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Research Article

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

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ABSTRACT

The present works were conducted to screen and semi-quantify a few secondary metabolites from the stem bark of *Erythropleum guineensis* (G.Don) on one hand and test the antibacterial and antifungal potential of the crude hydro-alcoholic extract on the other. The investigated chemicals included alkaloids, polyphenols, coumarins, anthocyanes, flavonoids, saponosids, terpenoids, steroids, tannins, anthraquinones, phlobotannins, mucilages and resins. The antimicrobial activity was tested by standard macro-dilution and disk diffusion techniques on twelve microbial types. The parameters investigated were the minimal inhibitory concentration (MIC), the minimal lethal concentration (MLC) and the diameter of the zone of growth inhibition. About 64 % of the secondary metabolites investigated were detected. Also all germs were susceptible within the range of extract concentrations used (200 - 0.781 mg/mL). Susceptibility at the MIC was not Gram-type dependent and, close to 67% of the most susceptible at this concentration were ESBL-expressing isolates. In addition in these ESBL-positive isolates the MBC/MIC values were the highest like in *C. albicans*. The crude extract activity on multiple phenotype-expressing microbes suggested possible action on multiple microbial targets. Overall, this activity on both bacterial Gram types and *C. albicans* expressing a variety of resistance traits is a special asset that can be used to argue and promote the use of the improved version of the *E. guineensis* extract in the management of some infectious diseases; consistent with traditional practices, the advocacy of the WHO about the relevance of traditionally improved drugs in healthcare and the necessity to provide basic healthcare for all with available and affordable resources. Optimal use might, therefore, represent an alternative to many conventional drugs including some C_3G, owing to its activity on ESBL-producing strains; but financial and motivated human resources are required for this goal to be achieved.

Keywords: *Erythropleum guineensis,* secondary metabolites, antimicrobial potential

INTRODUCTION

Prior to the discovery of the first antibiotics in the first half of the 20th century, infectious diseases (IDs) represented a major threat to human health with high morbidity and mortality rates in all settings. The use of Penicillin revolutionized human healthcare during the Second World War and provided hope in the control of IDs and baseline for the development of new antibacterial agents. This hope started fading within the decade that followed the first use of these antibiotics when penicillin-resistance bacterial stains were isolated. Resistance continued to be observed against other antimicrobial substances developed thereafter and became a paramount health concern in the 1980s when it was recognized as a worldwide threat[1,2]. IDs caused by multidrug-resistant bacteria also became a genuine cause of concern in all nations, with the highest burdens recorded in resource-limited communities were the numbers and types of resistance-selecting vehicles are larger[2]. In many technologically advanced countries in fact, resistance-control policies have been implemented to monitor the phenomenon in order to minimize the rates and spread of resistance phenotypes, key to optimizing therapeutic success. Worldwide it is estimated that approximately 17 millions of deaths are causes by microbial diseases every year. This issue is exacerbated by involvement of multidrug-resistant organisms (opportunistic and true human pathogens) owing to the extreme flexibility of their genome[3]. In most developing nations, especially in Africa, Asia and Latin America, three of the most important conducive factors are: low purchasing power, shortage of human resources for health and believes. These constraints contrast with the high diversity of plants used empirically in traditional medicine in these areas. Currently they are known to contain secondary metabolites which can play significant role in the control of human diseases and the production of novel conventional drugs[4,5,6]. Estimating that close to 80% of Africans rely on traditional medicine, the WHO[7] encouraged valorization of traditional drugs with research on, and production of Traditionally Improved Drugs (TIDs). Otherwise, increased resistance in microorganisms to the current antimicrobials has motivated the search for and evaluation of other agents. This search for new sources of antimicrobial is undertaken by many research teams throughout the world on various plant species that are used with acceptable therapeutic index in traditional medicine[8-14]. So far, most efforts have been concentrated on extracts activity and the relationship between this activity and that of conventional drugs. The relationship between the activity observed and the resistance phenotypes expressed by microbial strains against conventional drugs is poorly understood, although it deserves attention to address the mechanism(s) of action of the chemicals used and anticipate their choice in specific contexts.

