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In vitro antibacterial activity of *Baillonella toxisperma* (Pierre) extracts against *Staphylococcus aureus*, *Salmonella typhi*, *Proteus mirabilis* and *Bacillus cereus* F3748

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This study evaluated the *in vitro* antibacterial activity of the ethyl acetate, acetone, methanol and hydro-ethanol mixture (2: 8) extracts of the leaves and stem-barks of *Baillonella toxisperma* (Pierre), harvested in the East and center regions of Cameroon, on *Staphylococcus aureus*, *Salmonella typhi*, *Proteus mirabilis* and *Bacillus cereus* F3748. These bacteria are usually responsible for diarrheal diseases and in severe cases can lead to the dead of patients. The susceptibility of the bacteria to the extracts was evaluated by the well diffusion method and the inhibition parameters of the bacterial growth were determined by the micro-dilution assay according to the directives of document M27-A9 (2012) of the Clinical and Laboratory Standards Institutes (CLSI). The Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal concentrations (MBC) obtained were between 1.56 and 25.00 mg/ml. Stem-barks ethyl acetate extract from the East region, was most active on *S. aureus*, *S. typhi* and *P. mirabilis* with a MBC of 6.25 mg/ml. The leaves methanolic extracts from the center region was the most active with a MBC of 6.25 mg/ml on *S. aureus*. The ratio MBC/MIC shows that the majority of the extracts were bacteriostatic on the strains tested. The phytochimical screening revealed that the plant contained bioactive substances such as phenols, tannins, flavonoids, steroids, alkaloids, saponins, triterpenes and cardiac glycosides, reported by several authors for their antibacterial activity. The results obtained validate the traditional use of this plant in the treatment of affections of bacterial origin.

Key words: Cameroon, *Baillonella toxisperma* (Pierre), bioactive substances, antibacterial activity.

INTRODUCTION

Bacterial infections constitute a serious public health problem in the world. Among the causative agents, *Staphylococcus aureus*, *Salmonella typhi*, *Proteus mirabilis* and *Bacillus cereus* are cited in most clinical cases. *S. aureus* is one of the principal causes of food toxi-infections which are characterized by a severe appearance of diarrhoea. It is the almost-universal cause of furuncles, carbuncles, and skin abscesses and worldwide is the most commonly identified agent responsible for skin and soft tissue infections (McCaig et al., 2006). *S. typhi* is the causative agent of typhoid fever and diarrhoea. *B. cereus* is responsible for food poisoning and diarrheal syndromes

(Logan and Rodrigez-Diaz, 2006). *P. mirabilis* on its part is responsible for urinary tract, cutaneous, respiratory tract infections, septicemia and bacteremia (Ronald, 2003). These bacterial infections can in extreme cases, lead to the dead of the patient. Moreover, these bacteria have over time developed resistance to certain usual antibiotics. It is the case of *B. cereus* which has become resistant to penicillin, ampicillin, cephalosporines and trimethoprimes (Murray et al., 2007). There is an imperative need for the research and renewal of active ingredients which have become ineffective due to the emergence of the phenomenon of microbial multi-resistance to common antibiotics.

Medicinal plants via their secondary metabolites constitute a potential source of antimicrobial (Li et al., 2007). Many scientific studies have been undertaken in order to study the botanical and therapeutic aspects of the latter and to integrate their medicinal properties in a modern health system by exploiting their active ingredients (Biyiti et al., 2004). Many bioactive compounds isolated from plants such as flavonoids, phenolic alkaloids, saponins, tannins, coumarins, phenolic acids and terpenes, were been used for a long time as active ingredients in the development of anti-infectious drugs (Ghost et al., 2007).

