WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 9, Issue 3, 187-202. Research Article ISSN 2277– 7105

EXTRACTS FROM *Acmella caulirhiza* POTENTIATE THE ACTIVITY OF CIPROFLOXACIN AGAINST MULTIDRUGRESISTANT BACTERIA EXPRESSING EXTENDED-SPECTRUM BETA-LACTAMASES

Matchuenkam Gayap Sonia Raissa[1,5], Fotsing Kwetche Pierre René*[1,2], Mafo Fokam Marcelle Aude[3,5], Yawat Djogang Anselme Michel[1,2], Munvera Mfifen Aristide[3], Dzotam Tamgue Joachim[4], Fonkeng Sama Léonard[4], Kuiate Jules Roger[4]

[1]School of Pharmacy, Higher Institute of Health Sciences, Université des Montagnes; Bangangté-Cameroon, P.O BOX 208, Bangangté-Cameroon.

[2]Laboratory of Microbiology, Université des Montagnes Teaching Hospital. Bangangté; Cameroon.

[3]Department of Organic Chemistry, Faculty of Sciences; University of Yaoundé I P.O Box: 812 Yaoundé-Cameroon.

[4]Department of Biochemistry, Faculty of Sciences, University of Dschang, P.O BOX: 96 Dschang-Cameroon.

[5]Institute of Medical Research and Medicinal Plants, P.O BOX 6163 Yaoundé-Cameroon.

Article Received on
11 Jan. 2020,
Revised on 01 Feb. 2020,
Accepted on 21 Feb. 2020
DOI: 10.20959/wjpr20203-16941

***Corresponding Author**
Fotsing Kwetche Pierre René
School of Pharmacy, Higher Institute of Health Sciences, Université des Montagnes; Bangangté-Cameroon, P.O BOX 208, Bangangté-Cameroon.

ABSTRACT

Faced with the disturbing phenomenon of bacterial resistance, the search for new efficient and available natural antibacterial agents becomes primordial. In the present study we assessed the antibacterial potential of four extracts (hexane, methylene chloride, ethyl acetate and ethanol) from *Acmella caulirhiza* (a plant that belongs to the *Asteraceae* family) in restoring the activity of three common antibiotics on multidrug-resistant and extended spectrum beta-lactamase-positive bacteria. Extracts were primarily subjected to chemical screening according to standard protocols. The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) tests were conducted with a modified rapid *p*-iodonitrotetrazolium chloride (INT) colorimetric assay. Subsequently, association tests were performed between these extracts and two beta-

lactams (Amoxicillin, Ampicillin) on one hand and a fluoroquinolone (Ciprofloxacin) on the other. The phytochemical assays revealed that all crude extracts contained alkaloids, triterpenes and sterols. The ethyl acetate and ethanol extracts further contained all secondary metabolites investigated. Only the hexane extract displayed moderate activity on multidrug-resistant strains (*Klebsiella pneumoniae*, *Klebsiella oxytoca* and *Serratia odorifera*) with the MIC values found between 512 and 1024 µg/mL. After combination, improved amoxicillin activity was observed with all extracts though this was observed on a single clinical isolate (*Staphylococcus* spp.). Ciprofloxacin was potentiated on 5 out of 7 (71.43%) multidrug-resistant isolates by all the extracts, including those that did not have any activity when they were tested alone (FIC values: 0.125 through 0.5). These findings suggested that the extracts might contained inhibitors which act on other resistance mechanisms like efflux pumps. In addition, it appeared that constituents that are responsible for the antibacterial activity of the extracts when they were used alone are not the ones that induced the synergistic effects once used in combination. It might be legitimate to pay more attention on these combinations, especially in the caretaking of infections due to extended-spectrum β-lactamases-positive bacteria which also express resistance to Ciprofloxacin.

KEYWORDS: *Acmella caulirhiza*, multidrug-resistance, extended spectrum beta-lactamase, ciprofloxacin.

INTRODUCTION

Beta-lactam antibacterial agents belong to one of the most diverse and widely used families of antibiotics worldwide. The high utilization rates is partly due to their broad spectrum of action, low toxicity, and cost-effectiveness.[1] With their frequent use in therapeutics, bacterial resistance rapidly developed to become a major health challenge at the global scale, reducing the drug effectiveness of the available therapeutic arsenal.[2] Though a natural phenomenon,[3] bacterial resistance is known to be exacerbated by inappropriate use of antimicrobial agents in humans, animals and plants.[4–7] It is estimated that 90% of the overall deaths caused by infectious diseases were recorded in Africa**.** This rate is partly associated with drug resistance expressed by the microbes incriminated. In fact, multidrug resistance observed in Gram-negative bacteria are responsible for a large proportion of hospital acquired infection (60%) and can be masterminded by sets of bacterial strategies like those involving the production of inactivating enzymes such as beta-lactamases.[1] which are amongst the most common and most important resistance mechanisms against beta-lactams.[6,8–10] Broad

