or to treat coexisting diseases. However, drug synergism between known antibiotics and bioactive plant extracts is a new concept and could be beneficial (synergistic or additive interaction) or deleterious (antagonistic or toxic) (Ibezim et al., 2006). It is known that the particularity of herbal medicine lies in the use of combinations of plants and in the interactions (synergistic, additive, or antagonistic) between the components (Heinrich et al., 2012). Synergy is the effect of a combination of substances that is greater than would be expected by adding their separate contributions (Williamson 2001). Thus, the synergistic effect resulting from the combination of antimicrobial agents with crude extracts of the plants studied washas been verified in our work on certain combinations against certain bacterial strains. The application of *Trichoscypha acuminata* and *Ongokea gore* extracts with AMP, AMX, and DOX increased the antimicrobial activity of all tested antibiotics. However, an antagonistic effect was observed in the majority of bacteria when the antibiotics were associated with extracts of *Trichoscypha acuminata* and *Ongokea gore* plants. Our results show that the combination of the *Trichoscypha acuminata* and *Ongokea gore* plants with commercial antimicrobials has mainly an antagonistic effect. The antibiotic activity of Ampicillin against *Staphylococcus aureus* ATCC 25293 BHI (MeOH extract), *Escherichia coli* CIP 105182 and *Enterococcus faecalis* CIP 10397 (Et-Aq extract) was enhanced in the presence of *Trichoscypha acuminata* extracts. The result was demonstrated by an increase of 70.59, 26.58, 94.12, and 33.33% in the antibiotic activity of Ampicillin by MeOH extract and ethanol-aqueous extract, respectively. The antibiotic activity of Amoxicillin was improved on the same reference strains in the presence of extracts of *Trichoscypha acuminata*. The increase in antibiotic activity ranges from 24.60 to 157.87%. Only the combination of Doxycycline and the methanolic extract gave a synergistic effect (9.76% increases) on reference strains, in particular *Staphylococcus aureus* ATCC 25293 BHI. Extracts of *Ongokea gore* improve the activity of Ampicillin from 12.50 to 140.85% on all reference strains except *Pseudomonas aeruginosa* CRBIP 19249. Amoxicillin only improved *Escherichia coli* CIP 105182 and *Staphylococcus aureus* ATCC 25293 BHI strains (13.33 to 65.85%), whereas Doxycycline improved only the *Enterococcus faecalis* CIP 10397 strain (12.50%). Regarding the antibiotic activity of Ampicillin, Amoxicillin, and Doxycycline in combination with methanolic and ethanol-aqueous extracts of *Trichoscypha acuminata* and *Ongokea gore* plants on multidrug-resistant bacteria; generally, we have more than an antagonistic effect. The synergistic effect is greater on MDRs than on reference strains. Indeed, the increase in antimicrobial activity of antibiotics tested is generally higher on MDR (9.09-29026.88%) when combined with extracts of *Trichoscypha acuminata* and *Ongokea gore*. An exceptional result was obtained on the MDR strain *Klebsiella oxytocae* concerning the antibiotic Amoxicillin associated with extracts of *Trichoscypha acuminata*. Indeed, individually, Amoxicillin and extracts of the plant have no activity on the bacterium. But the combination of the antibiotic with these extracts gives a strong antibiotic activity with a halo inhibition of 20.67 to 21.67 mm (i.e. an increase in activity of more than 2067%). This result confirms that plant antimicrobials prove to be synergistic activators in that they may not have antimicrobial properties alone, but when taken together with standard drugs, they enhance the effect of that drug (Kamatou et al., 2006). Some researchers have observed the ability of crude extracts of plants to potentiate the activity of antibiotics. Furthermore, it has been proven that, in addition to the production of intrinsic antimicrobial compounds, plants also produce multidrug-resistance (MDR) inhibitors that enhance the activity of the antimicrobial compounds (Stermitz et al., 2000). In the treatment of drug-resistant infections, combinations of antibiotics have often been used, as this takes advantage of different mechanisms of action. The use of antimicrobial medicines with synergistic effects is one of the well-established indications for combination antimicrobial therapy (Rybak and McGrath, 1996).