Baillonella toxisperma (Pierre) is a plant of the Cameroonian pharmacopeia, traditional hailed for its medicinal virtues. Commonly called Moabi, this plant is used in treating more than 50 diseases among which are microbial infections (Laird, 2000). *B. toxisperma* (Pierre) develops in hot tropical forests and under wet climates (Louppe, 2005; Angerand, 2006). In Cameroon, it is found abundantly in the East and South regions. This plant is used traditionally to treat infections of microbial origin such as mycoses, rheumatism, hemorroides, diarrheal diseases, sexually transmissible diseases (Dibong et al., 2011; Ngueguim et al., 2009). In this respect, it constitutes a potential source of anti-infectious compounds. With the aim of valorizing this plant, we proposed in this study to evaluate the *in vitro* antibacterial activity of its extracts against *S. aureus*, *S. typhi*, *P. mirabilis* and *B. cereus* F3748. In order to determine the influence of the harvest site on the antimicrobial activity, we used botanical materials from
two regions: the East and Center regions of Cameroon.

MATERIALS AND METHODS

Preparation of the leaves and stem-barks extracts of *Baillonella toxisperma* (Pierre)

Extracts of the botanical material were extracted according to the protocol described by Prakash and Gupta (2005). The leaves and stem-barks were cut out into scraps then, dried at ambient temperature, free from moisture and light. The dried plant materials were finely crushed using an electric blender. The powder obtained was macerated in four solvents: ethyl acetate, acetone, methanol and ethanol-water (8: 2). 100 g of powdered stem-barks and leaves were macerated in 500 ml of each solvent for 48 h. The marcs obtained were filtered through Whatman N° 1 filter paper and the filtrates collected in conical flasks. This process was repeated thrice for complete exhaustion of the plant material and the filtrates obtained were concentrated in a rotarvapor. The dry extracts were preserved at +4°C in a refrigerator. The extraction yields expressed in percentage (%) were determined by the formula below:

Yield (%) = (Mass of macerated powder / Mass of the extract) × 100.

Phytochemical screening

Determination of the phytochemical composition of the various extracts was carried out according to standard methods described by Harbone (1998) and Sofowora (1993).

Preparation of the bacterial inoculum

For each tested micro-organism, overnight cultures of bacterial colonies seeded on Mueller Hinton Agar (Fortress Diagnostics Limited U.K) and incubated at 37°C were suspended in 5 ml saline water in test tubes. This suspension was read thereafter with a spectrophotometer at 625 nm. When the optical density was between 0.08 and 0.13, the bacterial load was 10^8 CFU/mL (0.5 McFarland). After a 100^{th} dilution, the bacterial load was 10^6 CFU/ml (Hernandez et al., 2000).

Preliminary sensitivity test of the strains to the extracts

The preliminary tests of sensitivity of the bacterial strains to the various extracts were carried out as recommended by CLSI (2005). 100 µl of each bacterial inoculum was inoculated on Mueller Hinton agar (Fortress Diagnostics Limited U.K). The Petri dishes were then allowed to dry at ambient temperature under a fumes cupboard for 15 min. 6 mm wells were bored in the agar and the bottom of each well plugged with a drop of Mueller Hinton agar to limit the diffusion of the extracts from below. Fixed volumes of 50 µl of the stock solutions of the extracts (50 mg/ml) and gentamicin (1 mg/ml) were then introduced into each well. After a pre diffusion time of 15 min of the antibacterial substances to be tested at ambient temperature, the Petri dishes were incubated at 37°C for 24 h. The inhibition diameters round each well was measured using a sliding caliper. Each test was carried out in triplicate and the inhibition diameters expressed mean ± standard deviation.

Determination of the inhibition parameters: MIC and MBC

The inhibition parameters of bacterial growth were evaluated according to the M27-A9 guideline described by CLSI (2012). This involved preparing double dilutions of tested substances in 100 µL of glucose supplemented nutrient broth (GNB) medium (Acumedia Manufacturers) into the wells of a microtiter. The range of final

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Table 1. Extraction yields and physico-chemical characteristics of leaves and stem-barks extracts of *Baillonella toxisperma* (Pierre).

