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Antimicrobial activity of extracts from *Crotalaria bernieri* Baill. (Fabaceae)

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This work was designed to study the antimicrobial activity of *Crotalaria bernieri* Baill. (Fabaceae). Extracts from leaf, root, pod and seed using hexane, ethyl acetate and methanol were tested *in vitro* for their activity against 17 bacteria, 5 fungi (3 yeasts and 2 molds) using disc diffusion and micro dilution methods. At the concentration of 1 mg/disc, all the extracts exhibited antimicrobial activity depending on the plant part and the extraction method used. The most sensitive germs were *Salmonella enteridis*, *Streptococcus pyogenes* and *Candida guilliermondii* with inhibition zone diameter (IZD) of 11 mm, 15 mm and 13 mm respectively. Most of extracts showed, broad activity spectrum varying from one extract to another. Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) of all extracts were recorded. Ten extracts displayed an excellent effect (MIC < 100 µg/ml), 8 a moderate effect (MIC from 100 to 500 µg/ml), 5 a weak effect (MIC from 500 to 1000 µg/ml) and the others were ineffective (MIC > 1000 µg/ml). Leaf methanol extracts were the most efficient and Gram positive bacteria the most sensitive. All extracts had bactericidal (MBC/MIC ≤ 4) or fungicidal action (MFC/MIC ≤ 4) in certain microorganisms and bacteriostatic (MBC/MIC > 4) or fungistatic action (MFC/MIC > 4) in others. Antimicrobial activity might be due to tannins, polyphenols, steroids, triterpenes and flavonoids that were present in most of the plant organs, but alkaloids in leaf and pod and saponosides in root might also be involved. *C. bernieri* with the effectiveness of all its parts, the variety of its secondary metabolites, the great number of sensitive pathogen microorganisms and its ubiquity make this plant species an interesting source of antimicrobial agents.

Key words: *Crotalaria bernieri*, antimicrobial activity, disc diffusion method, microdilution method, minimum inhibitory concentration, minimum bactericidal concentration, minimum fungicidal concentration.

INTRODUCTION

Antimicrobial resistance is one of the world's most serious public health problems. There is an urgent need

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Figure 1. *Crotalaria bernieri* (a) the whole plant; (b) flower; (c) fruits (Source: the authors).

to find new disposable and affordable remedies to face this problem (Zongo et al., 2011). Many studies led to systematic screening of plant extracts as a source of anti-bacterial compounds (Dalmarco et al., 2010; Stefanovic and Comic, 2011). Several *Crotalaria* species have been reported to display antimicrobial properties. For example, *Crotalaria madurensis* is active against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Candida albicans* (Bhakshu et al., 2008), *Crotalaria capensis* against *Salmonella typhimurium* (Dzoyem et al., 2014), *Crotalaria burhia* against *B. subtilis* and *S. aureus* (Sandeep et al., 2010; Mansoor et al., 2011), *Crotalaria juncea* against *S. aureus* (Chouhan and Sushil, 2010), *Crotalaria pallida* against *E. coli* and *Pseudomonas* sp (Pelegrini et al., 2009), and *Cladophora trichotoma* against *Alternaria solani* (Ravikumar and Rajkumar, 2013).

The purpose of this study was to assess the antimicrobial activity of *C. bernieri* by testing plant part's extracts obtained in different methods on pathogen bacteria and molds. *C. bernieri* is one of the 53 *Crotalaria* species growing in Madagascar, an annual herb which is found in open vegetation, grassy places and roadsides in most regions of Madagascar (Peltier, 1959). It flowers on July to October and December to March (Polhill, 1982; Dupuy et al., 2002).

MATERIALS AND METHODS

Plants

C. bernieri (Figure 1) were harvested in Ibity, District of Antsirabe, Region of Vakinankaratra, 200 km from Antananarivo region. The plant was collected in April and July, 2013 and was identified by Polhill R.M. Voucher specimens (Herizo R. 010) of *C. bernieri* were deposited in the herbarium of Plant Biology and Ecology Department of the Faculty of Sciences of the University of Antananarivo.

Microorganisms strains

The microorganisms used in this study consisted of 17 strains of bacteria (10 Gram (-) and 7 Gram (+)), 3 yeasts and 2 molds (Table 1). These strains were obtained from the collections of Laboratoire de Chimie des Substances Naturelles et Sciences des aliments (LCSNSA) of La Réunion University. They were maintained on agar slant at 4°C and cultured on a fresh appropriate agar plate during 24 h prior to antimicrobial tests.

Chemicals for antimicrobial assay

Commonly used pre-impregnated discs, from Bio-Rad F 92430 Marnes-la-Coquette were chosen as antimicrobial references

Table 1. Bacterial, yeast and mold strains used to study antimicrobial activities.