The present works were conducted in that vein and targeted the phytochemical screening and antimicrobial potential of the hydro-alcoholic extract from the stem bark of *Erythropleum guineensis*, a plant grown in Cameroon and traditionally used for the treatment of sexually transmissible and skin infections in some communities. Specifically the presence of a few secondary metabolites was investigated in the crude extract that was tested on twelve microbial types (eleven bacterial isolates, some of which expressed multiple resistance phenotypes and a dimorphic fungal species, *Candida albicans* that expressed resistance against two common antifungal drugs). Activity was assessed through the minimal inhibitory and minimal lethal concentrations (MIC and MLCs, respectively) and the inhibition zone diameters.

MATERIALS AND METHODS

Plant identification and study sites:

The plant material was collected in November 2014 in Akounou, a neighborhood in the Center Region of Cameroon. Identification was conducted at the National Herbarium of Cameroon under collection reference number 45750/HNC.

Experimental works were performed in the premises of two laboratories: the Laboratory of Pharmacognosy and Pharmaceutical Chemistry of the Faculty of Medicine and Biomedical Sciences (University of Yaoundé I, Center-Cameroon) for phytochemical screening; and the Laboratory of Microbiology of the Cliniques Universitaires des Montagnes, Université des Montagnes (West-Cameroon) for investigation into the antimicrobial potentials of the extract.

Extraction and Phytochemical screening:

Subsequent to harvesting, the stem bark was allowed to dry at room temperature for a week. The dried material was then grinded into a powder which underwent the maceration process. For this, a total mass of 6500 g of the powder was put into 19.5 L of a mixture of water and 96% alcohol (70/30 v/v), then incubated for 72 h. The product of maceration was thereafter filtered through a Whatman filter paper N° 1 to have the first filtrate. The residue was further macerated in the appropriate volume of solvent for 24 additional hours then, filtered (second filtrate). Both filtrates were mixed and allowed to evaporate at 70°C under a rotavapor to have the product which, after

dehydration at 45°C in an incubator was used in the chemical screening and microbiological testing.
Investigation into the secondary metabolites was performed with a gallery of tests that characterized those that were present in the extract. The original solution used in this series of tests was prepared at 1% in distilled water (1 g of the powdered extract into 100 mL of distilled water).

Test for alkaloids
Mayer test
Two labeled tubes were used in this essay: one as 'test' and the other as 'control'. 2 mL of the extract were dispensed into the test tube and 2 mL of distilled water into the control. Into each of these tubes, 3 to 5 drops of the Valse Mayer reagent were added. Development of a whitish-yellow creamy precipitate in the test tube indicated the presence of alkaloids.

Hager test
Two tubes were used. 2 mL of the extract were dispensed into the first (test) and 2 mL of distilled water into the second (control). Into each of them, 3 to 5 drops of Hager reagent were added. Development of a reddish precipitate in the test tube was evidence of the presence of alkaloids.

Wagner test
Two tubes were used. 2 mL of the extract were dispensed into the first (test) and 2 mL of distilled water into the second (control). Upon addition of 3 to 5 drops of Wagner reagent a creamy whitish precipitate developing in the test tube indicated the presence of alkaloids.

Test for Polyphenols
Perchloride test
To about 3 mL of the extract, 2 to 3 drops of $FeCl_3$ were added. The presence of polyphenols was evidenced by the development of a greenish color.

Leather acetate test
Two tubes were used to perform this test. About 2 mL of the extract and distilled water were dispensed into the 'test' and 'control' tubes, respectively. If a whitish precipitate developed in the test tube upon addition of a few drops of 10% leather acetate, the test was regarded as positive for the presence of polyphenols in the extract.

Test for Coumarins
Into two tubes (labeled 'test' and 'control') 2 mL of the extract and 2 mL of distilled water were dispensed, respectively. If a blue or green color developed in the test tube upon addition of a few drops of a 10% $FeCl_3$ and changed into yellow with addition of HNO_3, it indicated that coumarins were present in the extract.

Test for Anthocyanes
To 2 mL of the crude sample, 2 mL of H_2SO_4 were added. A pink-red color that turned into purple-blue with addition of ammonia indicated the presence of anthocyanins[4].