Sites of harvest	Extraction Solvents	Parts of the plant	Extraction yield (%)	Physical properties of the extracts	
				Color	State
Dimako (East Region -Cameroon) 21/01/15	Hexane	Stem-barks	2,42	Yellow	Fatty
		Leaves	2,59	Dark-green	Pasty
	Ethyl-acetate	Stem-barks	1,53	Brown	Powdery
		Leaves	6,24	Dark-green	Pasty
	Methanol	Stem-barks	14,02	Thick-red	Pasty
		Leaves	8,88	Dark-green	Pasty
	Ethanol-water (8/2)	Stem-barks	18,34	Thick-red	Powdery
		Leaves	13,95	Brown	Pasty
	Acetone	Stem-barks	8,32	Red	Powdery
		Leaves	7,92	Dark-green	Pasty
Mbalmayo (Center Region-Cameroon) Le 09/12/14	Hexane	Stem-barks	2,75	Yellow	Fatty
		Leaves	3,16	Dark-green	Pasty
	Ethyl-acetate	Stem-barks	1,08	Yellowish	Powdery
		Leaves	2,40	Dark-green	Pasty
	Methanol	Stem-barks	4,72	Brown	Pasty
		Leaves	6,80	Light-green	Pasty
	Ethanol-water(8/2)	Stem-barks	6,85	Thick-red	Powdery
		Leaves	10,7	Greenish	Powdery
	Acetone	Stem-barks	4,10	Red	Powdery
		Leaves	4,57	Dark-green	Pasty

concentrations tested were 25 to 0,097 mg/ml for each plant extract and 0,250 to 0,00097 mg/ml for gentamicin (Brunhild Pharmaceutical Private Limited). Each serial dilution was performed in triplicate. The bacterial inoculum was prepared at 10^6 CFU/mL using McFarland. Volumes of 100 µL of this inoculum were distributed to all the wells of the microtiter. A line of the plate without plant extract served as a control for the growth of the organism (negative control) and another (without plant extract and without inoculum) served as sterility testing medium (positive control). The microtitre plates were thereafter sealed with aluminum foil and incubated at 37°C for 24 h. After incubation, 40 µl of 2,3,5-triphenyl tetrazolium chloride (Sigma-Aldrich) (0,2 mg/mL) were introduced into each well (Burdock et al., 2011) . The MIC was defined as the smallest concentration of the extract for which there was no change in the initial yellowish color of the medium to red. The MBC were determined by subculture. 50 µL of the contents of wells greater than or equal to the MIC was introduced into 150 µL of fresh GNB. The microtitre plates were incubated for 48h at 37°C, thereafter revealed as earlier done. The smallest concentration for which no color change was observed was regarded as the minimum bactericidal concentration.

RESULTS

Extraction yield

The extraction yield of the leaves and stem-barks of *B. toxisperma* (Pierre) are shown in Table 1. It is observed that the extraction yields are comprised between 1.08 % (stem-barks ethyl acetate) and 10.07% (leaves hydro-ethanolic extract) for the plant material harvested in the Center and between 1.53 % (stem-barks ethyl acetate) and 18.34% (hydro-ethanolic stem-barks) for the plant material harvested in the East region.

Phytochemical screening

The phytochemical screening revealed the presence of several groups of secondary metabolites such as tannins, the flavonoids, steroids, saponins, terpenoids and phenols in both extracts of *Baillonella toxisperma* (Pierre) harvested from the East and Center regions of Cameroon. Table 2 summarizes the results obtained from the screening depending on the extracts considered.

Susceptibility test

The results obtained from the susceptibility test (Table 3) show that the bacterial strains were sensitive to the leaves and stem-barks extracts of *B. toxisperma* (Pierre). For the plant material harvested in the East, the inhibition diameters of the leaves crude extracts was between 8.66 ± 0.57 mm (hydro-ethanolic extract on *B cereus*) and 11.33 ± 0.57 mm (methanolic extract on *B cereus*), and for the botanical material harvested in the Center, the inhibition diameters ranged from 9.00 ± 1.00 mm (acetone extract) to 11.66 ± 0.57 mm (methanolic extract on *S. typhi*). The inhibition diameters of the stem-barks crude extracts was comprised between 9.66 ± 0.57 mm (hydro-ethanolic extract on *S. aureus*) and 19.66 ± 0.57 mm

Table 2 . Phytochemical screening.