spectrum beta-lactamases encompass a group of enzymes that confer resistance to penicillins, 1st, 2nd, 3rd and 4th generations of cephalosporin and Aztreonam. In addition, they do not, confer resistance to Cephamycime and Carbapenems and are inhibited by beta-lactamase inhibitors like clavulanic acid.[1] The selection, growth and spread of antibiotic resistance therefore, render healthcare unaffordable to many populations around the world, especially in low-income countries where traditional medicine practices are common.[11,12] According to the WHO, more than 80% of African populations rely on traditional medicine for their health issues. Otherwise the higher infectious rates in most low-income countries, especially in Africa couples with natural resources that can be valorised and used sustainably for the neediest communities. Valorising this natural inheritance requires researches for standards protocols that could be recommended to traditional healers in their daily activity. In this vein, several initiatives on medicinal plants are on the way in Cameroon.[12,13] More specifically, some of these investigations focus on the phytochemical,[14] the ethnobotanical and pharmacological aspects[15] of the extracts from *Acmella caulirhiza* (*Asteraceae*). From the pharmacological point of view, promising results against infectious agents have been recorded on bacteria. According to Sinei *et al.*, (2013), *Acmella caulirhiza* is widely spread in Cameroon.[16] The ethnobotanical survey conducted in the Ndé Division (in West Cameroon) reported the use of parts of *Acmella caulirhiza* in the caretaking of several human disorders, including infectious diseases).[17] Another one undertaken two years later (Tatah *et al.*, 2015) revealed the potential of its extracts on antibiotic-resistant bacteria that are aetiologies of urinary tract infections and on *Salmonella* Typhi.[18] To further investigate the antibacterial potential of this plant, the present survey was carried out to address the extended spectrum beta-lactamase (ESBL) inhibitor potential of some of *Acmella caulirhiza* extracts. Confirming this inhibitory potential could guide associating the extracts to conventional antibacterial agents in healthcare for infections due or related to ESBL-positive bacteria. In other words, the present work was designed to evaluate the ability of *Acmella caulirhiza* extracts to restore the activity of common antibiotics by inhibiting broad-spectrum beta-lactamases expression in multidrug-resistant bacteria.

MATERIAL AND METHODS

Plant material and extracts

Material used in this work consisted of the whole *Acmella caulirhiza*, collected in Bangangté (West Cameroon) in June 2015. Identification was thereafter conducted at the National Herbarium under reference Voucher number 42 040 HNC. Each specimen type was air dried

and powdered. For 72 hours at room temperature, 300 g of each powder was used separately in the extraction process (1: 10 m/v) with ethyl acetate, methylene chloride, ethanol and hexane. The extraction products were then concentrated under reduced pressure to have the necessary crude extracts. All extracts were subsequently kept at 4°C until use.

Bacteria strains

Eight bacterial types were chosen either for their frequent involvement in human pathologies and/or ESBL positivity. These included 5 clinical isolates (*Staphylococcus* spp., *Klebsiella oxytoca*, *Bacillus* spp., *Serratia odorifera* and *Citrobacter* spp.) and 3 reference strains (*Klebsiella pneumoniae* ATCC 700603, *Escherichia coli* ATCC 35218 and *Staphylococcus aureus* ATCC 25923). The clinical isolates were provided the Laboratory of Microbiology of the "Université des Montagnes" Teaching Hospital and the reference strains by the Unit of Microbiology and Research in Natural Antimicrobial Agents, University of Dschang.

Pure powers of conventional antibacterial agent consisted of Ampicillin (1 g), Amoxicillin (500 mg), Ciprofloxacin (500 mg) and Amoxicillin + clavulanic acid (625 mg). These were used as references for antibiotics during the investigations. Also, 0.2 % *p*-Iodonitrotetrazolium chloride (INT) and Dimethylsulfoxide (DMSO) (Sigma-Aldrich (St. Quentin Fallavier, France) were used respectively as microbial growth indicator and surfactant for compound dissolution, respectively.