Phenolic Compounds in Antimicrobial Therapy

The observed enhancement of antibiotic activity could be explained by the presence of biologically active compounds in these extracts. Since phenolic compounds contribute significantly to the overall antibacterial activity (Mandal et al., 2017), it was reasonable to determine their total amount in the extracts tested. It is found that, of the two plants, *Trichoscypha acuminata* contains more phenolic compounds (3111 ± 0.63 mg of GAE/g of extract than 2516 ± 0.09 mg of GAE/g of methanol extract). On extracts of *Trichoscypha acuminata*, there is a correlation between the activities of the combinations on reference strains and the antibiotics. Indeed, in the association with Ampicillin, the higher the flavonoid amount, the higher the synergistic activity is. Amoxicillin has the same profile when combined with the extracts in correlation with the amount of tannin. *Ongokea gore* extracts show no descriptive correlation because the synergistic activity changes according to the antibiotic and the bacterial strain. The literature tells us that phenolic compounds are responsible for interesting biological activities, including antibacterial, antiviral, anti-inflammatory, antidiabetic, cardioprotective, and antitumor. They have been reported to modulate intracellular signaling pathways, altering the activity of target enzymes and affecting gene expression (Kumari and Jain, 2012; Stefanovic and Comic, 2012). These bioactive phytocompounds inhibit the growth of bacteria and their joint activity with antibiotics leads to an enhanced antibacterial effect and a continuation of the useful life time of antibiotics (Stefanovic and Comic, 2012).

Conclusion

The results of this study suggest that antimicrobial resistance is a growing and worrying problem. Our data show that infection with a single, multidrug-resistant bacterium can lead to treatment failure. The resistance profile exhibited by these isolates corresponds to the type of antibiotic commonly used in hospitals. Therfore, health authorities in Gabon should intensify their efforts to search for new antibiotics that can be more effective against infections caused by multidrug-resistant organisms. The results obtained by combinations show that *Trichoscypha acuminata* and *Ongokea gore* extracts potentiate Ampicillin, Amoxicillin and Doxycycline against some bacterial strains, including multidrug-resistant strains. The antimicrobial effects of these antibiotics against the tested bacteria probably suggest the possibility of simultaneous use of these antibiotics and plant extracts in the treatment of diseases caused by these multidrug-resistant bacteria. However, before using these combinations in multidrug-resistant antibacterial therapy, an antibiogram of these combinations would be necessary.

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Conflicts of interest

The authors declare that they have no conflicts of interest

Ethical considerations

The study protocol was approved by the Ethic Committee of Gabon (N°005 march 2011). Additional, informed consent was obtained each time.

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References

Abiramasundari, P., Priya, V., Jeyanthi, G.P., and Gayathri, D. (2011). Evaluation of the Antibacterial activity of Cocculus hirsutus. J Drugs Med. 3, 26-31.

Abubakar, A.R., and Haque, M. (2020). Preparation of medicinal plants: basic extraction and fractionation procedures for experimental purposes. J Pharm Bioallied Sci. 12(1), 1-10. Doi: 10.4103/jpbs.JPBS_175_19.

Aiyegoro, O.A., and Okoh, A.I. (2009). Use of bioactive plant products in combination with standard antibiotics: implications in antimicrobial chemotherapy. J Med Plants Res. 3(13), 1147-52. doi: 10.5897/JMPR.9001277.

Ampaire, L., Muhindo, A., Orikiriza, P., Mwanga-Amumpaire, J., Bebell, L., and Boum, Y. (2016). A review of antimicrobial resistance in East Africa. Afr J Lab Med. 5(1):1-6. doi: 10.4102/ajlm.v5i1.432.

Angelini P. (2024). Plant-Derived Antimicrobials and Their Crucial Role in Combating Antimicrobial Resistance. Antibiotics 8, p.746. DOI : 10.3390/antibiotics13080746

Aron, P.M., and Kennedy, J.A. (2008). Flavan-3-ols: Nature, occurrence and biological activity. Mol Nutr Food Res. 52(1), 79-104. doi: 10.2165/00003495-199652030-00005.