Phytochemical groups	Plant extract															
	East-region								Center-region							
	E1	F1	E2	F2	E3	F3	E4	F4	E'1	F'1	E'2	F'2	E'3	F'3	E'4	F'4
Phenols	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tanins	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Steroïds	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+
Triterpenes	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-
Saponines	+	-	+	+	+	+	+	+	+	-	+	+	+	+	+	+
Cardiacglycosids	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-
Alcaloids	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

E1 and F1, Acetone and ethyl acetate stem-barks and leaves from the East; E2 and F2, Methanol stem-barks and leaves from the East; E3 and F3, hydro-ethanolic stem-barks and leaves from the East ; E4 and F4, acetone stem-barks and leaves extracts from the East; E'1 and F'1, ethyl acetate stem-barks and leaves from the Center; E'2 and F'2, Methanol stem-barks and leaves extract from the Center; E'3 and F'3, Hydro-ethanolic extracts from the Center; E'4 and F'4, Acetone stem-barks and leaves from the Center; +, Presence of compound; -, absence of compound.

(acetone extract on *S. typhi*) for the plant material harvested in the East and between 7.33± 0.57 mm (ethyl acetate and acetone extracts on *S. aureus* and *P. mirabilis*) and 17.00 ± 0.00 mm (acetone extract on *S. typhi*) for the plant material harvested in the Center.

Determination of the inhibition parameters

The results obtained for the inhibition parameters (Table 4) show that the MIC of the plant material from the East was comprised between 1.56 and 25 mg/ml and that of the Center, between 3.12 and 25 mg/ml. The MBC was between 6.25 and 25 mg/ml. According to Fauchère and Avril (2002) when the MBC of an antibiotic on a given strain is close to the MIC (1 ≤ MBC/MIC ≤ 2), the antibiotic is described as being bactericidal. On the other hand, when these values are relatively distant, (4 ≤ MBC/MIC ≤ 16), the antibiotic is known to be bacteriostatic. Lastly if the MBC/MIC >16, it is described tolerant.

DISCUSSION

The extraction yields of the leaves and barks show that the extraction yields (Table 1) were between 1.08% (ethyl acetone stem-barks extracts) and 10.07 % (Hydro-ethanolic leaves extracts) for the plant material from the Center region and between 1.53 % (ethyl acetate stem-barks extracts) and 18.34% (Stem-barks ethanol-water extract) for the plant material collected in the East region. For the same solvent and plant organ (leaves or bark), variations in the extraction yields could be due to edaphic and climatic factors. Globally, methanol and hydro-ethanol extracts gave the best extraction yields. This could be explained by the fact that the secondary metabolites extracted are more soluble in alcohols (Bruneton, 1999).

Results obtained from the phytochemical screening (Table 2) of the extracts of *B. toxisperma* (Pierre) show that this plant is endowed with secondary metabolites such as phenols, saponins, tannins, flavonoids, triterpenes, steroids and cardiac glycosids. These bioactives substances have been reported by several authors for their antibacterial activity. These bioactive compounds have long been used in modern medicine for drug developement (Dawang and Datup, 2012). Several molecules isolated from plants such as pinocembrine, ponciretine, sophora flavanone G and naringine significantly showed antimicrobial activities in both Gram positive and Gram negative bacteria (Tim and Andrew, 2005). For the same solvent and the same plant organ (leaves or bark), variations in the phytochemical composition were observed. This could be due to ecological parameters, which generally differ from one area to another depending on geographic distant. These differences can strongly influence the biology and the physiology of the plants, in particular their composition in secondary metabolites (Etchiké *et al.*, 2011).

The results obtained from the susceptibility test (Table 3) show that at a concentration of 50 mg / ml, the inhibition diameters of the bacterial growth were between 6.66 ± 0.57 and 19.66 ± 0.57 mm. For a given strain, these inhibition diameters were however lower than those of gentamicin (13.00 ± 0.00 to 28.66 ± 0.57 mm). The distinct sensitivity of the strains with regards to the extracts could be due to the intrinsic features specific to each micro-organism (permeability of the cell wall, presence of an external membrane) and with the phytochemical profile of the extracts (Takeo et al., 2004; Achraf et al., 2012). The Gram positive bacteria (*S. aureus* and *B. cereus*) were more sensitive to the toxic effect of the extracts than their Gram negative (*S. typhi*

Table 3. Susceptibility test.