Susceptibility tests

All MIC values throughout the study were assessed with the rapid INT colorimetric assay methods as described by Eloff (1998) with some modifications.[19,20] All extracts primarily underwent dissolution in DMSO/Mueller Hinton Broth (MHB) to the final concentration of DMSO lower than 2.5%.[21] The resulting mixture was thereafter, added to Mueller Hinton Broth, and serially diluted two folds (in a 96-wells microplate). One hundred microliters (100 µL) of the bacterial inoculum (≈ 1.5×10^6 CFU/mL) prepared in appropriate broth was subsequently added to the preparation.[20,21] The plates containing these preparations were covered with a sterile sealer, thoroughly mixed by gentle agitation with a plate shaker and incubated for 18 h at 37°C. The assay was repeated three times in each case. Wells containing adequate broth, 100 µL of inoculum and DMSO to a final concentration of 2.5% served as negative control. The MIC were recorded upon completion of incubation with addition of 40 µL of the INT that was followed by additional incubation at 37°C for 30 min. Viability was indicated by the dye change from yellow into pink. MIC was defined as the concentration at

which no color change developed, testifying complete inhibition of microbial growth.[19] For the MBC, 50 µL from the well were no growth was reported in the MIC essay was added to 150 µL of MHB and re-incubated for 48 h in convenient bacterial growth environment. Upon completion, the MBC was regarded as the lowest concentration of extract at which no color change appeared upon addition of INT and incubation, as done for the MIC.

To evaluate the potentiating effect of extracts, their sub-inhibitory concentrations (MIC/2 and MIC/4) were combined with antibiotics and used on the subjected bacterial isolates. The fractional inhibitory concentration (FIC) of each combination was thereafter calculated as the ratio of MIC of antibiotic in combination *versus* the MIC of the antibiotic used alone (MICAntibiotic in combination/MICAntibiotic alone) and the association were regarded as synergistic when FIC values were ≤ 0.5. It was said to be indifferent for FIC values found between 1 and 4 and antagonistic when they were larger than 4.[22,23] All assays were performed in triplicate.

RESULTS

The yield and phytochemical screening of the four extracts tested provided ranges of indications that could help in attesting their activity. Summary of these results was presented as shown in table I.

Table I. Yield and phytochemical screening of each extract (qualitative estimate).

Yield/ Phytochemical categories	Extracts			
	Ethyl acetate	Methylene chloride	Ethanol	Hexane
Yield	1.46	2.06	2.97	0.65
Alkaloids	+	+	+	+
Anthraquinones	+	+	+	-
Flavonoids	+	-	+	-
Polyphenols	+	-	+	-
Saponins	+	+	+	-
Sterols	+	+	+	+
Tannins	+	+	+	-
Triterpenes	+	+	+	+

(-): Absent; (+): Present;

The overall picture highlighted the presence of all secondary metabolites investigated in the ethyl acetate and ethanol extracts. In addition, alkaloids, sterols, and triterpenes were detected in all extraction products.

***In vitro* antibacterial potential**

When tested, the antibacterial potential varied from one extract to the other on the isolates subjected to the experiment. The MIC, MBC and the MBC/MIC ratio (R) recorded from various essays were tabled as displayed below (Table II).

Table II. Antibacterial activity of the extracts from *Acmella caulirhiza* (µg/mL).

Bacterial isolates	Hexane Extract			Methylene chloride extract			Ethyl acetate extract			Ethanol extract		
	MIC	MBC	R	MIC	MBC	R	MIC	MBC	R	MIC	MBC	R
Staphylococcus spp.	>1024	>1024	-	>1024	>1024	-	>1024	>1024	-	>1024	>1024	-
Citrobacter spp.	>1024	>1024	-	>1024	>1024	-	>1024	>1024	-	>1024	>1024	-
K. oxytoca	512	1024	2	>1024	>1024	-	>1024	>1024	-	>1024	>1024	-
Serratia odorifera	1024	>1024	-	>1024	>1024	-	>1024	>1024	-	>1024	>1024	-
Bacillus spp.	>1024	>1024	-	>1024	>1024	-	>1024	>1024	-	>1024	>1024	-
K. pneumoniae ATCC 700603	512	1024	2	>1024	>1024	-	>1024	>1024	-	>1024	>1024	-
S. aureus ATCC 25923	512	1024	2	1024	-	-	>1024	>1024	-	>1024	>1024	-
E. coli ATCC 35218	>1024	>1024	-	>1024	>1024	-	>1024	>1024	-	>1024	>1024	-

R: MIC/MBC; -: not determined.

It appeared that the hexane extract was most active, as shown in 50% of bacteria under study. The methylene chloride extract exhibited a slightly weak potential on a single strain (*S. aureus* ATCC 25923). Further insights indicated that the MIC values for other extracts were greater than 1024 µg/mL, while the bactericidal effects were recorded when the hexane extract was used on *K. oxytoca*, *K. pneumoniae* and *S. aureus* ATCC 25923.

***In vitro* antibacterial potential of common antibiotics on the studied bacterial isolates**

The test was carried out to determine on one hand, the inherent MIC values for the antibiotics in order to attest the resistant phenotype expressed by the tested bacterium, and evaluate the effect of their association with the plant extracts on the same isolate on the other. For this test, antibacterial agents from two families were used: beta-lactams and fluoroquinolones. The findings recorded were displayed as shown in table III.

Table III. Antibacterial activity of some antibiotics (µg/ml).

