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Resistance Profile of Bacteria Isolated from Diabetic Wounds: Phytochemicals and Antibacterial Studies of *Eriosema robustum* Leaf Extracts

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Abstract

Diabetics are susceptible to severe trophic problems and wounds, which are frequently referred to as "unhealable wounds". Untreated diabetic wounds provide an ideal habitat for the growth of bacteria, which can eventually develop resistance to standard treatments and need amputation. This study aimed to assess the *in vitro* antibacterial activity of ethanolic and aqueous extracts of *Eriosema robustum* leaves on the same bacterium, as well as the resistance profile of bacteria isolated from diabetic wounds to conventional antibiotics. Thirty samples from diabetic wounds were collected at the Baleveng Hospital, subjected to bacteria isolation and antibiotics resistance tests using the disk diffusion method. On the other hands, leaves of *Eriosema robustum* were collected, hydroethanolic extraction were performed. The extract obtained was used for the antibacterial (by determination of the MIC) and antioxidant (DPPH and FRAP tests) activities as well as phytochemicals composition (using HPLC methods) assessment. Amon the 30 samples analyzed, 19 (63.33 %) showed bacterial infections and *Staphylococcus aureus* was the most common pathogen (48 %), followed by *Pseudomonas aeruginosa* (24 %), *Klebsiella pneumoniae* (19 %), and *Streptococcus agalactiae* (9 %). Fifty percent (50 %) of *S. aureus* isolates were MRSA (methicillin-resistant *S. aureus*). The MIC value obtained 64 µg/mL against *S. aureus*, *Streptococcus agalactiae* and *Pseudomonas aeruginosa*. Phytochemicals analysis of fractions from the 70 ° hydroethanolic leaves of *Eriosema robustum* revealed the presence of coumarin, gallic acid, quinoline, vanillin, ascorbic acid, caffeic acid, kaempferol, and cinnamic acid. The antibacterial and antioxidant potential of the extracts highlighted in this study could make this plant a good avenue for the discovery of new molecules effective against infected diabetic wounds.

Keywords: Diabetic wound, *Eriosema robustum*, Antibacterial activity, Resistance profile, Alternative medicine

Introduction

Diabetic wounds are a major public health concern worldwide [1], and are generally caused by trophic disorders and poor diabetes management. They are also responsible for amputation and have a major impact on the quality of life of patients. The annual incidence of diabetic ulcers worldwide is 9.1 and 26.1 million [2]. The prevalence of diabetic wounds in Africa is 7.2 % in general, and 9.9 % in Cameroon [1]. Notably, 85 % of amputations in diabetics come directly from infections [3]. These

infections can be caused by microorganisms, such as bacteria, that produce toxins that damage tissues and cause inflammation, making the wound difficult to heal. Antibiotics have long been used in the treatment of diabetic wounds to reduce infection and amputation rates and improve patients' quality of life [4-6]. However, recent decades have marked the development of multidrug resistant bacteria that reduce or eliminate the effectiveness of antibiotic therapy with the immediate consequence of therapeutic failure. Therefore, the search for new substances capable of eliminating bacterial infections, particularly those caused by *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and many other bacteria, is becoming essential.

Plants have long been used as food, ornaments, and natural preservatives against the development of microorganisms and for the treatment of diseases [7]. Previous studies have shown that plant extracts are rich in certain substances called secondary metabolites, which give them antibacterial capacity [8].

Eriosema robustum is a non-climbing shrub plant with yellow flowers of the Fabaceae family, found particularly in Burundi, Ethiopia, Kenya, Rwanda, Tanzania, Uganda, the Democratic Republic of Congo, and Cameroon [9]. He is traditionally used to treat coughs in East Africa [10] and skin diseases in the Western Region of Cameroon [11]. The genus *Eriosema* has been the subject of forty-nine publications over the past 25 years covering phytochemical, toxicological, ethnopharmacological, and pharmacological studies; hence, there is a very low publication frequency of 2 articles per year, despite this being a type of plant full of therapeutic virtues [12]. As a result, *E. robustum* has not yet been the subject of an antibacterial study on bacteria isolated from diabetic wounds and on multidrug resistant bacteria, also, studies on the phytochemical composition of the extract fraction are lacking. This study aimed to determine the resistance profile of bacteria isolated from diabetic wounds to common antibiotics and to evaluate the *in vitro* antibacterial activity of ethanolic and aqueous extracts of *Eriosema robustum* leaves on the same bacteria.

Materials and methods

Materials

Study population

This cross-sectional study was conducted at the Baleveng Wounds Hospital, Menoua Division, West Cameroon region, from February to May 2023. Patients with type 2 diabetes mellitus were included in this study, and those who had just completed their wound dressing were excluded. Ethical clearance was obtained from the Regional Ethics Committee under authorization NO/373/2023/02/2023/CE/CRERSH-OU/VP.

Collection of data

After receiving the consent of patients, informations including age, sex, profession and clinical data were collected using a structured questionnaire. Thereafter, samples were collected for bacterial isolation.

Plant material

The plant material consisted of the leaves of *Eriosema robustum* collected in the village of Balepo (Geographic coordinates: 5°43′08″N, and 10°09′26″E), located in the Babadjou Sub-Division, West-Cameroon in July 2023.

Bacterial strains

Four reference strains namely *Staphylococcus aureus* ATCC 25923 and ATCC 29213, *Pseudomonas aeruginosa* ATCC 27853 and *Klebsiella pneumoniae* ATCC 10031 obtained from the URMSA (Research Unit of Microbiology and Antimicrobial Substances) Laboratory at the University of Dschang were used.

Antibiotics

Antibiotics use or commonly prescribed by Medical Doctor were selected and included amoxicillin (AX, (25µg)), amoxicillin-clavulanic acid (AMC, (30 µg)) and imipenem (IMP, (10 µg)) as beta- lactams; gentamycin (GN, (30 µg)) as aminoglycosides; vancomycin (VA, (30 µg)) as glycopeptide; doxycycline (DO, (30 µg)) as tetracycline; erythromycin (E, (15 µg)) as macrolides; ciprofloxacin (CIP, (5 µg)) as Fluoroquinolones.