Bacterial strains	Inhibition diameters (mm)																Gen
	East-region										Center-region						
	E1	F1	E2	F2	E3	F3	E4	F4	E'1	F'1	E'2	F'2	E'3	F'3	E'4	F'4	
Bacillus cereus	15.33± 0.57	/	16.66 ± 0.57	11.33 ± 0.57	16.00± 0.00	8.66± 0.57	16.00± 0.00	10.33± 0.57	13.33± 0.57	/	14.00± 1.00	10.00± 1.00	14.00± 0.00	10.33± 0.57	13.33± 0.57	9.33± 0.57	23.33± 0.57
Staphylococcus aureus	12.00 ± 0.57	/	10.33 ± 0.57	/	9.66 ± 0.57	/	10.00± 1.00	/	10.33± 0.57	/	6.66 ± 0.57	/	/	/	7.33 ± 0.57	10.33± 0.57	13.00± 0.57
Salmonella typhi	18.66 ± 0.57	/	18.00 ± 1.00	11.00 ± 0.00	19.00 ± 1.00	/	19.66± 0.57	11.00± 0.00	11.00± 0.00	/	13.66± 1.15	10.33± 0.57	13.66± 0.57	11.66± 0.57	17.00± 0.00	9.00 ± 1.00	28.66± 0.57
Proteus mirabilis	15.00 ± 1.00	/	10.33 ± 0.57	/	14.00 ± 1.00	/	14.33± 0.57	/	7.33 ± 1.15	/	10.66± 0.57	/	11.33± 1.15	/	10.66± 0.57	/	15.00± 1.00

E1 and F1, Ethyl acetate stem-barks and leaves from the East; E2 and F2, Methanol stem-barks and leaves extracts from the East; E3 and F3, Hydro-ethanolic stem-barks and leaves from the East; E4 and F4, Acetone stem-barks and leaves extracts from the East; E'1 and F'1, Ethyl acetate stem-barks and leaves extracts from the Center; E'2 and F'2, Methanol stem-barks and leaves extracts from the Center; E'3 and F'3, Hydro-ethanolic stem-barks and leaves extracts from the Center; E'4 and F'4, Acetone stem-barks and leaves extracts from the Center; Hu ,Oil; Gen, Gentamicin.

and P. mirabilis) counterparts. This could be due to the significant differences in the outer layer of Gram positive and Gram negative bacteria. Gram negative bacteria possess an external membrane and a periplasmic space which is absent in Gram positive bacteria (Duffy and Power, 2001). The surface of Gram-negative bacteria is largely composed of the glycolipid lipopolysaccharide (LPS), serving as one of the initial barriers against extracellular stresses. Specifically, LPS is a major constituent of the outer leaflet of the outer membrane phospholipid bilayer, which envelops the peptidoglycan containing periplasm and the inner membrane (Band and Weiss, 2015).

With regards to the inhibition parameters (Table 3), the MIC ranges from 1.56 to 25.00 mg/ml, and the MBC between 6.25 to 25.00 mg/ml. The ratio MBC/MIC was determined and according to the classification made by Fauchère and Avril (2002), the acetone extracts and the hydro-ethanolic extracts of the leaves and stem-barks harvested in the Center and East were bactericidal on *S. aureus* (1 ≤ CMB/CMI ≤ 2). The antibacterial activity of these extracts can be ascribed to the presence of phenols, terpenoids, tannins and flavonoids whose mechanisms of actions on bacteria are: the destruction of the membrane of the micro-organism through a lipophilic action (Cowan, 1999), bacterial and viral protein precipitation as well as heavy metals (Kansole, 2009), complexing property to soluble extracellular proteins and to the bacterial cell wall (Cowan, 1999) and the inactivation of microbial adhesion, enzymes and extracellular proteins respectively (Ghestem et al., 2001). From the classification of Fauchère and Avril (2002), the extracts presented a bacteriostatic action on most of the strains (4 ≤ MBC/MIC ≤ 16) except acetone and hydro-ethanolic extracts which were bactericidal.