Bacterial isolates	Amoxicillin			Ampicillin			Amoxicillin/ clavulanic acid			Ciprofloxacin		
	MIC	MBC	R	MIC	MBC	R	MIC	MBC	R	MIC	MBC	R
Staphylococcus spp.	4	8	2	>256	>256	-	4	32	8	4	16	4
Citrobacter spp.	>256	>256	-	256	>256	-	32	256	8	32	128	4
K. oxytoca	>256	>256	-	>256	>256	-	32	64	2	32	32	1
S. odorifera	>256	>256	2	>256	>256	-	2	8	4	4	16	4
Bacillus spp.	>256	>256	-	>256	>256	-	32	32	1	4	128	32
K. pneumoniae ATCC 700603	>256	>256	-	32	128	4	16	64	4	8	128	16
S. aureus ATCC 25923	<0.5	>256	-	32	>256	-	<0.5	>256	-	4	4	1
E. coli ATCC 35218	>256	>256	-	128	256	2	32	32	1	16	64	4

R: MIC/MBC; -: not determined.

Tests on *S. aureus* ATCC 25923 (the susceptible reference) yielded the expected results. This strain is known to be devoid of resistance mechanisms. At the same level of susceptibility MIC and MBC) for *Staphylococcus* spp (a multidrug-resistant-positive isolates), the values recorded were approximately 32-fold higher than those obtained with *S. aureus* ATCC 25923 when Amoxicillin, Amoxicillin/clavulanic acid were used, and 8-fold higher when Ampicillin and Ciprofloxacin were used.

For *K. pneumoniae* ATCC 700603 (an ESBL-positive strain), the MIC values with amoxicillin were similar to those observed for 3 out of the 4 isolates that belonged to the *Enterobacteriaceae* family. With Ampicillin, the MIC for the other isolates were very large compared to the value obtained with the reference ESBL-positive strain (32 for ≥ 256 µg/mL). With Ciprofloxacin, all MIC values were equal or larger than four (≥ 4). A significant change was recorded when the MIC's documented with amoxicillin and amoxicillin/clavulanic acid were compared. This significant decrease (by a factor of at least 8) was likely related to the action of ESBL inhibitor (clavulanic acid).

Antibacterial activities of crude extracts in combination with common antibiotics.

When conventional antibiotics were associated with the plant extracts, sets of findings were gathered. Subtle details on these results with respect to bacterial isolates and extracts/antibiotics combinations used were summarized and displayed as shown in table IV.

Table IV. Potential of the extracts/antibiotics combinations

Bacteria isolates	Antibiotics	MIC value	Extracts (µg/mL)							
			Ethanol		Ethyl acetate		Methylene chloride		Hexane	
			MIC/2	MIC/4	MIC/2	MIC/4	MIC/2	MIC/4	MIC/2	MIC/4
K. neumoniae ATCC 700603		8	1(0.125)^{S}	2(0.25)^{S}	1(0.125)^{S}	1(0.125)^{S}	1(0.125)^{S}	1(0.125)^{S}	2(0.25)^{S}	2(0.25)^{S}
	Amoxicillin	>256	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}
	Ampicillin	>256	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}
.E. coli ATCC 5218	Ciprofloxacin	16	>256(≥16)^{A}	>256(≥16)^{A}	>256(≥16)^{A}	>256(≥16)^{A}	>256(≥16)^{A}	>256(≥16)^{A}	>256(≥16)^{A}	>256(≥16)^{A}
	Amoxicillin	>256	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}
	Ampicillin	128	>256(≥2)^{I}	>256(≥2)^{I}	>256(≥2)^{I}	>256(≥2)^{I}	>256(≥2)^{I}	>256(≥2)^{I}	>256(≥2)^{I}	>256(≥2)^{I}
S. aureus ATCC 25923	Ciprofloxacin	4	1(0.25)^{S}	2(0.5)^{S}	1(0.25)^{S}	1(0.25)^{S}	1(0.25)^{S}	1(0.25)^{S}	1(0.25)^{S}	1(0.25)^{S}
	Amoxicillin	<0.5	>256(≥512)^{A}	>256(≥512)^{A}	>256(≥512)^{A}	>256(≥512)^{A}	>256(≥512)^{A}	>256(≥512)^{A}	>256(≥512)^{A}	>256(≥512)^{A}
	Ampicillin	32	>256(≥8)^{A}	>256(≥8)^{A}	>256(≥8)^{A}	>256(≥8)^{A}	>256(≥8)^{A}	>256(≥8)^{A}	>256(≥8)^{A}	>256(≥8)^{A}
S.phylococcus spp.	Ciprofloxacin	4	1(0.25)^{S}	2(0.5)^{S}	0.5(0.125)^{S}	1(0.25)^{S}	0.5(0.125)^{S}	0.5(0.125)^{S}	1(0.25)^{S}	1(0.25)^{S}
	Amoxicillin	4	0.5(0.125)^{S}	0.5(0.125)^{S}	0.5(0.125)^{S}	0.5(0.125)^{S}	0.5(0.125)^{S}	1(0.25)^{S}	1(0.25)^{S}	1(0.25)^{S}
	Ampicillin	>256	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}
Serrata odorifera	Ciprofloxacin	4	4(1)^{I}	4(1)^{I}	2(0.5)^{S}	2(0.5)^{S}	1(0.25)^{S}	2(0.5)^{S}	2(0.5)^{S}	4(1)^{I}
	Amoxicillin	>256	128(≥0.5)^{S}	>256(≥1)^{I}	128(≥0.5)^{S}	128(≥0.5)^{S}	128(≥0.5)^{S}	128(≥0.5)^{S}	128(≥0.5)^{S}	128(≥0.5)^{S}
	Ampicillin	>256	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}
Citrobacter spp.	Ciprofloxacin	32	>256(≥8)^{A}	>256(≥8)^{A}	>256(≥8)^{A}	>256(≥8)^{A}	>256(≥8)^{A}	>256(≥8)^{A}	>256(≥8)^{A}	>256(≥8)^{A}
	Amoxicillin	>256	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}