Culture media

The following culture media were used: Mueller Hinton Agar (Mueller Hinton Agar: MHA), Mueller Hinton Broth (Mueller-Hinton Broth), Chapman medium (mannitol salt agar), Columbia, McConkey, and Cetrimide. All media were prepared according to the manufacturer's instructions.

Methods

Collection of samples

Thirty samples were obtained from the wounds of diabetic patients (type 2) at the Baleveng Wound Hospital. The wounds were superficially cleaned with saline water (0.9 %; *w/v*) and then delicately massaged to express exudation. Samples were collected using a sterile swab (previously wetted with sterile saline water) from the center to the edges of the wound. Samples collected were immediately transported to the Laboratory for culture and analysis.

Culture and identification of bacteria

Each sample was cultured in 4 different culture media, namely, mannitol salt agar specifically for *Staphylococcus*, blood agar (Columbia) for *Streptococcus*, Cetrimide medium on which *Pseudomonas* grew with a green color, and McConkey selective and differential agar for the Gram-negative bacteria. After inoculation, all Petri dishes were incubated at 37 °C for 24 h. The bacteria obtained were subjected to macroscopic, microscopic, and additional identification tests such as coagulase, catalase, and oxidase.

Antimicrobial susceptibility testing

The susceptibility of isolates was determined using the disk diffusion method in accordance with the Clinical and Laboratory Standard Institute (CLSI) guidelines [13]. Bacterial suspension (0.5×10^8 CFU/mL) was prepared using 0.5 McFarland standard, cultured on surface of the Muller-Hinton agar using a sterile swab and kept dry at room temperature. Thereafter, the antibiotic disks were placed on the surface of the agar and cultures were incubated at 37 °C for 16 - 18 h. Inhibition diameters were determined and the breakpoints proposed by CLSI allowed us to assign different sensitivity profiles.

Preparation of different extracts

After harvested, the *E. robustum* leaves were washed under running water and dried away from the sun for 14 days. They were then crushed using an electric moulinex (SINBO) to obtain a fine powder. Fifty grams of this powder was macerated individually in 250 mL of 90, 70, 30 ° ethanol/water (*v/v*) and 0 ° (water) for 48 h with stirring 3 to 4 times per day. The macerates were filtered using Wattman No.1 paper, then concentrated in a rotary evaporator at 60 °C before being dried in a thermostatically controlled oven at 45 °C. The dry extracts were stored in tightly closed glass jars in a refrigerator at 4 °C [14].

Preparation of bacterial inoculum

Bacterial colonies aged 18 - 24 h were used to prepare the suspension. Therefore, 2 - 3 colonies were diluted in sterile physiological water. McFarland standard was obtained until turbidity as identical to that of point 0.5, equivalent to a concentration of 1.5.108 CFU/mL. These suspensions were subsequently diluted to 1/100 using Mueller-Hinton broth to obtain a bacterial concentration of $1.5.10^6$ CFU/mL. Stock solutions of the extract were prepared in 5 % DMSO at a concentration of 4,096 µg/mL.

Determination of Minimum Inhibitory Concentrations (MIC)

The bacterial growth inhibitory capacity of each extract was determined using the microdilution method [15]. One hundred microliters (100 µL) of culture broth (MHB) was introduced into all 96 wells of the microplate. Then, 100 µL of each extract was introduced into the first 3 wells of the first line, and serial dilutions were carried out following a geometric progression with a ratio of 2. Then, 100 µL of the bacterial inoculum was introduced into each well, and the plates were incubated at 37 °C for 18 h. The wells containing inoculum, 5 % DMSO, and culture broth (MHB) were considered negative controls, and those containing only the culture medium (200 µL) were used as negative controls. After incubation, 50 µL of an aqueous solution of 0.2 % deparaffinitrotetrazolium bromide chloride (INT) was added to each well and incubated for 30 min. Thus, wells that turned pink after INT addition indicated bacterial growth. All concentrations that prevented the appearance of pink color were considered inhibitory concentrations, and the lowest was noted as the MIC.

Determination of the Minimum Bactericidal Concentration (MBC)

The MBCs was determined by introducing 50 µL of the contents of the wells with no bacterial growth into new microplates containing 150 µL of MHB culture broth, followed by incubation for 48 h at 37 °C. After 30 min of INT addition, the concentrations for which no pink coloring was observed were considered bactericidal, and the lowest was noted as MBC. MBC/MIC ratios were calculated.

***In vitro* evaluation of the antioxidant activity of extracts**

Study of antiradical activity using the DPPH test

DPPH (2,2 diphenyl-1-picrylhydrasyl) is the commonly used substrate for assessment of antioxidant activity because of its stability in the free radical form. This was evaluated as previously described by Mensor [16].

Study of antioxidant activity using the ferric reducing antioxidant power method

The FRAP reducing powers of the samples were determined according to the protocol described by Benzie and Strain [17]. This was based on the chemical reaction of the reduction of Fe^{3+} present in the $K_3Fe(CN)_6$ complex with Fe^{2+}. The antioxidant power of the samples was calculated using the calibration curve of the $FeSO_4$ solution.

Phytochemical analysis of *Eriosema robustum* leaf extracts

Quantitative analysis

The total phenolic content was determined using the method described by Ramde-Tiendrebeogo [18], the flavonoid content was determined using the aluminum chloride colorimetric method [19], and that of tannins was determined using the Folin-Ciocalteu method as described by Govindappa [20].

Identification of secondary metabolites by HPLC

High-pressure liquid chromatography was carried out on a Perkin Elmer-Flexor-type HPLC chromatograph coupled to a UV-visible diode detector and a C18 column. The mobile phase consisted of a gradient of solvents A (5 % acidified water) and B (methanol). The flow rate was 1 mL/min, and the wavelength was 254 nm, which was chosen depending on the nature of the extract or the fraction analyzed.

Statistical analyzes

The results obtained in this study are expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA) with post hoc Waller-Duncan multiple range tests using SPSS 26.0 for Windows. $p < 0.05$ was considered significant.