Asokan, G.V., Ramadhan, T., Ahmed, E., and Sanad, H. (2029). WHO Global Priority Pathogens List: A Bibliometric Analysis of Medline-PubMed for Knowledge Mobilization to Infection Prevention and Control Practices in Bahrain. Oman Med J. 34(3), 184-193. doi:10.5001/omj.2019.37.

Bhatia, P., Sharma, A., George, A.J., Anvitha, D., Kumar, P., Dwivedi, V.P., and Chandra, N.S. (2021). Antimicrobial activity of medicinal plants against ESKAPE: An update. Heliyon. 7: e06310. https://doi.org/10.1016/j.heliyon.2021.e06310.

Billerbeck, V.G. (2007). Essential oils and antibiotic-resistant bacteria. Phytotherapie. 5(5), 249. doi: 10.1007/s10298-007-0265-z.

Cantón, R., and Morosini, M.I. (2011). Emergence and spread of antibiotic resistance following exposure to antibiotics. FEMS Microbiol Rev. 35(5), 977-91. doi: 10.1111/j.1574-6976.2011.00295.x.

Donkor, A.-M. .; Ahenkorah, B. .; Yakubu, A. .; Donkor, M. N. (2024) Enhancing Antibiotic Efficacy: Exploring Synergistic Interactions Between Plant Extracts and Conventional Antibiotics. *Fine Chemical Engineering*, *5*, 73-87.

European Communities. (1984). Standard method for determining the tannins in sorghum. Off J Eur Communities N° L 197/ 19. Available from: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:31984R2159&from=FR.

Freitas, E., Aires, A., Rosa, E.A. de S, and Saavedra, M.J. (2013). Antibacterial activity and synergistic effect between watercress extracts, 2-phenylethyl isothiocyanate and antibiotics against 11 isolates of Escherichia coli from clinical and animal source. Lett Appl Microbiol. 57(4), 266-73. doi: 10.1111/lam.12105.

Garé, M., Chesnais, C.B., Campillo, J.T., Ngogang, M.P., Fonkoua, M.C., Le Moing, V., Boullé, C. (2025). Antimicrobial resistance of Enterobacterales in Central Africa: a systematic review and meta-analysis. Commun Med (Lond) 4;5(1):453. doi: 10.1038/s43856-025-01148-3.

Harborne, A.J. (1998). Phytochemical methods a guide to modern techniques of plant analysis. 3rd ed. Chapman and Hall, editor. New York: springer science & business media; 191-198 p.

Heinrich, M., Barnes, J., Gibbons, S., and Williamson, E. (2012). Fundamentals of pharmacognosy and phytotherapy. 2nd ed. Churchill Livingstone, editor.

Ibezim, E.C., Esimone, C.O., Nnamani, P.O., Onyish, I.V., Brown, S.A., and Obodo, C.E. (2006). In vitro study of the interaction between some fluoroquinolones and extracts of kola nitida seed. African J Biotechnol. 5(19): 1781-1784.

Kamatou, G.P.P., Van Zyl, R.L., Van Vuuren, S.F., Viljoen, A.M., Figueiredo, A.C., Barroso, J.G., Pedro L.G. and Tilney, P.M. (2006). Chemical composition, leaf trichome types and biological activities of the essential oils of four related Salvia species indigenous to Southern Africa. J Essent Oil Res. 18(1), 72-9. doi: 10.1080/10412905.2006.12067125.

Krucke, G.W., Grimes, D.E., Grimes, R.M., and Dang, T.D. (2009). Antibiotic resistance in Staphylococcus aureus-containing cutaneous abscesses of patients with HIV. Am J Emerg Med. 27(3), 344-7. doi: 10.1016/j.ajem.2008.03.046.

Kumari, M., and Jain, S. (2012). Tannins: An antinutrient with positive effect to manage diabetes. Res J Recent Sci. 2277, 2502.