Based on the *in vitro* antibacterial activity obtained with the extracts of *B. toxisperma* (Pierre), this plant englobes a set of criteria which could justify the renewed interest for the exploitation of this natural resource in the development of antibacterial drugs order to mitigate the narrow activity spectrum which the usual molecules pose.

Conclusion

The results obtained in this study bring scientific justification as to the use of *B. toxisperma* (Pierre) in traditional medicine for the treatment of microbial infections, in particular those of bacterial origin. Depending on the harvesting site of the plant (East and Center regions of Cameroon), more or less significant variations in the antibacterial activity was observed. These variations were attributed to edaphic and climatic factors which influenced the qualitative and quantitative chemical composition of the secondary metabolites in the plant at their site of growth. Phytochemical

Table 4. Inhibition parameters: MIC, MBC, MBC/MIC.

Bacterial strains	Inhibition parameters (mg/ml)	Plant extracts																
		East-region								Center-region								
		E1	F1	E2	F2	E3	F3	E4	F4	E'1	F'1	E'2	F'2	E'3	F'3	E'4	F'4	Gen
Bacillus cereus	MIC	1.56	12.5	1.56	6.25	3.12	12.5	1.56	25	12.5	25	3.12	3.12	6.25	12.5	3.12	6.25	0.125
	MBC	12.5	ND	12.5	ND	25	12.5	12.5	ND	25	25	12.5	12.5	25	25	12.5	ND	0.25
	MBC/MIC	8	ND	8	ND	8	1	8	ND	2	1	4	4	4	2	4	ND	2
Staphylococcus aureus	MIC	1.56	6.25	3.12	6.25	3.12	12.5	3.12	12.5	12.5	12.5	3.12	6.25	3.12	6.25	6.25	12.5	0.125
	MBC	6.25	6.25	12.5	ND	6.25	25	6.25	25	25	ND	12.5	6.25	ND	12.5	12.5	25	0.125
	MBC/MIC	4	1	4	ND	2	2	2	2	2	ND	4	1	ND	2	2	2	1
Salmonella typhi	MIC	1.56	6.25	1.56	12.5	1.56	12.5	1.56	6.25	6.25	25	3.12	6.25	3.12	3.12	3.12	6.25	0.0625
	MBC	6.25	ND	12.5	25	6.25	ND	6.25	ND	25	ND	12.5	ND	ND	25	12.5	ND	0.0625
	MBC/MIC	4	ND	8	2	4	ND	4	ND	4	ND	4	ND	ND	8	4	ND	1
Proteus mirabilis	MIC	1.56	6.25	1.56	6.25	1.56	25	3.12	12.5	12.5	25	3.12	12.5	3.12	6.25	3.12	12.5	0.0312
	MBC	6.25	25	12.5	25	6.25	ND	12.5	ND	ND	ND	12.5	ND	ND	12.5	12.5	25	0.25
	MBC/MIC	4	4	8	4	4	ND	4	ND	ND	ND	4	ND	ND	4	4	2	8

E1 and F1, Ethyl acetate stem-barks and leaves from the East; E2 and F2, Methanol stem-barks and leaves extracts from the East; E3 and F3, Hydro-ethanolic stem-barks and leaves from the East; E4 and F4, Acetone stem-barks and leaves extracts from the East; E'1 and F'1, Ethyl acetate stem-barks and leaves extracts from the Center; E'2 and F'2, Methanol stem-barks and leaves extracts from the Center; E'3 and F'3, Hydro-ethanolic stem-barks and leaves extracts from the Center; E'4 and F'4 ,Acetone stem-barks and leaves extracts from the Center; Hu ,Oil; Gen, Gentamicin; ND, not determined.

screening of the extracts of the plant material from the East region and that from the Center region showed that no matter the place of harvest, the two samples were rich in terpenoids, tannins, flavonoids, phenols, saponins, steroids and cardiac glycosides. These bioactive molecules can isolated from this plant and used in the development of pharmaceutical specialties capable of ensuring the treatment of many infectious diseases.

Conflict of interests

The author(s) did not declare any conflict of interest.

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