Results and discussion

Sociodemographic characteristics of patients

Table 1 shows that diabetic wounds were most prevalent among individuals aged 56 - 65 years (33.3 %), followed by those aged 46 - 55 years (23.3 %) and those older than 65 years (13.4 %). This is similar to the results obtained in Tunisia, where the average age was 56.6 ± 11.8 years [21]. We also observed that individuals aged ≥ 56 years constituted the majority (46.7 %), which is similar to a previous study conducted in Morocco [22]. It is well known that older adults are at a higher risk of developing diabetic wounds due to several reasons. First, as people age, the risk of developing diabetes increases, thereby increasing the likelihood of developing complications, such as diabetic wounds. Second, older adults may have issues with blood circulation and nerve sensation in their feet, which makes it difficult to detect wounds early. Finally, older adults may have underlying medical conditions such as neurological, arterial, and immune senescence; reduced collagen production; and a reduced ability of the skin to regenerate, which can make managing diabetic wounds more challenging. However, it is important to note that a long duration of diabetes, rather than age, may be a risk factor for foot complications. In this study, most patients had diabetes for at least 10 years. These results are consistent with a previous study conducted in Ivory Coast, which found an average duration of 8.2 ± 3.4 years [23]. This may be because prolonged diabetes can cause damage to the blood vessels and nerves, making it difficult for wounds to heal.

Sex differences have emerged as critical factors in numerous health aspects [24]. As shown in **Figure 1**, 57 % of the patients were men, while 43 % were women. This male predominance is comparable to the findings of Vanherwegen *et al.* [25], who reported a 72 % predominance of males.

Men's lower disease awareness and reluctance to seek medical attention may contribute to this disparity [26]. In contrast, women are more likely to recognize symptoms, seek professional care sooner, and visit healthcare providers more frequently than men [27,28]. Women also demonstrate greater accuracy in foot self-care when dealing with diabetic wounds, whereas men tend to wear inappropriate footwear [27]. However, a study in Nigeria found that women were disproportionately affected by diabetic sores [29]. This discrepancy could be due to the small sample size of our study, as we only collected data from a single hospital, which may have limited generalizability compared to the larger multi-clinic study conducted in Nigeria.

Table 1 Distribution of patients according to age group.

Ages (Years)	Frequency	Percentage
26 - 35	3	10.0
36 - 45	6	20.0
46 - 55	7	23.3
56 - 65	10	33.3
> 65	4	13.4
Total	30	100

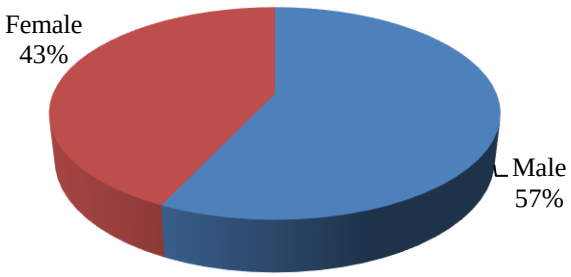

Figure 1 Distribution of patients by gender.

Table 2 presents information about the professions of the patients. The socio-professional categories mentioned are farmers, housewives, and traders, with percentages of 50, 20, and 16.7 %, respectively. Civil servants accounted for the lowest percentage at 13.3 %. The prevalence of farmers in this study may be attributed to their lack of awareness about the unique hazards that farming poses to their legs. They often neglect to change their boots for extended periods and continue to wear them even when they are wet, unaware that this creates an ideal environment for the growth of microorganisms, such as bacteria, which can lead to wounds in diabetic patients. To address any injury or pressure sore, it is essential to act promptly by washing the affected area with soap and clean water, drying it, and protecting it with a bandage [30].

Table 2 Distribution of patients according to their profession.

Profession	Frequency	Percentage
Civil servant	4	13.3
Traders	5	16.7
Farmers	15	50.0
Housewives	6	20.0
Total	30	100

Patient clinical data on wound location

Table 3 provides information on the location of the wounds in the 30 diabetic patients. This analysis showed that the sole of the foot was the most affected (53.4 %), followed by the leg and/or thigh (26.6 %), and whereas the dorsal side of the foot represented only 3.3 %. The findings of this study align with those of Abrogoua *et al.* [23], who discovered that foot involvement was the most pronounced at 57.70 %, with an additional 20.4 % located primarily at the soles of the feet. This correlation can be attributed to the distinct mechanical pressures exerted by the body on the soles of the feet, such as external aggression, barefoot walking, and trauma from falling objects, which ultimately result in neuropathy and arteriopathy. These conditions serve as the initiating factors for diabetic wounds.

Table 3 Distribution of patients based on wound location.

Location	Frequency	Percentage
Toes	3	10.0
sole	16	53.4
dorsal side of the foot	1	3.3
Hands	2	6.7
Leg and or thigh	8	26.6
Total	30	100

Microbiological characteristics of diabetic wounds

Out of the 30 samples analyzed, 19 (63.33 %) were found to contain bacteria. Two of these samples contained 2 distinct bacteria, resulting in a total of 21 isolates. The distribution of the various bacteria isolated during the study is illustrated in **Figure 2**. It was observed that *S. aureus* was the most commonly detected bacterium (47.61 %, 10/21), followed by *P. aeruginosa* (23.8 %, 5/21), *K. pneumoniae* (19.04 %, 4/21), and *S. agalactiae* (9.52 %, 2/21). These findings are similar with those reported by Fomba, who found an infection rate of 71.95 %. This could be attributed to the fact that our study only focused on facultative and aerobic bacteria, thereby excluding the possibility of identifying anaerobic bacteria [31].

In this study, gram-positive bacteria were found to be predominant (12/21, 57.14 %), with *S. aureus* being the most frequently isolated pathogen (10/21, 48 %). This result is consistent with other studies, which have reported *S. aureus* as the most commonly isolated bacterium from infected diabetic wounds [32]. Among the remaining 42.86 % of gram-negative bacteria, *P. aeruginosa* was the most frequently detected (24 %). These findings are supported by [33,34], who also identified *P. aeruginosa* as the primary gram-negative bacterium responsible for diabetic wound infections. The high prevalence of *S. aureus* and *P. aeruginosa* in diabetic wounds can be attributed to their ability to utilize sugar as an energy source and also their resistance to various antibiotic classes.