	Ampicillin	256	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}
K. oxytoca	Ciprofloxacin	32	8(0.25)^{S}	8(0.25)^{S}	4(0.125)^{S}	4(0.125)^{S}	4(0.125)^{S}	4(0.125)^{S}	8(0.25)^{S}	8(0.25)^{S}
	Amoxicillin	>256	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}
	Ampicillin	>256	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}
Bacillus spp.	Ciprofloxacin	4	8(2)^{I}	8(2)^{I}	2(0.5)^{S}	4(1)^{I}	8(2)^{I}	4(1)^{I}	4(1)^{I}	4(1)^{I}
	Amoxicillin	>256	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}
	Ampicillin	>256	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}	>256(≥1)^{I}

(): FIC (Fractional Inhibitory Concentration) of the antibiotics after association with plants extract; S: Synergy, I: Indifference; The values in bold represent the cases of synergy between extract and antibiotic; A: antagonist

Amongst beta-lactams, amoxicillin was the only one that was potentiated by all extracts on *Staphylococcus* spp. All others isolates remained "indifferent" to the drug antibiotic/extract combination.

Ciprofloxacin was also potentiated by the extracts on 5 strains which previously expressed resistance to Ciprofloxacin when it was used alone. Also fascinating, the ethyl acetate and methylene chloride extracts displayed relatively stronger synergistic effects when they were used in combination with Ciprofloxacin compared to ethanol and hexane extracts.

Comparing the MIC values of Amoxicillin/clavulanic acid (MIC = 4 µg/mL) and Amoxicillin/plant extract (MIC < 0.25 µg/mL) for the *Staphylococcus* spp., it was obvious that the MIC's of the antibiotic /extract combination was relatively lower, indicating a higher inhibitory potential.

DISCUSSION

This investigation on the phytochemical screening and anti-β-lactamase potential of *Acmella caulirhiza* extracts on ESBL-positive isolates generated questionable results in several ways. The phytochemical screening revealed that the chemical composition of extracts were solvent-dependent. Thus, with ethyl acetate and ethanol, all the target metabolites were detected, consistent with the polarity and indicating their suitability as the most effective extraction solvents. However, the extraction yield with ethanol was twice higher than the one obtained with ethyl acetate. Otherwise, ethanol would extract more polar compounds than ethyl acetate. The yield with methylene chloride that was also better than the one obtained with ethyl acetate could have in addition to its polar characteristic, a greater corrosive effect that allows better fractionation, which in turn, facilitates release of the plant cell's contents.[24] However, the fact that, unlike ethyl acetate, hexane did not extract polyphenols that are polar compounds is yet to address comprehensively. Overall on this issue, availability of ethanol is a great asset in developing cost-effective techniques that could be used in traditional medicine.

With a glance on the antibacterial potential of extracts on target bacteria isolates, it was observed that only the hexane and methylene chloride extracts expressed a certain degree of activity. Further insight indicated that the hexane extract was more potent than the methylene chloride's. It could therefore, be anticipated that the chemicals that are present in the extract have preferentially been extracted with hexane. The fact that the poorest extract in terms of metabolites (the hexane extract) has the highest antibacterial potential could be consistent with anticipation that its overall antibacterial potential strongly correlated its contents in secondary metabolites, more specifically, the sterols and the triterpenes. This extract was in fact, the poorest in target secondary metabolites, but exhibited the highest antibacterial activity compared to the others on 3 out of 7 multidrug-resistant isolates (MIC values ranging from 512 to 1024), with moderate activity from one isolate to the other. According to former authors, a plant extract is regarded as very active when the MIC <100 µg/mL, moderate when 100 ≤ MIC <625 µg/mL and low when the MIC > 625 µg/mL. Accordingly, the methylene chloride and hexane extracts exhibited moderate activity on *S. aureus* ATCC 25923, a susceptible reference strain. This agrees with the findings by previous investigations (Ramsewak *et al.*, 1999; Tatah *et al.*, 2015) which reported the antibacterial potential of the hexane and methylene chloride extracts of *Acmella caulirhiza.*[18,25] According to Tatah *et al.* (2015), however, this activity observed with *Acmella caulirhiza* is likely associated with the