Test for flavonoids
Sodium hydroxyde test
2 mL of the plant extract and 2 mL of distilled water were dispensed into one tube (test) and the other (control), respectively. Into each of these, about 1 mL of a 1 N Na0H was added. A yellow-orange color developing in the test tube indicated the presence of flovonoids. This color changing into colorless upon addition of a few drops of HCl acid further confirmed their presence in the extract[15].

Sulfuric acid test
Into two tubes containing respectively 2 mL of the extract solution (test) and 2 mL of distilled water (control), a few drops of concentrated H_2SO_4 were gently allowed through the tube wall. A yellow color developing in the test tube was regarded as a positive test, indicating the presence of flavonoids in the extract.

Saponosids test (Frothing test)
2 mL of the test solution were introduced into a test tube containing 2 mL of distilled water. The tube was stopped and shaken vigorously for about 15 seconds. Allowed to stand for 15 min, persistent frothing indicated the presence of saponosids[16].

Test for terpenoids
To 2 mL of the extract 2 mL of chloroform were added. Concentrated H_2S0_4 (3 mL) was also gently added to form a layer. A reddish-brown coloration at the interface indicated the presence of terpenoids[4].

Test for steroids
Liebermann-Burchard Test
About 2 mL of the extract were mixed with chloroform. 1-2 mL acetic anhydride and 2 drops concentrated H_2SO_4 were further gently added from the wall of the test tube. First red, then blue and finally green color indicated the presence of sterols[17].

Test for Tannins
To 2 mL of the test solution a few drops of ferric chloride test reagent were added. An intense green,

purple, blue or black color that developed was regarded as a positive test, indicating the presence of tannins in the extract[18].

Test for anthraquinones

About 2 mL of chloroform were added to 2 mL of the extract solution. The resulting mixture was shaken for 5 minutes then filtered. The filtrate was once again shaken with equal volume of 10 % ammonia solution. The presence of a bright-pink color in the aqueous layer indicated the presence of anthraquinones.

Test for phlobotannins

In a test tube, 2 mL of the test solutions were mixed with 2 mL of 1% HCl. A red precipitate developing after 10 minutes of incubation in a water bath at 80°C was evidence of the presence of phlobotannins in the extract[15].

Test for mucilages

To 2 mL of the test solutions, 4 mL of absolute alcohol were added and constantly stirred.
Development of air bubbles indicated the presence of mucilages[16].

Test for resins

Into one tube (test) 2 mL of the extract were dispensed while 2 mL of distilled water were dispensed into the other (control). About 2 mL of anhydric acetic acid and 1 mL of H_2SO_4 were thereafter added to each of these preparations. Development of a yellow color indicated the presence of resins in the extract. For all positive tests, semi-quantification based on the intensity of the test indicator was made.

Microbial isolates and antimicrobial tests:

Test organisms and choice motivation

The twelve microbial isolates used in the present survey included one reference (*Pseudomonas aeruginosa* Qc 76110), ten clinical isolates wherein one Gram-positive cocci *(Staphylococcus aureus*), eight Gram negative bacilli and *C. albicans* with or without specified resistance trait to conventional drugs (table 1).
The reference and clinical strains were kindly provided by the 'Centre Pasteur du Cameroun' and the Clinique Universitaires des Montagnes, respectively. Selection of useful organisms was based on their frequent implication in both nosocomial and community-acquired IDs on one hand, and their ability to develop resistance to conventional antimicrobial agents on the other.

Antimicrobial tests

Minimal inhibitory concentration (MIC)