Microorganisms		Strains	References
Bacteria	Gram(-)	*Campylobacter jejuni*	ATCC 29428
		Enterobacter aerogenes	ATCC 13048
		Enterobacter cloacae	ATCC 13047
		Escherichia coli	ATCC 25922
		Pseudomonas aeruginosa	ATCC 10145
		Salmonella enteridis	ATCC 13076
		Shigella flexneri	ATCC 12022
		Vibrio parahaemolyticus	ATCC 17802
		Yersinia enterocolitica	ATCC 23715
		Proteus mirabilis	ATCC 35659
	Gram(+)	*Bacillus cereus*	ATCC 14579
		Clostridium perfringens	ATCC 13124
		Enterococcus faecalis	ATCC 29121
		Listeria monocytogenes	ATCC 19114
		Staphylococcus aureus	ATCC25923
		Streptococcus pneumoniae	ATCC 6305
		Streptococcus pyogenes	ATCC 19615
Fungi	Yeasts	*Candida albicans*	ATCC 10231
		Candida guilliermondii	ATCC 6260
		Candida krusei	ATCC 14243
	Molds	*Aspergillus fumigatus*	ATCC 204305
		Aspergillus niger	ATCC 16888

Table 2. Abbreviations designating the different extracts tested.

Extracts	Hexane Extract	Ethyl acetate Extract	Methanol Extract
Leaves	LHE	LEA	LME
Seeds	SHE	SEA	SME
Pods	PHE	PEA	PME
Roots	RHE	REA	RME

(Camara et al.,2013; Rakholiya et al., 2014): amoxicillin 25 µg, chloramphenicol 30 µg, penicillin 6 µg as antibiotics and miconazole 50 µg as antifungal.

Preparation of extracts

The dried leaves, seeds, seed pods, and roots of the plant were grounded into powder. The resulting powder (100 g) was extracted successively with 4x500 mL of hexane, ethyl acetate and methanol for 24 h under stirring at room temperature. After filtration using a Whatman filter paper, each combined extract was evaporated under reduced pressure to dryness. The dry residues, dissolved in hexane, ethyl acetate and sterile distilled water, constituted hexane, ethyl acetate and methanol extracts respectively (Table 2).

Phytochemical screening

The reactions for the detection of chemical groups were those developed by Fong et al. (1977) and Marini-Bettolo et al. (1981).

Antimicrobial assays

Antimicrobial activity test

The *in vitro* antimicrobial activity of the extracts was determined using disc diffusion method described by Pyun and Shin (2006) and Ngameni et al. (2009). Two mL of inoculum corresponding to 0.5 MacFarland (10^8 CFU/ml) was uniformly spread on the surface of Columbia Agar medium (for *Streptococcus*); Mueller-Hinton Agar (MHA) for the other bacteria and Potato Dextrose Agar (PDA) for yeasts. Sterilized filter paper discs 6 mm diameter (BioMérieux, REF 54991) were impregnated with 10 µL of each extract to the concentration of 100 mg/mL (1 mg/disc). The soaked discs were then placed on the surface of the agar and incubated at 37°C during 24 h for bacteria, or at 25°C for yeasts. The inhibition zone diameter (IZD) was measured and the results were interpreted by

Table 3. Extraction yields of *C. bernieri* extracts.

Extracts		Yield %
	LHE	14.6
Leaf	LEA	22.5
	LME	12.0
	SHE	18.4
Seed	SEA	12.1
	SME	10.0
	PHE	15.1
Pod	PEA	11.2
	PME	4.2
	RHE	13.7
Root	REA	15.3
	RME	24.1

means of the scale used by Ponce et al. (2003) and Celikel and Kavas*,* (2008) stating that bacteria are not sensitive for IZD less than 8 mm, sensitive for IZD of 9 to 14 mm, very sensitive for IZD of 15 to 19 mm and extremely sensitive for IZD larger than 20 mm. Antifungal activity was evaluated by a method described by Favel et al. (1994). One ml of each extract was added to 19 ml of medium culture of PDA and maintained at 45°C. The mixture is then poured into Petri dishes and dried for 15 min at 37°C. 10 µl of each tested microorganism corresponding to 0.5 MacFarland were spread on the medium surface. IZD were measured after incubation at 25°C for 72 h. Negative controls were prepared by using the same solvents employed to dissolve the plant extract samples while the reference antibiotics were used as positive controls. All the experiments were performed in triplicate. The results were expressed as mean values ± standard deviations (mm ± SD).