presence of soluble lipid and more specifically β-stigmasterol and hexadecanoic acid (palmitic acid).[18] Hexane is a nonpolar solvent that can easily extract the soluble lipids such as essential oils, sterols and triterpenes in the plant.[18] This is once again, consistent with findings from the present investigation and the above discussion on the detection of sterols and triterpenes implying that these chemicals (sterols and triterpenes), β -stigmasterol and hexadecanoic acid might possess the antibacterial potential. Extracts from ethyl acetate and ethanol were richer in secondary metabolites but did not express any antibacterial activity, also in line with earlier investigation.[26] Unlike that study however, MIC values were obtained by agar dilution technique for aerial parts of *Acmella caulirhiza.* Inactivity of these extract could be attributed to several factors including negative interactions that might develop amongst the chemicals in the crude product. Flavonoids detected in higher concentration in the ethyl acetate and ethanol extract could also play critical roles in the overall activity observed and beyond.

An investigation undertaken by Ngoupayo *et al.* in 2015 disclosed that Flavonoids possess anticancer, antioxidant, antiviral, and antimicrobial potentials.[27] In addition, metabolic contents in plants is dependent upon both biotic and abiotic influences that interact with that plant. Otherwise, plant from the same species that are grown in dissimilar environments would likely have slightly different chemical compositions. Concentration of secondary metabolites may also vary from one part of the plant to the other and, from one plant to the other, the same metabolite may have slightly different characteristics. Frequently shown to display good antibacterial activity on both major bacteria Gram categories [27–29], observing that flavonoids were inactive in the present study also still to be addressed, acknowledging that their potential can also be altered by the presence of other polyphenols, 2010).[24] In their roles in general, polyphenols are known for their antibacterial potential through increased permeabilization of the cell envelope that eventually results in cell lysis like polypeptides, disrupts the super-coiling of bacterial genome like quinolones or acts as anti-metabolite like sulfonamides.[29] In all cases, the activity was actually observed only with the hexane extract. The present investigation further focused on combinations of antibacterial agents that would inhibit ESBL expression. In this regards, the extract/antibiotic combination appeared to have a positive effect in ESBL expression. It is, therefore, likely that the compounds responsible for the antibacterial activity of the extracts tested alone were not the same ones that induced the synergistic effect that resulted in inhibition of ESBL expression. One might anticipate that these compounds are polar. Further steps into the synergistic studies disclosed that

Ciprofloxacin was potentiated (5/7 strains) by all extracts, including those which did not have any antimicrobial action when they were used alone. The fractional inhibitory concentrations related ranged between 0.125 and 0.5.

This potential might be related to the higher cell membrane permeability facilitated by flavonoids that allowed easy access to the antibiotic target, the topoisomerase. It is also clear that many other mechanisms might be involved, since β-lactam-resistant isolates that are positive for ESBL became susceptible to various drug combinations. Some resistance mechanisms might also make bacteria more susceptible to other antibacterial agents. In this and with regards to *Staphylococcus aureus* ATCC 25923, it would not be exaggerated to allege that the Amoxicillin/extract combination reduced amoxicillin concentration which became less available at the Penicillin Binding Protein (PBP) target in the periplasmic space then, responsible for the inactivity recorded.

With regards to β-lactams, only Amoxicillin was potentiated by all extracts in a single ESBL-positive clinical isolate (*Staphylococcus* spp.), with a FIC lower than 0.25. One could also anticipate that *Staphylococcus* spp., was the only isolate equipped with receptors for inhibitors that were present in the plant extract, or that the ESBL present in this *Staphylococcus* spp., differed from those expressed by the others isolates. The differences might also be related to the localization of β-lactamases in Gram-positive (extracellular) and Gram-negative (periplasmic), or their biogenesis (inducible in *Staphylococcus aureus* and constitutive in Gram-negative).[30] Comparing the effect of the Amoxicillin/extract combination with that of the Amoxicillin/clavulanic acid association on this clinical isolate it was observed that the MIC of the combination with the extract was lower (MIC < 0.25) than the one recorded with the association with clavulanic acid (MIC = 4). This finding was key evidence that some of the extracts' secondary metabolites had improved inhibitory potential than clavulanic acid.