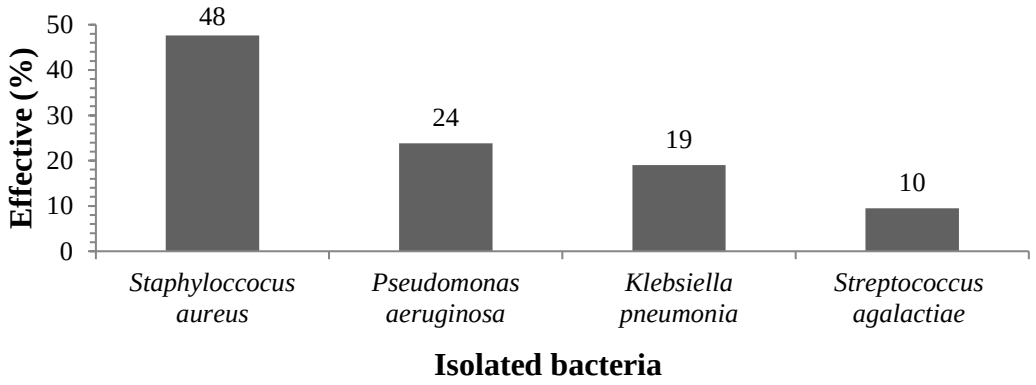

Figure 2 Frequency of bacteria isolated from diabetic wounds.

Antimicrobial susceptibility testing

The current challenge faced by the medical world is the emergence of multidrug-resistant organisms and their associated complications in developing countries [35]. Isolated bacteria were subjected to

antimicrobial susceptibility testing and the inhibition diameters are illustrated in **Table 4**. We noted that all staphylococci were multidrug resistant due to resistance to at least 3 antibiotic families, and 50 % were (5/10) methicillin-resistant *Staphylococcus aureus* (MRSA), including S. au 1, S. au 5, S. au 6, S. au 7, and S. au 10. Isolate S. au 1 was sensitive only to ciprofloxacin and vancomycin, with inhibition diameters of 25 and 20 mm, respectively. It should also be noted that *S. aureus* strains 2, 3, and 4 were sensitive to all other antibiotics tested, except amoxicillin, erythromycin, doxycycline, and gentamicin. S.au 5 was sensitive only to erythromycin, gentamicin, and vancomycin, with diameters of 20, 18, and 30 mm, respectively. S.au 6, for its part, was sensitive only to erythromycin with an inhibition diameter of 30 mm. S.au 7 was sensitive only to gentamicin and ciprofloxacin with diameters of 16 and 25 mm, respectively. Isolate S.au 8 was resistant to amoxicillin-clavulanic acid, amoxicillin, erythromycin, and ciprofloxacin, while isolate S.au 9 in turn was susceptible to ciprofloxacin, vancomycin, and imipenem with inhibition diameters of 26, 26, and 25 mm, respectively. However, isolate S.au 10 was susceptible to gentamicin, ciprofloxacin, and vancomycin with inhibition diameters of 20, 28, and 24 mm, respectively. This result is similar to [34], who reported that 96 % of *S. aureus* strains were resistant to tetracycline and methicillin, with varying degrees of resistance to other antibiotics. Regarding *P. aeruginosa*, P.ae1 and P.ae4 were sensitive to all antibiotics tested, while P.ae2 was resistant to 3 antibiotics, namely amoxicillin, gentamicin, and erythromycin, with inhibition diameters of 06, 10, and 08 mm, respectively. P.ae3 was resistant to 3 antibiotics, amoxicillin-clavulanic acid, ciprofloxacin, and doxycycline, with inhibition diameters of 8, 12, and 8 mm, respectively. And finally, P.ae5 was sensitive to doxycycline, vancomycin, and imipenem, with inhibition diameters of 19, 15, and 16 mm, respectively. It should be noted that all isolates were sensitive to vancomycin and imipenem. These results are in perfect alignment with Yakout [34], who obtained 100 % sensitivity to imipenem. This can be explained by the fact that imipenem is costly and therefore not accessible to all social classes, and the probability that bacteria will be resistant to it is very low. All *K. pneumoniae* isolates from diabetic wounds were sensitive to all antibiotics tested, except for amoxicillin and amoxicillin-clavulanic acid. However, Klebsiella are intrinsically resistant to amoxicillin so this result is not surprising. for *S. agalactiae*, Isolate S.ag1 was sensitive to all antibiotics tested, except gentamicin and amoxicillin, with inhibition diameters of 6 and 4 mm, respectively. The analysis also showed that S.ag 2 was sensitive to all antibiotics tested. The majority of the bacteria presented resistance to amoxicillin, which has already been reported in Texas [36], with 64 % resistance to *P. aeruginosa* and 100 % of *K. pneumoniae* to amoxicillin. This resistance can be explained by the inappropriate and abusive use of antibiotics, which results in mutations that confer resistance.

Table 4 Antibiotics resistance profile of bacteria isolated from diabetic wounds.

Isolates			**Antibiotics**							
			AMC	**AX**	**GN**	**E**	**CIP**	**DO**	**VA**	**IMP**
Klebsiella pneumoniae	BP	S	≥ 15	≥ 18	≥ 15	≥ 23	≥ 21	≥ 14	≥ 12	≥ 16
		I	-	14 - 17	13 - 14	14 - 22	16 - 20	11 - 13	10 – 11	14 - 15
		R	≤ 10	≤ 13	≤ 12	≤ 13	≤ 15	≤ 10	≤ 9	≤ 13
K.pn1	Diameters		08	00	16	26	25	24	18	18
K.pn2			10	06	13	28	18	22	16	20
K.pn3			05	00	16	25	22	16	15	20
K.pn4			08	04	15	20	19	14	13	15
Pseudomonas aeruginosa	BP	S	≥ 15	≥ 18	≥ 15	≥ 23	≥ 21	≥ 14	≥ 12	≥ 16
		I	-	14 - 17	13 - 14	14 - 22	16 - 20	11 - 13	10 – 11	14 - 15