All isolates were seeded on Mueller Hinton agar (Liofilchem®) and incubated aerobically at 37°C for 24 h. From the resulting pure culture, a bacterial suspension equal to 0.5 McFarland ($\approx 10^6$-10^8 CFU/mL) was prepared and adjusted to the final density required for susceptibility tests according to the ''Comité de l'Antibiogramme de la Société Française de Microbiologie'or, CA-SFM (2014), in short. To assess the MIC, the macro-dilution technique in liquid medium[19] was used with slight modifications. Briefly, in each of a series of twelve glass test tubes (12x75 mm), 2 mL of Mueller Hinton broth (MHB) were dispensed. The stock solution was prepared at 400 mg of the crude extract per mL of MHB with DMSO at 10% (v/v). From this solution, a serial dilution was performed to make solutions with extract concentrations ranging from 200 mg/mL through 0.781 mg/mL. Into each of these concentrations, 10-15 µL of the above bacterial suspension was added. The same procedure was followed with *C. albicans* on Sabouraud agar and broth. The quality control solutions were prepared as followed: broth + microbial inoculums for the positive control; the broth alone for the broth sterility control; and extract + broth for extract sterility test. The preparations were allowed to incubate aerobically at 37°C (or 30°C) overnight. When incubation was completed, the MIC was projected first by broth turbidity prior to centrifugation, then after centrifugation at 5000 rpm for five minutes from the first tube in which no whitish deposit was observed. To assess and attest reproducibility, this experiment was repeated three times.

Determination of minimal lethal concentration (MLC)

To assess the MLC, 5 µL of the 24 h suspensions from the tubes where no growth was recorded for the MIC test were seeded on fresh Mueller Hinton (or Sabouraud) agar and incubated aerobically at 37°C (or 30°C) overnight. At the end of this time, the MBCs (or MFCs) were determined from the first (the least concentrated) suspension from which no visible growth was obtained. The MLC/MIC ratios were then assessed for the bacteriostatic or the bactericidal potential of the extract on test organisms. Otherwise, when it was higher than 4, the extract was said to have a bacteriostatic action while equal to or lower than 4, it was regarded as bactericidal. The extract was said to have absolute bactericidal action when the MIC was equal to the MLC (MLC/MIC= 1).

Inhibition zone diameter

The dried extract was dissolved in a convenient broth/DMSO (10% of DMSO) to the concentration of 400 mg/mL. This original solution was used to prepare a serial dilution (d/2 step) of the extract in the appropriate broth as to, eventually obtain extract concentrations ranging from 200 mg/mL through 0.78125 mg/mL, as for the MIC test. About 15 µL of each of these solutions at the MIC and MLC was used to inoculate five paper disks (Whatman N°2) of six millimeters of diameter previously deposited on the MH (or Sabouraud) agar on which the microbial inoculums (bacterial or the fungal) prepared according to the CA-SFM (2014) were lawn to confer a monolayer confluent growth after overnight incubation. The results were read by measuring the diameter of the zone of inhibition (in mm) that developed around the impregnated disks. The negative control consisted of a disk impregnated with sterile distilled water.

RESULTS

The extract from the barks of the trunk of *Erythrophleum guineense* yielded an output of 10.7%. Investigation into the secondary metabolites contained in it resulted in data summarized and presented in table 2.

It indicates that 64% of the secondary metabolites investigated were found in the crude extract. More specifically it had high contents in tannins, polyphenols, flavonoids and alkaloids. Others like steroids, resins, muscillages and phlobotannins were not detected. As concerned terpenoids, saponins, coumarins and anthraquinons, the contents were approximately half that of tannins, polyphenols, flavonoids and alkaloids.

The techniques used for detection and semi-quantification of flavonoids and polyphenols yielded similar results; while detection of alkaloids was shown to be method/regent-dependent as their sensitivity differed greatly.

About the antimicrobial potentials of the extract, data recorded were brought together and displayed in table 3.

Within the range of concentrations used, all microbial types were susceptible, although at varied levels. In fact the MIC values were found between 0.781 mg/mL (ESBL-positive *S. aureus*, *P. mirabilis*, and ESBL-positive *E. coli)* and 6.25 mg/mL (*Serratia* spp.).

About 90% of these values were lower than /or equal to 3.125 mg/mL. As regard the MLC, the related values ranged from 3.125 mg/mL (IRP-positive *E. coli*) through 200 mg/mL (*C. albicans*).

Still other intermediate concentrations (100 mg/mL) were recorded when the tests were conducted on *S. aureus, Serratia* spp., and *Citrobacter* spp. In the large majority of isolates, however, the MLC's were very high compared to the MIC's as evidenced by the R values (MLC/MIC). These results also indicated that R was highest in the ESBL-producing bacterial isolates.