The fact that the extract did not potentiate the activity of Amoxicillin on ESBL-expressing rods from the *Enterobacteriaceae* family could further imply that the mechanisms enacted by the extracts are different than the one used by clavulanic acid, but these allegations are yet to be fully demonstrated. Subtle focus on the MIC values revealed that the isolates under study expressed resistance to β-lactams.[31] Furthermore, it was shown that resistance to β-lactams through expression of ESBL is generally associated with resistance to fluoroquinolones.[32] This latest clue would justify, at least partly, the resistance expressed by isolates to

Ciprofloxacin. However, a synergistic effect was recorded between the extracts and this Ciprofloxacin for certain isolates which were resistant to it, when it was used alone. This might suggest that Ciprofloxacin could be associated with extracts of *Acmella caulirhiza* for the caretaking in some instances where resistance to Ciprofloxacin is observed. This point however, requires further researches to determine the necessary accurate concentrations and resulting contraindications.

CONCLUSION

Acmella caulirhiza is rich in secondary metabolites for which extraction is solvent dependent. The hexane extract was the poorest in secondary metabolites but paradoxically expressed the highest potential on bacteria subjected to susceptibility tests. It might be legitimate to pay more attention on these combinations, especially in the caretaking of infections that involve extended-spectrum β-lactamases-positive bacteria which also express resistance to Ciprofloxacin.

ACKNOWLEDGEMENTS

This piece of work is a contribution to a large-scale program on the search for safer healthcare with improved traditional drugs. Special tribute is paid here to Sitcheping Kuetche Claude, engineer in Water Facility who strongly advocated the use of natural resources in the management for human welfare. The authors are also thankful to the “Association pour l’Education et le Développement (AED)” for logistic support through the Université des Montagnes.

REFERENCES

1. Pieboji JG. Caractérisation des beta-lactamases et leur inhibition par les extraits de plantes médicinales. Thèse présentée en vue de l’obtention du diplôme de Doctorat ès Sciences en Biochimie. Université de Liège, 2007; 1-127.
2. Carle S. La résistance aux antibiotiques: un enjeu de santé publique important! Pharmactuel., 2009; 42.
3. Martínez JL, Baquero F. Emergence and spread of antibiotic resistance: Setting a parameter space. Ups J Med Sci., 2014; 30.119(2): 68–77.
4. Pradeau S, Schrive I, Charbit M, Balansard G. Evaluation de la prescription nominative des antibiotiques dans un service de chirurgie générale et urgences. J Pharm Clin., 1997; 16(4): 249–53.
5. Tchapdie Ngassam FR, Megne Tantse, Fotsing Kwetche PR, Noukela Noumi DP,

Kouamouo J Simo Louokdom J et al. Multicenter study on antibiotic susceptibility/resistance trends in the western region of Cameroon. Int J Biol Chem Sci., 2017; 11(1): 131–43.

6. Simo Louokdom J, Fotsing Kwetché PR, Yawat Djogang AM, Gamwo Dongmo S, Nankam Nguekap WL, Tchoukoua SH et al. Antibiotic Susceptibility / Resistance profile of bacteria from farm wastes : findings in excreta from four poultries of West Cameroon. World J Adv Healthc Res., 2018; 2(4): 213–21.
7. Yawat Djogang AM, Fotsing Kwetché PR, Simo Louokdm J, Gamwo Dongmo S, Nankam Nguekap WL, Tchoukoua SH et al. Antibiotic susceptibility profile of bacteria from farm wastes : findings in chicken excreta , food and water from four poultries versus trend in a non-exposed exposed community of West Cameroon. Int J Curr Res., 2018; 10(11): 75629–38.
8. Galleni M, Amicosante G, Frère J-M. A survey of the kinetic parameters of class C β-lactamases. Cephalosporins and other β-lactam compounds. Biochem J., 1988; 255(1): 123–9.
9. Weiss LDK. La résistance bactérienne la nouvelle guerre froide., 2002; 37: 41–8.
10. Fotsing Kwetche PR, Simo Louokdom J, Kamga C, Kaba K, Kouamouo J. ß-Lactamase-Associated resistance phenotypes amongst multidrug resistant Bacteria isolated in a School Hospital of West Cameroon. Integr J Br., 2015; 2(8): 1–70.
11. Voukeng IK, Kuete V, Dzoyem JP, Fankam AG, Noumedem JAK, Kuiate JR, et al. Antibacterial and antibiotic-potentiation activities of the methanol extract of some cameroonian spices against Gram-negative multi-drug resistant phenotypes. BMC Res Notes., 2012; 5(1): 299.
12. Dzotam JK, Touani FK, Kuete V. Antibacterial and antibiotic-modifying activities of three food plants (Xanthosoma mafaffa Lam., Moringa oleifera (L.) Schott and Passiflora edulis Sims) against multidrug-resistant (MDR) Gram-negative bacteria. BMC Complement Altern Med., 2015; 16(1): 9.
13. Wamba B, Mbaveng AT, Nayim P, Dzotam JK, Ngalani OJT, Kuete V. Antistaphylococcal and Antibiotic Resistance Modulatory Activities of Thirteen Cameroonian Edible Plants against Resistant Phenotypes. Int J Microbiol., 2018.
14. Dzotam JK, Konga Simo I, Bitchagno GTM, Çelik İ, Ekti's SF, Kammogne Wackam V, et al. Further antibacterial compounds from Myristica fragrans. Investig Med Chem Pharmacol., 2018; 1(2): 1–5.
15. Kuete V. Potential of Cameroonian plants and derived products against microbial