Isolates			Antibiotics							
			AMC	**AX**	**GN**	**E**	**CIP**	**DO**	**VA**	**IMP**
		R	≤ 10	≤ 13	≤ 12	≤ 13	≤ 15	≤ 10	≤ 9	≤ 13
P.ae1			15	20	18	20	25	16	15	22
P.ae2			20	06	10	08	25	16	15	25
P.ae3	Diameters		08	20	18	14	12	08	13	20
P.ae4			22	20	20	24	28	17	13	18
P.ae5			06	00	08	10	13	19	15	16
		S	≥ 29	≥ 29	≥ 15	≥ 23	≥ 21	≥ 16	-	≥ 23
Staphylococcus aureus	BP	I	-	-	13 - 14	14 - 22	16 - 20	13 - 15	-	22 - 17
		R	≤ 28	≤ 28	≤ 12	≤ 13	≤ 15	≤ 12	-	≤ 16
S.au 1			10	00	00	10	25	00	20	10
S.au 2			30	16	10	05	22	07	26	25
S.au 3			30	08	00	06	22	08	26	23
S.au 4			30	00	10	08	26	00	30	28
S.au 5	Diameters		12	10	18	20	14	08	30	10
S.au 6			10	00	00	30	08	01	12	12
S.au 7			08	10	16	01	25	01	10	15
S.au 8			00	12	15	10	08	29	28	24
S.au 9			10	15	12	01	26	01	26	25
S.au 10			14	12	20	05	28	00	24	14
		S	-	-	-	≥ 21	-	≥ 23	≥ 17	-
Streptococcus agalactiae	BP	I	-	-	-	16 - 20	-	19 - 22	-	-
		R	-	-	-	≤ 15	-	≤ 18	-	-
S.ag 1	Diameters		24	04	06	24	25	26	22	20
S.ag 2			26	24	24	32	22	28	29	25

BP = Breaking Point, S.au = *Staphylococcus aureus*, S.ag = *Streptococcus agalactiae*, P.ae = *Pseudomonas aeruginosa*, K.pn = *Klebsiella pneumoniae*, S = Susceptible, R = Resistant, I = Intermediate, AX = amoxicillin, AMC = amoxicillin- clavulanic acid, IMP = imipenem, GN = gentamycin, VA = vancomycin, DO = doxycycline, E = erythromycin, CIP = ciprofloxacin.

***In vitro* antibacterial activity of *Eriosema robustum* extracts on bacteria isolated from diabetic wounds**

Plants have always occupied a place in the treatment of many infectious diseases [37]. The results of the antibacterial activity of hydroethanolic (Heth) at 30, 70, and 90 ° and aqueous extract of *E. robustum* leaves were evaluated by determining the MIC and MBC against different bacterial strains and those isolated from diabetic wounds at the Baleveng Wound Hospital, as presented in **Tables 5** and **6**. We noted that all extracts from the leaves of *E. robustum* had antibacterial activity against at least one isolate tested, with MICs ranging from 64 to 1,024 µg/mL while the MBCs were between 256 and 1,024 µg/mL. In general, all extracts showed bactericidal activity (antibacterial power equal to MBC/MIC) against all tested bacteria (MBC/MIC ≤ 4) [38]. Considering the different antibacterial activities of *K. pneumoniae*, all the extracts were active against all the isolates tested. The MIC results vary between 128 and 1,024 µg/mL. Heth 70 ° extract showed the most effective activity (MIC = 128 - 512 µg/mL), with the lowest MIC (128 µg/mL) obtained for K.pn4. Antibacterial activity against *P. aeruginosa* showed varying MICs between 64 and 1,024 µg/mL, with the most effective MIC of 64 µg/mL observed for the Heth 70 ° extract of P. ae1. For the Heth 90 ° extract, the MICs were 128, 512, and 512 µg/mL against P.ae1, P.ae2, and P.ae5, respectively. The hydroethanolic extract of *E. robustum* leaves was found to be highly active against the reference strains K. pn ATCC 10031 and isolates S.au1, S.ag1, and P.ae1, with a MIC of 64 µg/mL. It also demonstrated significant activity against all tested strains and isolates, including S.au2, K.pn1, K.pn3, K.pn4, S.ag4, S.au5, S.ag6, S.au8, and S.ag9, with MICs ranging from 128 to 512 µg/mL. This may be attributed to the extraction solvent's polarity, which allows for the extraction of bioactive components that water cannot. The antibacterial activity of *E. robustum* was confirmed in Cameroon by Awouafack *et al.* [11], who showed that the ethanolic extract of *E. robustum* stems was highly active against *S. aureus* (20 µg/mL) and significantly active (310 µg/mL) against *P. aeruginosa* [39]. The stems' antibacterial activity can be compared to that of the leaves, as secondary metabolites are not equally distributed in the plant and may be more abundant in some parts than others. Previous studies on plants of the genus Eriosema have also shown interesting antibacterial properties. The ethanolic extracts of *E. chinense* roots showed significant antibacterial activity against *S. aureus*, *P. aeruginosa* and *K. pneumoniae* [40]. The methanol extract of *E. glomeratum*'s stems and leaves demonstrated antibacterial properties against *S. aureus*, *K. pneumoniae,* and Mycobacterium aurum, with MICs ranging from 50 to 1,000 µg/mL [41]. These findings indicate the richness of antibacterial molecules in plants of the genus Eriosema. All the extracts exhibited bactericidal activity against isolates of *S. aureus*, *P. aeruginosa*, and, *S. agalactiae*. This may partly explain the plant's use in traditional medicine to treat ailments such as cough and skin diseases [10,11].

Table 5 Antibacterial activity of *E. robustum* extracts against bacteria isolated.

Isolates	Parameters	Extracts (µg/mL)			
		Aqueous	Heth (30 °)	Heth (70 °)	Heth (90 °)
K.pn1	MIC	1,024	512	512	512
	MBC	NA	1,024	512	512
	AP	NA	2	1	1
K.pn2	MIC	1,024	1,024	256	512
	MBC	1,024	NA	1,024	1,024
	AP	1	NA	4	2
K.pn3	MIC	512	1,024	256	512
	MBC	512	NA	512	1,024
	AP	1	NA	2	2
K.pn4	MIC	1,024	256	128	512
	MBC	NA	512	512	1,024
	AP	NA	2	4	2