MIC, MBC and MFC determination

For extracts showing antibacterial activity in the disc diffusion method (IZD ≥ 8 mm), MIC (minimum inhibitory concentration), MBC (minimum bactericidal concentration) MFC (minimum fungicidal concentration) were determined by microdilution method (Kuete et al., 2009).

The concentration of each extract was adjusted to 25 mg/ml. This was serially diluted two-fold to obtain concentration ranges of 0.024 to 25 mg/ml. Each concentration was added in a well (96-well microplate) containing 95 µl of Mueller-Hinton broth (MHB) and 5 µl of inoculum (standardized at 0.5 MacFarland). A positive control containing bacterial culture without the extract and a negative control containing only the medium, were also analyzed. The plates were covered with sterilized aluminum foil, and then incubated at 25°C (yeasts and molds) or at 37°C (bacteria) for 24 h. The MIC of each extract was detected following addition 40 µl of 0.2 mg/ml *p*-iodonitrotetrazolium chloride and incubation at 25°C (yeasts and molds) or at 37°C (bacteria) for 30 min (Kuete et al., 2009). Viable bacteria reduced the yellow dye to a pink color. MIC was defined as the lowest sample concentration that prevented this change and exhibited complete inhibition of bacterial growth. For the determination of MBC and MFC, 5 µl from each well that showed no change in color was transferred on MHA or PDA plate and incubated at 25°C (yeasts and molds) or at 37°C (bacteria) for 24 h. The lowest concentration at which no growth occurred on the agar plates corresponded to the MBC or MFC.

The ratios MBC/MIC and MFC/MIC were calculated for each extract, to determine the nature of the effect. The extract is bactericidal or fungicidal for MBC/MIC or MFC/MIC ≤ 4 and bacteriostatic or fungistatic when these ratios are >4 (Djeussi et al., 2013; Bouharb et al., 2014; Chamandi et al., 2015).

Statistical analyses

Results were expressed as mean values ± standard deviations of three separate determinations. One-way analysis of variance (ANOVA) which was followed by Newman Keuls comparison test with Staticf® software was used for statistical analysis. Statistical estimates were made at confidence interval of 95%.

RESULTS

Extraction yields

The extractive yield of different parts of *C. bernieri* with different solvents varied from 4.2 (PME) to 24.1% (RME) (Table 3).

Qualitative phytochemical analysis

The major secondary metabolites identified in the different organ extracts are presented in Table 4. Tannins, polyphenols, steroids, triterpenes and unsaturated sterols occurred in all the *C. bernieri* organs. Flavonoids were found in all organs except root. Alkaloids were present only in leaf and pod while saponins only in root. Iridoïds, leucoanthocyanins, and quinones were not detected in all parts of *C. bernieri*.

Antimicrobial activity

At 1 mg/disc, a concentration generally used for antimicrobial activity assessment in plants (Sandeep et al., 2010; Govindappa et al., 2011; Linthoingambi and Mutum, 2013; Marimuthu et al., 2014), the large majority of *C. bernieri* extracts (16 of 22) inhibited the microorganism growth with IZD ranging from 8 to 15 mm (Tables 5 to 7). However, activity depended on the microorganism, the plant parts and extraction method used. The most sensitive germs were *S. enteridis* (IZD=11 mm), *S. pyogenes* (IZD=15 mm) and *C. guilliermondii* (IZD=13 mm) in Gram (-) bacteria, Gram (+) bacteria and yeasts, respectively. Gram (-) strains *C. jejuni* and *E. coli*, *E. faecalis,* Gram (+) *L. monocytogenes* and the two molds *A. fumigatus* and *A. niger* were resistant to all the extracts. REA, with an IZD of 15 mm against *S. pyogenes,* displayed the highest antibacterial activity.

Table 4. Phytochemical screening of *C. bernieri* extracts.

Chemical groups	Tests	Leaf			Seed			Pod			Root		
		a	b	c	d	e	f	g	h	i	j	k	l
Alkaloids	Mayer	-	-	+	-	-	-	-	-	+	-	-	-
	Wagner	-	-	+	-	-	-	-	-	+	-	-	-
	Dragendorff	-	-	+	-	-	-	-	-	+	-	-	-
	Confirmatory test (solubility in ethanol)	-	-	+	-	-	-	-	-	+	-	-	-
Saponins	Foam test	-	-	-	-	-	-	-	-	-	-	-	+
	Confirmatory test (hemolytic test)	-	-	-	-	-	-	-	-	-	-	-	+
Flavonoids	Willstätter	-	+	+	-	+	+	-	-	+	-	-	-
Leucoanthocyanins	Bate-Smith	-	-	-	-	-	-	-	-	-	-	-	-
Tannins and Polyphenols	Gelatin 1%	+	-	+	-	+	+	-	-	-	+	+	+
	Gelatin-salt 10%	+	-	+	+	+	+	-	+	-	+	+	+
	$FeCl_3$	-	+	+	-	-	+	-	-	+	-	-	+
Quinones	Borntrager	-	-	-	-	-	-	-	-	-	-	-	-
Steroids	Liebermann-Burchard	+	+	-	+	+	+	+	+	-	+	+	-
Iridoïds	Hot HCl	-	-	-	-	-	-	-	-	-	-	-	-
Triterpenes	Liebermann-Burchard	+	-	-	+	+	+	+	+	-	+	+	-
Unsaturated sterols	Salkowsky	+	-	-	+	+	+	+	+	-	+	+	-