The MIC and MBC also appeared to be isolate-dependent in *Pseudomonas* and *E. coli* unlike *Klebsiella* in which the extract concentration associated with the MIC was the same while those at the MBC differed.

Inhibition zone at the MIC and MLC values for a few stains were recorded, summarized and presented in table 4.

The inhibition zones at the MIC values ranged from 6±0 mm with *S. aureus* through 8.4±2 mm with *P. aeruginosa.* They were larger at the MLC with the values comprised within 8.2±0 mm for *E. coli* and 16.4±1 mm for *C. albicans.*

Overall, in close to 36% of cases, the diameters recorded were larger than the minimal expected (6 mm) as far as the MICs were concerned, while at the MLCs, up to 73 % of tests resulted in inhibition zone larger than 10 mm. Among bacteria, the largest MBC-MIC value was recorded for ESBL-positive *S. aureus* (≈5.2 mm).

DISCUSSION

The present survey targeting the phytochemical screening and antimicrobial potential of *Erythropleum guineensis* (*E. guineensis*) revealed that the plant was rich in several chemical compounds some of which are kwon as potent antimicrobial agents. In fact, 64% of the secondary metabolites investigated were detected. The most abundant included alkaloids and total polyphenols (more importantly tannins and flavonoids). In some cases, detection was method-dependent; implying the necessity to use for this plant the most sensitive technique in order to rule out falsely negative results. It is clear that detection techniques cannot be generalized in all plants because each one would have specific sets of characteristics associated with local biotic and abiotic surrounding factors. Although above chemicals have been identified in different proportions in large numbers of plants before[12,19] it is known that similar or related molecules from different origins may virtually not exhibit the same potential[5]. In addition, parts of the same plant may contain different amounts of a specific chemical[20] that may more or less be associated with the expected potential. Present in several groups of plants for instance, tannins were reported to interfere with

mutagenic activity of mutagens in animals while its antimicrobial potential was acknowledged to be in part responsible for increasing the shelf-life of perishable food items like fruits[6].

In previous surveys flavonoids and alkaloids were recognized as antibacterial, antiviral and anticancer agents[4,21,22]. Flavonoids from different plant species were shown to act differently on Gram-positive and Gram-negative bacteria[20,23]. As these former authors did not discuss the phenotypic profiles of the isolates they used, it is likely that expression of one or more resistance phenotypes might have played relevant roles in the results they obtained. Activity on ESBL-producing isolates from both major bacteria Gram types (as observed in the present study) is consistent with the empiric use of the extract by traditional practitioners.

The *E. guineensis* richness in other polyphenol molecules is likely to have played a relevant role in the activity of the extract. Earlier, Mandalari *et al.*[24] observed that combined action of different groups of polyphenol could have either synergistic or antagonistic effect on microorganisms. Polyphenols in general were reported to interfere with the microbial cell functions by: causing leakage of the bacterial cell content like lipopeptides; impairing the supercoiling process of bacterial DNA like quinolones; acting as anti-metabolites like sulfonamides or inhibiting the sterol synthesis in fungal cell envelop like the azoles[11].