Isolates	Parameters	Extracts (µg/mL)			
		Aqueous	**Heth (30 °)**	**Heth (70 °)**	**Heth (90 °)**
P.ae1	MIC	512	1,024	64	128
	MBC	1,024	NA	256	256
	AP	2	NA	4	2
P.ae2	MIC	1,024	1,024	128	512
	MBC	1,024	1,024	512	512
	AP	1	1	4	1
P.ae3	MIC	NA	1,024	512	NA
	MBC	NA	1,024	1,024	NA
	AP	NA	1	2	NA
P.ae4	MIC	NA	1,024	512	NA
	MBC	NA	NA	1,024	NA
	AP	NA	NA	2	NA
P.ae6	MIC	512	1,024	128	512
	MBC	512	1,024	256	1,024
	AP	1	1	2	2
S.au1	MIC	NA	512	64	128
	MBC	NA	1,024	256	512
	AP	NA	2	4	4
S.au 2	MIC	NA	1,024	128	512
	MBC	NA	NA	512	1,024
	AP	NA	NA	4	2
S.au 3	MIC	1,024	256	1,024	256
	MBC	NA	1,024	1,024	512
	AP	NA	4	1	2
S.au 4	MIC	NA	256	256	NA
	MBC	NA	512	512	NA
	AP	NA	2	2	NA
S.au 5	MIC	1,024	1,024	128	128
	MBC	1,024	NA	256	512
	AP	1	NA	2	4
S.au 6	MIC	1,024	256	256	256
	MBC	1,024	512	512	512
	AP	1	2	2	2
S.au 7	MIC	NA	NA	1,024	1,024
	MBC	NA	NA	1,024	1,024
	AP	NA	NA	1	1
S.au 8	MIC	1,024	512	512	1,024

Isolates	Parameters	Extracts (µg/mL)			
		Aqueous	Heth (30 °)	Heth (70 °)	Heth (90 °)
	MBC	1,024	512	NA	NA
	AP	1	1	NA	NA
S.au 9	MIC	1,024	NA	512	NA
	MBC	1,024	NA	1,024	NA
	AP	1	NA	2	NA
S.au 10	MIC	512	256	512	256
	MBC	1,024	512	1,024	512
	AP	1	2	2	2
S.ag 1	MIC	1,024	512	64	128
	MBC	1,024	1,024	256	512
	AP	1	2	4	4
S.ag 2	MIC	512	1,024	128	512
	MBC	1,024	NA	512	512
	AP	2	NA	4	1

MIC = Minimum Inhibitory Concentration, MBC = Minimum Bactericidal Concentration, AP = Antibacterial Power, S.au = *Staphylococcus aureus*, S.ag = *Streptococcus agalactiae*, P.ae = *Pseudomonas aeruginosa*, K.pn = *Klebsiella pneumoniae.*, Heth = Hydroethanolic, NA = no activity.

Table 6 Antibacterial activity of *E. robustum* leaf extracts against reference strains.

Strains	Parameters	Extracts (µg/mL)				Antibiotic (µg/mL)
		Aqueous	Heth (30 °)	Heth (70 °)	Heth (90 °)	Ciprofloxacin
K.pn ATCC 10031	MIC	1,024	512	64	64	1
	MBC	NA	1,024	512	256	
	AP	NA	2	4	4	
P.ae ATCC 27853	MIC	NA	1,024	256	512	4
	MBC	NA	NA	512	1,024	
	AP	NA	NA	2	2	
S.au ATCC 25923	MIC	512	1,024	128	256	2
	MBC	NA	NA	512	512	
	AP	NA	NA	4	2	
S.au ATCC 29213	MIC	512	512	256	256	4
	MBC	512	512	512	512	
	AP	1	1	2	2	

MIC = Minimum Inhibitory Concentration, MBC = Minimum Bactericidal Concentration, AP = Antibacterial Power, S.au = S*taphylococcus aureus*, S.ag = *Streptococcus agalactiae*, P.ae = *Pseudomonas aeruginosa*, K.pn = *Klebsiella pneumoniae.*, Heth = hydroethanolic, NA = no activity.

Phytochemicals analysis

The current literature offers no information regarding the phytochemical characteristics that exhibit antioxidant activity through the DPPH test, FRAP, total flavonoids, total tannins, and total phenols. As of yet, no HPLC analysis has been conducted on the fractions of the 70 ° hydroethanolic extract of *E. robustum* leaves. Thus, the data obtained in this study represents an initial source of information.

Antioxidant activities

Study of antiradical activity using DPPH test and FRAP test

Antioxidants play a crucial role in the body's defense system against reactive oxygen species (ROS), which are harmful by products generated during normal cell aerobic respiration [42]. Additionally, various environmental stress factors, such as pollution, drought, temperature, excessive light intensities, and nutritional limitation, can increase the production of ROS [43]. **Table 7** shows the results of the DPPH and FRAP anti free radical tests conducted on various *E. robustum* extracts, with vitamin C serving as the reference molecule in both tests. Our findings indicate that *E. robustum* exhibits DPPH antiradical activity ranging from 11.31 ± 0.19 to 27.92 ± 0.29 µg/mL for the different extracts tested, and FRAP antiradical activity ranging from 114.28 ± 0.85 to 137.64 ± 0.15 mmol FeSO4/g for the different extracts tested. With vitamin C displaying antiradical capacity of 2.19 ± 0.11µg/mL for DPPH and 160.52 ± 0.55 mmol FeSO4/g for FRAP. The Heth 70 ° extract showed the lowest antioxidant activity using the DPPH test with an EC50 of 27.92 ± 0.29 µg/mL, while the aqueous extract showed the lowest antioxidant activity using the FRAP test with a value of 114.28 ± 0.85 mmol FeSO4/g. The results obtained from the DPPH test in this study confirm the scavenging activity of different *E. robustum* leave extracts and can be compared to those obtained by Awouafack *et al.* [11], using the twigs of *E. robustum*. Furthermore, the *E. chinense* Roots/Heth extract FRAP assay showed a result of 0.263 µg/mL and DPPH assay, with IC50 values of 146 µg/mL [40]. The different antioxidant activity of all the extracts can be justified by the presence of bioactive compounds such as phenolic acid and flavonoids, as well as the age of the plant, with younger plants containing more antioxidants than older ones.

Table 7 Antioxydant activity of *E.robustum* extract using the FRAP and DPPH methods.