Magiorakos, A-P., Srinivasan, A., Carey, R.B., Carmeli, Y., Falagas, M.E., Giske, C.G., Harbarth, S., Hindler, J.F., Kahlmeter, G., Olsson-Liljequist, B., Paterson, D.L., Rice, L.B., Stelling, J., Struelens, M.J., Vatopoulos, A., Weber, J.T., and Monnet, D.L. (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 18(3), 268–281. 10.1111/j.1469-0691.2011.03570.x.

Mandal, S.M., Dias, R.O., and Franco, O.L. (2017). Phenolic compounds in antimicrobial therapy. J Med Food. 20(10), 1031-8. doi: 10.1089/jmf.2017.0017.

Ngoua-Meye-Misso, R.L., Ndong, J.D.L.C., Sima-Obiang, C., Ondo, J.P., Ndong-Atome, G.R., Abessolo, F.O., and Obame-Engonga, L.C. (2018). Phytochemical studies, antiangiogenic, anti-inflammatory and antioxidant activities of Scyphocephalium ochocoa Warb. (Myristicaceae), medicinal plant from Gabon. Clin Phytoscience. 4(15), 1-13. doi: 10.1186/s40816-018-0075-x.

Ngoua-Meye-Misso, R.L., Ondo, J.P., Assam-Evoung, J.N., Orango-Bourdette, J.O., Sima-Obiang, C., Ndong, J.D.L.C, Ovono Abessolo, F., and Obame-Engonga, L.C. (2017). Phytochemical screening, antioxidant and antiangiogenic properties of Oncoba welwitschii (Oliv.) Gilgn. and Tetrorchidium oppositifolium (Pax. and Khoffm.), medicinal plants from Gabon. Int J Innov Res Sci Eng Technol. 6(1):1-10. 25.

Ngoua-Meye-Misso, R.L., Sima-Obiang, C., Ndong, J.D.L.C., Ondo, J.P., Ovono Abessolo, F., and Obame-Engonga, L.C. (2018). Phytochemical screening, antioxidant, anti-inflammatory and antiangiogenic activities of Lophira procera A. Chev. (Ochnaceae) medicinal plant from Gabon. Egypt J Basic Appl Sci. 5(1): 80-6. doi: S2314808X17303779.

Prigent, S.V.E. (2005). Interactions of phenolic compounds with globular proteins and their effects on food-related functional properties. Wageningen University, Wageningen, The Netherlands. doi: 10.3390/foods10112798.

Quettier-Deleu, C., Gressier, B., Vasseur, J., Dine, T., Brunet, C., Luyckx, M., Cazin, M., Cazin, J.C., Bailleul, F., and Trotin, F. (2000). Phenolic compounds and antioxidant activities of buckwheat (Fagopyrum esculentum Moench) hulls and flour. J Ethnopharmacol. 72(1-2), 35-42. doi: 10.1016/S0378-8741(00)00196-3.

Rello, J., Kalwaje Eshwara, V., Lagunes, L., Alves, J., Wunderink, R.G., Conway-Morris, A., Rojas, J.N., Alp, E., and Zhang, Z.. (2019). A global priority list of the TOp TEn resistant Microorganisms (TOTEM) study at intensive care: a prioritization exercise based on multi-criteria decision analysis. Eur J Clin Microbiol Infect Dis. 38(2), 319-323. DOI:10.1007/s10096-018-3428-y.

Rybak, M.J., and McGrath, B.J. (1996). Combination antimicrobial therapy for bacterial infections. Drugs. 52 (3): 390-405. doi: 10.2165/00003495-199652030-00005.

Sima Obiang, C., Ondo, J.P., Ndong Atome, G.R., Obame Engonga, L.C., Djoba Siawaya, J.F., and Nsi Emvo, E. (2016). Phytochemical screening, antioxidant and antimicrobial potential of stem barks of Coula edulis Baill. Pseudospondias longifolia Engl. and Carapa klaineana Pierre. from Gabon. Asian Pacific J Trop Dis. 6(7), 557-63. doi :10.1016/S2222-1808(16)61086-1.