Although in the present investigation the raw product contained a variety of chemical compounds with no predictable interaction, its *in vitro* effectiveness on *C. albicans* and other bacterial isolates expressing varied resistance phenotypes can be, at least partially understood based on its multi-potential on the tested microbes. The multi-potency of polyphenols is further supported by reports on their modulatory role on endogenous intestinal microbial flora growth[25,26]. Mainly as glycosides in plants in fact, polyphenols are converted into aglycones prior to absorption, according to these authors. Endowed with inhibitory potential, aglycones can, therefore, modulate (either directly or indirectly) the activity of the gut microbial flora. Owing to the fact that this class of chemical is also abundant in foodstuff and has proven beneficial for human[11], it can reasonably be anticipated that very high concentrations are required for significant adverse effects on the bacterial growth on one hand and/or that typical endogenous flora that have become adapted to this chemical group use some of them to be rid of invading microorganisms (most incriminated in microbe-related gastro-intestinal disorders). This assertion is consistent with Chung *et al.*[6] findings on the role of polyphenols in fruits protection against microbes. Otherwise, the inhibitory action of polyphenols is a selective one. It was observed that in their presence, pathogens were negatively selected while growth was promoted in probiotic bacteria[27]. Traditionally, *E. guineensis* extract is use in case syphilis, gonorrhea and skin infections. Syphilis, for instance, is caused by the Gram-negative flexible spiral-shaped bacterial species, *Treponema pallidum*. Members of this species are known to be susceptible to former generations of antibiotics belonging to the family of β-lactams (penicillins), also recognized as potent antibiotics against Gram-positive bacteria through attachment to penicillin-binding proteins that eventually results in the inhibition of bacterial cell wall synthesis. Özçelik *et al.*[28,29] reported activity of flavonoids on β-lactamase-producing *K. pneumoniae* before. ESBLs are potent hydrolyzing enzymes that interfere with the action of β-lactams; easily identified by their inhibitory action on third generation cephalosporins (C_3G), privileged line drugs from the group in many settings. In this, its action on ESBL-positive *S. aureus* could readily be understood. Owing, however, to the versatile antimicrobial potential of the secondary metabolites contained in the extract, other mode of action or putative combinations of action cannot be ruled out to the current knowledge. In general, ESBL-positive strains proved susceptible to the extract. Whether this extract actually inhibits ESBL synthesis is likely, supported by highest R values observed and implying that this ESBL inhibitory potential may be stronger than the bactericidal's. But this potential is yet to better address.

Higher effectiveness of the *E. guineensis* extract on Gram-positive organisms could further explained its use by traditional practitioners in healing wounds. *S. aureus* was susceptible in the present survey, in agreement with previous findings with other Gram-positive bacteria[11,27,30,31]. If fact, members of the genus *Staphylococci* are most common etiologies of skin and other disorders in immune-depressed individuals[32-34] followed by fungi (most of which are polymorphic like *C. albicans*), *P. aeruginosa*[35] and *K. pneumoniae* in what susceptibility was recorded in the present works.

This multivariate action of products from other plants was observed before[36]. But as stated earlier, authors were rarely interested in the resistance phenotype expressed by bacteria they studied. The use of C_3G and other antimicrobial agents is believed to be the major engine responsible for the selection of resistant strains that disseminate rapidly in communities were the hygiene standards are low. Effectiveness of the extract on bacteria expressing ESBLs and other

resistance phenotypes could be pointed out as evidence of its action on multiple targets and likely largely associated with the complexity of the extract composition on one hand and the isolated action of each constituent on the other. Yet to fully address is, therefore, also whether or not this complexity actually potentiates its antimicrobial action or not. Antagonism may be projected but as plants develop these chemical substances to ensure their fitness upon exposure to stressors, it is most likely that beneficial combinations (potentiating) would overwhelm the antagonistic ones.

Table 1
Microbial isolates and resistance phenotypes

N°	Micro-organisms	Resistance Phenotype	N°	Micro-organisms	Resistance Phenotype
1	*S. aureus*	ESBL	7	*K. pneumoniae*	IC/ LLC
2	*E. coli*	IC/ LLC/ HLP/ IRP	8	*K. pneumoniae*	ESBL/ IC
3	*E. coli*	ESBL/ LLC/ HLP	9	*P. aeruginosa*	IC/ HLC
4	*Serratia* spp.	ON	10	*P. aeruginosa* (76110) ^{QC}	ON
5	*Citrobacter* spp.	ESBL/ IC	11	*S.* Typhimurium	ON
6	*P. mirabilis*	HLP/ LLC/IC/ IRP	12	*C. albicans*	NyR/ FlyR

QC: Quality control strain; ON: Obviously none of the phenotypes; NyR: Nystatine-resistant; FlyR: Flucytosin-resistant; ESBL: Extended Spectrum β-lactamase; IC: Inducible cephalosporinase; LLP: Low level penicillinse; HLP: High level penicillinase; LLC: Low level cephalosporinase; HLC: High level cephalosporinase; IRP: Inhibitor-resistant penicillinase