Plant extract	FRAP Test (mmol $FeSO_4$/g)	DPPH EC_{50} (µg/mL) Test
HEth 30 °	137.64 ± 0.15^{d}	13.67 ± 0.19^{c}
HEth 70 °	123.62 ± 0.52^{c}	27.92 ± 0.29^{e}
HEth 90 °	115.73 ± 0.23^{b}	11.31 ± 0.33^{b}
Aqueous extract	114.28 ± 0.85^{a}	21.74 ± 0.25^{d}
Vitamin C	160.52 ± 0.55^{e}	2.19 ± 0.11^{a}

The values assigned to the letters (a, b, c, d, and e) are significantly different at the 5 % probability threshold. Each value represents the mean ± standard deviation. HEth = Hydro-ethanolic.

Phytochemical characteristic of plant extracts

Various studies have shown that phenolic compounds have high antioxidant and antimicrobial potential, resulting in a beneficial effect to human health [44]. Quantitative chemical analysis helped us to determine the content of total flavonoids, phenols, and tannins in the different *E. robustum* extracts. The contents of the different groups of phytochemical compounds in the 4 plant extracts tested are presented in **Table 8**. There was a significant difference in the total phenol content between all extracts. The aqueous extract of *E. robustum* leaves had a significantly higher total phenol content ($p < 0.05$) than all other extracts. Then comes the 70 ° hydroethanolic extract. On the other hands, there was no significant difference in the total tannin content of the extracts ($p \geq 0.05$). Concerning flavonoids contents, the 70 ° hydroethanolic extract of *E. robustum* leaves presented significantly higher total flavonoid content ($p < 0.05$) than the other extracts. The 90 ° hydroethanolic and aqueous extracts showed no significant differences, and the 30 ° hydroethanolic extract had the lowest content. The presence of flavonoids, Tannins and phenols in the various extracts could be responsible for the various antioxidant and antibacterial activity reported in this present study.

Table 8 Quantitative composition of flavonoids, tannins, and phenols in *E. robustum* leaves.

Extracts	Flavonoids content (mg EQ/g d'extrait)	Tannins content (mg EQ/g d'extrait)	phenols content (mg EQ/g d'extrait)
HEth 30 ° extract	4.38 ± 0.33^{a}	1.94 ± 0.62^{a}	13.24 ± 0.16^{a}
HEth 70 ° extract	7.46 ± 0.12^{c}	2.09 ± 0.13^{a}	16.51 ± 0.34^{c}
HEth 90 ° extract	5.72 ± 0.34^{b}	2.25 ± 0.26^{a}	14.81 ± 0.25^{b}
Aqueous extract	5.53 ± 0.44^{b}	2.12 ± 0.34^{a}	22.24 ± 0.54^{d}

Values assigned to the letters (a, b, c, and d) are significantly different at the 5 % threshold. Each value represents the mean ± standard deviation. HEth = hydro-ethanolic.

Identification of phenolic compounds by HPLC in different fractions of the 70 ° hydro-ethanolic extract of *E.robustum* leaves

Many studies on other species in the Eriosema genus have found that flavonoids are responsible for their biological activity. In some cases, researchers have discovered new flavonoids, such as Kraussianones 1 - 7, found in the roots of *E. kraussianum* [45], Erioschalcones A and B, isolated from *E. glomerata* [46], Robusflavones A and B, extracted from the aerial parts of *E. robustum* [11], and Khonklonginols A - H, isolated from the roots of *E. chinense* [47]. The possibility of *E. robustum* containing novel molecules supports the need for further studies on its bioactive compounds. Unlike previous studies, our work focused on identifying polyphenols in different fractions of the 70 ° hydro-ethanolic extract of *E. robustum* leaves using HPLC, making this study the first of its kind. **Table 9** provides information on the different phenolic compounds (8 in total) obtained from the analysis of the different fractions of the 70 ° hydroethanolic extract of the *E. robustum* leaves. **Figure 3** shows the presence of 3 phenolic compounds identified with different retention times (RT) in the chloroform fraction: Ascorbic acid (1.76 min), gallic acid (2.41 min), and kaempferol. (15.94 min). The unique phenolic compound identified in the ethyl acetate fraction was ascorbic acid (1.78 min) (**Figure 4**). Ascorbic acid (1.75 min), caffeic acid (9, 47 min), quinoline (11.94 min), coumarin (12.30 min), and cinnamic acid (15.15 min) were present in the n-butanol fraction (**Figure 5**). Ascorbic acid (1.74 min), vanillin (10.17 min), and quinoline (11.85 min) were detected in the aqueous fraction (**Figure 6**). The presence of potential antimicrobial and antioxidant substances was demonstrated by phytochemical analysis of total tannins, total flavonoids, and total phenols (**Table 8**). Antimicrobial activity of medicinal plants is correlated with the presence of one or more classes of bioactive secondary metabolites in their extracts [48].

Table 9 Identification of Phenolic compounds by HPLC in different fractions of the 70 ° hydroethanolic extract of *E. robustum* leaves.

Fractions	N ° peak	Retention time (min)	% in fraction (µg/100µg)	Concentration (µg/mg extract)	References in retention	Compound names
Chloroform	1	1.76	0.89	0.9	1.78	Ascorbic acid
	2	2.41	0.23	0.23	2.40	Gallic acid
	3	15.94	0.46	0.47	15.90	Kaempferol
Ethyl acetate	1	1.78	0.18	0.16	1.78	Ascorbic acid
n- Butanol	1	1.75	1.14	4.45	1.78	Ascorbic acid
	2	9.47	17.66	68.64	9.55	Caffeic acid
	3	11.94	6.81	26.46	11.90	Quinoline
	4	12.30	8.34	32.41	12.34	Coumarine
	5	15.15	2.2	8.5	15.19	cinnamic acid
Aqueous	1	1.74	5.67	23.28	1.78	Ascorbic acid
	2	10.17	7.69	31.58	10.22	Vanillin
	3	11.85	1.78	7.33	11.90	Quinoline