infections: a review. Planta Med., 2010; 76(14): 1479–91.

16. Sinei KA, Okalebo FA, Mugo HN, Mwalukumbi JM. An Investigation of the Antimicrobial Activity of Acmella caulirhiza. African J Pharmacol Ther., 2013; 2(4).
17. Foutse Y. Contribution à l‟étude ethnopharmacologique dans le département du Ndé (Cameroun). [Thèse]. Pharmacie: Bangangté., 2009; 214.
18. Akoachere J-FTK, Suylika Y, Mbah AJ, Ayimele AG, Assob JCN, Fodouop SPC, et al. In vitro antimicrobial activity of agents from Spilanthes filicaulis and Laportea ovalifolia against some drug resistant bacteria. J Pharm Res Int., 2015;76–87.
19. Eloff JN. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Med., 1998; 64(08): 711–3.
20. Kuete V, Nana F, Ngameni B, Mbaveng AT, Keumedjio F, Ngadjui BT. Antimicrobial activity of the crude extract, fractions and compounds from stem bark of Ficus ovata (Moraceae). J Ethnopharmacol., 2009;124(3): 556–61.
21. Kuete V, Mbaveng AT, Tsaffack M, Beng VP, Etoa F-X, Nkengfack AE, et al. Antitumor, antioxidant and antimicrobial activities of Bersama engleriana (Melianthaceae). J Ethnopharmacol., 2008; 115(3): 494–501.
22. Braga LC, Leite AAM, Xavier KGS, Takahashi JA, Bemquerer MP, Chartone-Souza E, et al. Synergic interaction between pomegranate extract and antibiotics against Staphylococcus aureus. Can J Microbiol., 2005; 51(7): 541–7.
23. Coutinho HDM, Vasconcellos A, Freire-Pessôa HL, Gadelha CA, Gadelha TS, Almeida-Filho GG. Natural products from the termite Nasutitermes corniger lowers aminoglycoside minimum inhibitory concentrations. Pharmacogn Mag., 2010; 6(21): 1.
24. Mandalari G, Bisignano C, D'Arrigo M, Ginestra G, Arena A, Tomaino A, et al. Antimicrobial potential of polyphenols extracted from almond skins. Lett Appl Microbiol., 2010; 51(1): 83–9.
25. Ramsewak RS, Erickson AJ, Nair MG. Bioactive N-isobutylamides from the flower buds of Spilanthes acmella. Phytochemistry., 1999; 51(6): 729–32.
26. Prachayasittikul V, Prachayasittikul S, Ruchirawat S, Prachayasittikul V. High therapeutic potential of Spilanthes acmella: a review. EXCLI J., 2013; 12: 291.
27. Ngoupayo J, Njigou Mawouma AR, Fotsing Kwetché PR, Matchawe C, Ngadjui Tchaleu B. Phytochemical analysis and in vitro antimicrobial potential of the hydro alcoholic extract from the stem bark of Erythropleum guineensis: test on known resistance phenotypes-expressing strains. Int J Adv Pharmacy, Biol Chem., 2015; 4(3): 719–28.
28. Ayoola GA, Coker HA, Adesegun SA, Adepoju-Bello AA, Obaweya K, Ezennia EC, et

al. Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria. Trop J Pharm Res., 2008; 7(3): 1019–24.

29. Savithramma N, Rao ML, Suhrulatha D. Screening of medicinal plants for secondary metabolites. Middle-East J Sci Res., 2011; 8(3): 579–84.
30. Bradford PA. Extended-spectrum β-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. Clin Microbiol Rev., 2001; 14(4): 933–51.
31. Institute CLS. Performance Standards for Antimicrobial Susceptibility testing: Twenty Second Informational Supplement. M100 – S22: 32(3).
32. Vora S, Auckenthaler R. Que signifie «bêtalactamases à spectre élargi» en pratique. Rev Med Suisse., 2009; 5: 1991–4.