Table 2
Secondary metabolites in the hydro-alcoholic crude extract

Chemical categories	**Reagent /Method**	**Indicator Intensity**
Alkaloides	Hager	-
	Mayer	+
	Wagner	++++
Anthocyanins	10% H_2SO_4 + NH_4OH	+
Anthraquinones	CHloroform +ammonia	++
Coumarins	$FeCl_3$ 10% + $HN0_3$	++
Flavonoids	NaOH	++++
	H_2SO_4	++++
Polyphenols	$FeCl_3$	++++
	Lead acetate	++++
Saponosids	Vigorous shaking	++
Mucilage	95% Ethanol	-
Resins	CH_3COOH + H_2S0_4	-
Tannins	$FeCl_3$	++++
Phlobotanins	1% HCl	-
Terpenoids	Chloroform + H_2SO_4	++
Steroids	Acetic Anhydride + H_2SO_4	-

+ + + +: very intensely positive reaction; + +: intensely positive reaction; +: positive reaction; +: unclear reaction; -: negative reaction

Table 3
Values for MIC, MLC and R associated with microbial types

Micro-organisms	Strain characteristics	MIC (mg/mL)	MLC (mg/mL)	R*
S. aureus	ESBL	0.781	100	128
E. coli	IC, LLC, HLP, IRP	3.125	3.125	1
E. coli	ESBL, LLC, HLP	0.781	12.5	16
Serratia spp.	ON	6.25	100	16
Citrobacter spp.	ESBL, IC	3.125	100	32
P. mirabilis	HLP, LLC,IC, IRP	0.781	6.25	8
K. pneumoniae	IC, LLC	3.125	12.5	4
K. pneumoniae	ESBL, IC	3.125	50	16
Salmonella Typhimurium	ON	1.562	12.5	8
P. aeruginosa (76110) ^{QC}	ON	3.125	25	8
P. aeruginosa	IC, HLC	1.562	6.25	4
C. albicans	NyR, FlyR	1.562	200	128

** : R= MLC/MIC; ^{QC}: Quality control strain; ON: Obviously none of the phenotypes; NyR: Nystatine Resistant; FlyR: Flucytosin resistant; ESBL: Extended Spectrum β-lactamase; IC: Inducible cephalosporinase; LLP: Low level penicillinse; HLP: High level penicillinase; LLC: Low level cephalosporinase; HLC : High level cephalosporinase; IRP: Inhibitor-resistant penicillinase*

Table 4
Average diameter of growth inhibition

Microorganisms (phenotype)	Microorganisms Growth inhibition zone (mm)	
	CMI	CML
Staphylococcus aureus (ESBL)	6 ±0	11.2 ±1
Escherichia coli (ESBL, LLC, HLP)	6.8 ±2	8.2±3
Serratia spp.	6±0	10.2±2
Citrobacter spp. (ESBL, IC)	6±0	10.2±1
Proteus mirabilis (HLP, LLC,IC, IRP)	6±0	9.6±1
Klebsiella pneumoniae (IC, LLC)	8±2	10.2±0
Klebsiella pneumoniae (ESBL, IC)	6±0	10.8±1
Salmonella Typhimurium	7.2±1	8.2±0
Pseudomonas aeruginosa (76110)	6±0	10.4±0
Pseudomonas aeruginosa (IC, HLC)	8.4±2	10.4±1
Candida albicans (NyR, FlyR)	6 ±0	16.4±1

NyR: Nystatine Resistant; FlyR: Flucytosin resistant; ESBL: Extended Spectrum β-lactamase; IC: Inducible cephalosporinase; LLP: Low level penicillinse; HLP: High level penicillinase; LLC: Low level cephalosporinase; HLC: High level cephalosporinase; IRP: Inhibitor-resistant penicillinase

CONCLUSION

The present study provided evidence that the extract from *Erythropleum guineensis* could be used to manage bacterial and fungal infections caused by microorganisms expressing several resistance phenotypes. Common points with other plant extracts are that sustainable use requires high production (extraction outputs generally low), refined technologies and motivated human resources. But the optimal use may appear as alternative to many conventional drugs, including some C_3G owing to its activity on ESBL-producing strains.

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