Sima-Obiang, C., Ngoua-Meye-Misso, R., Ndong-Atome, G., Ondo, J.P., Obame-Engonga, L.C., and Nsi-Emvo, E. (2019). Phytochemical analyses, antimicrobial and antioxidant activities of stem bark extracts of Distemonanthus benthamianus H. Baill. and fruit extracts of Solanum torvum Sw. from Gabon. Asian Pac J Trop Biomed. 9(5) : 209-216 . doi 10.4103/2221-1691.259001.

Sima-Obiang, C., Ngoua-Meye-Misso, R.L., Ndong-Atome, G.R., Ondo, J.P., Obame-Engonga, L.C., and Nsi-Emvo, E. (2018). Phytochemical screening, phenolic compounds content, antioxidant, anti-inflammatory, and antimicrobial properties of Pachylobus balsamifera Guillaum from Gabon. Phytotherapie. 16, 65-73. doi: 10.3166/phyto-2018-0059.

Singleton, V.L., Orthofer, R., and Lamuela-Raventós, R.M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. In: Methods in enzymology. 299, 152-78. doi: 10.1016/S0076-6879(99)99017-1.

Sofowora, A. (1993). Screening plants for bioactive agents. Med plants Tradit Med Africa. 2, 134-56.

Stefanovic, O., and Comic, L. (2012). Synergistic antibacterial interaction between Melissa officinalis extracts and antibiotics. J Appl Pharm Sci. 2(1), 1-5.

Stermitz, F.R., Lorenz, P., Tawara, J.N., Zenewicz, L.A., and Lewis, K. (2000). Synergy in a medicinal plant: Antimicrobial action of berberine potentiated by 5'-methoxyhydnocarpin, a multidrug pump inhibitor. Proc Natl Acad Sci. 97(4), 1433-7. doi: 10.1073/pnas.030540597.

Tadesse, B.T., Ashley, E.A., Ongarello, S., Havumaki, J., Wijegoonewardena, M., González, I.J., and Dittrich S. (2017). Antimicrobial resistance in Africa: a systematic review. BMC Infect Dis. 17(1), 616. doi: 10.1186/s12879-017-2713-1.

Tyers, M. and Wright, G.D. (2019). Drug combinations: a strategy to extend the life of antibiotics in the 21st century. Nat Rev Microbiol. 17(3), 141-155. https://doi.org/10.1038/s41579-018-0141-x.

Veras, H.N.H., Rodrigues, F.F.G., Colares, A.V., Menezes, I.R.A., Coutinho, H.D.M., Botelho, M.A., and Costa, J.G.M. (2012). Synergistic antibiotic activity of volatile compounds from the essential oil of Lippia sidoides and thymol. Fitoterapia. 83 (3), 508-12. doi: 10.1016/j.fitote.2011.12.024.

Venne, D.M., Hartley, D.M., Malchione, M.D., Koch, M., Britto, A.Y., Goodman, J.L. (2023). Review and analysis of the overlapping threats of carbapenem and polymyxin resistant *E. coli* and *Klebsiella* in Africa. Antimicrob Resist Infect Control 12(29) :1-40. https://doi.org/10.1186/s13756-023-01220-4

Walker, R., and Sillans S. (1961). Plantes utiles du Gabon. Paul Leche. Sepia, editor. Paris; 614 p.

Wikler, M.A. (2006). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: approved standard. CLSI. 26: M7-A7.

Williamson, E.M. (2001). Synergy and other interactions in phytomedicines. Phytomedicine. 8(5), 401-9. doi: 10.1078/0944-7113-00060.

WHO. (2025[a]). Global Antibiotic Resistance Surveillance Report 2025. https://www.who.int/publications/i/item/9789240116337

WHO. (2024). WHO BPPL 2024 update https://www.who.int/publications/i/item/9789240093461

WHO. 2025[b]. Analysis of antibacterial products in clinical development (2025). https://www.who.int/health-topics/antimicrobial-resistance/02-10-2025-who-releases-new-reports-on-new-tests-and-treatments-in-development-for-bacterial-infections?utm_source=chatgpt.com (Accessed on 29/12/2025)