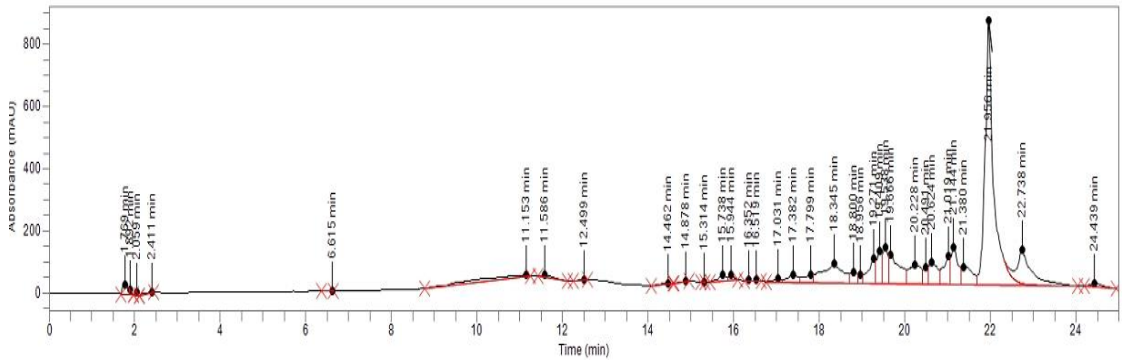

Figure 3 HPLC profile of polyphenols present in the chloroform fraction of 70 ° ethanolic extract of *E.robutum* leaves.

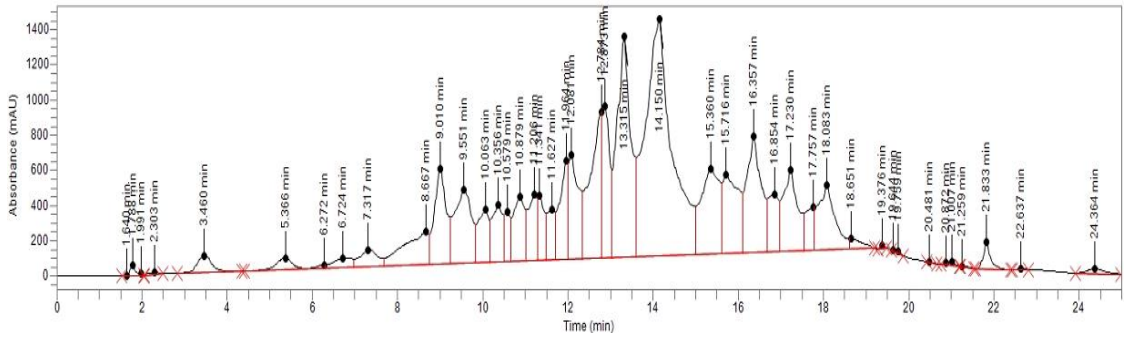

Figure 4 HPLC profile of polyphenols present in the acetate ethyl fraction of 70 ° ethanolic extract of *E.robutum* leaves.

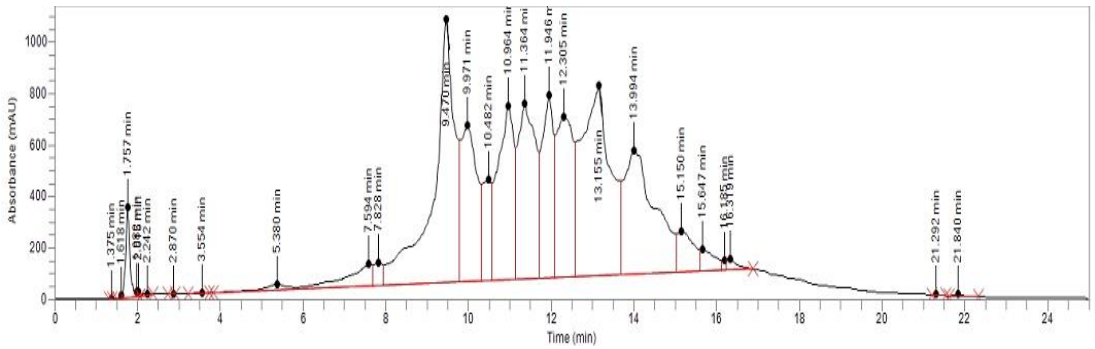

Figure 5 HPLC profile of polyphenols present in n-butanol fraction of 70 ° ethanolic extract of *E.robutum* leaves.

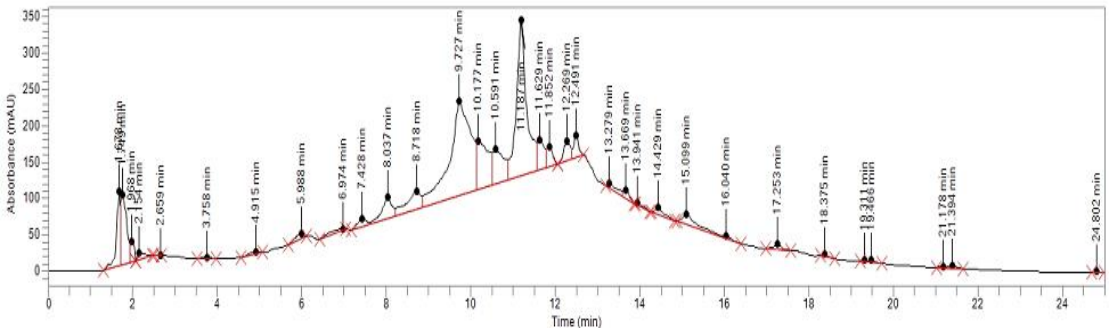

Figure 6 HPLC profile of polyphenols present an in aqueous fraction of 70 ° ethanolic extract of *E.robutum* leaves.

Conclusions

This study demonstrated that many of the bacteria isolated from diabetic wounds were multidrug-resistant which may result in prolonged debility of the patient and increased healthcare costs. The various extracts of *E. robustum* displayed antibacterial activity against different reference strains and isolates from diabetic wounds, ranging from 64 to 1,024 µg/mL, confirming its traditional use in treating various health issues. The phytochemical analysis also revealed the presence of numerous bioactive metabolites, including total tannins, phenols, and flavonoids. Additionally, the HPLC analysis showed that the HEth 70 ° extract fraction of *E. robustum* leaves contained several phenolic compounds, such as coumarin, gallic acid, quinoline, vanillin, ascorbic acid, caffeic acid, kaempferol, and cinnamic acid, which may be responsible for the plant's antioxidant and antibacterial properties. These findings suggest new opportunities for *in vivo* studies on the antibacterial and wound healing activities of *E. robustum* in diabetic wounds in rats.

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