+: positive result; -: negative result a: LHE; b: LEA; c: LME; d: SHE; e: SEA; f: SME; g: PHE; h: PEA; i: PME; j: RHE; k: REA; l: RME.

In yeasts, most of leaf extracts were active against the three *Candida* strains tested, but seed and pod extracts were active only against *C. guilliermondii*. Antibiotics used as references in this study (amoxicillin 25 µg, chloramphenicol 30 µg, penicillin 6 µg and miconazole 50 µg) were more effective than most of *C. bernieri* extracts. MIC, MBC, MFC and MBC or MFC/MIC ratio values are presented in Tables 8 to 10. MIC ranged from 0.048 to 25 mg/ml. MIC maximum values registered was 12.5 mg/mL except for RHE on *S. pyogenes* (MIC=25 mg/ml). Concerning MBC or MFC, maximum values for all extracts were 25 mg/ml except for root extracts on some Gram (+) bacteria and *C. guilliermondii* (MBC>25 mg/ml). The ratio MBC or MFC/MIC varied from 1 to more than 100.

The most sensitive microorganism were *P. mirabilis* in Gram (-) bacteria (MIC=MBC=0.097 mg/ml), *B. cereus* (MIC=0.048 mg/ml, MBC=0.195 mg/ml) and *S. pyogenes* (MIC=MBC=0.048 mg/ml) in Gram (+) bacteria and *C. guilliermondii* (MIC=MFC=0.048 mg/ml) in yeasts.

All methanol extracts were active. This is also the case for ethyl acetate extracts except LEA. As to hexane extracts, PHE and RHE were efficient but not LHE and SHE. Pod extracts had the broadest spectrum of activity with 10 sensitive microorganisms and seed extracts the narrowest ones with 8 sensitive microorganisms.

DISCUSSION

The present study shows that the *C. bernieri* extracts inhibited the growth of most tested microorganisms, indicating the presence of antimicrobial compounds in all parts of the plant. Phytochemical screening showed the presence of diverse secondary metabolites, reported to have antimicrobial property. At this stage of the work, results did not yet allow to state whether the same or different compounds are involved in the different parts of the plant. However, they suggested that *C. bernieri* antimicrobial activity might be mainly due to tannins, polyphenols, steroids, triterpenes and flavonoids, which were present in all or most of the plant organs. Alkaloids might also be concerned in leaves and pods and saponosides in root.

C. bernieri extracts showed generally a broad antimicrobial spectrum. They were capable of inhibiting the growth of different Gram (-) and Gram (+) bacterial strains as well as some yeasts. However, each extract

Table 5. *In vitro* Antimicrobial Activity (IZD in mm) of extracts (1 mg/disc) on Gram (-) bacteria.

Extracts/ controls		*Cj*	*Ea*	*Ec*	*Esc*	*Pa*	*Se*	*Sf*	*Vp*	*Ye*	*Pm*
Leaf	LHE	-	-	-	-	-	-	-	-	-	-
	LEA	-	-	-	-	-	-	-	-	-	-
	LME	-	10.00±0.01	-	-	9.33±0.47	-	-	7.00±0.01	-	8.33±0.47
Seed	SHE	-	-	-	-	-	-	-	7.00±0.01	-	-
	SEA	-	-	10.00±0.01	-	-	-	-	9.00±0.01	-	8.00±0.01
	SME	-	-	-	-	-	-	-	-	8.00±0.01	-
Pod	PHE	-	9.67±0.47	-	-	-	-	-	-	-	-
	PEA	-	-	9.00±0.01	-	-	-	-	10.67±0.01	-	-
	PME	-	-	-	-	-	11.00±0.82	8.00±0.01	9.00±0.82	8.00±0.01	8.00±0.01
Root	RHE	-	-	-	-	10±0.01	8.00±0.01	-	-	-	-
	REA	-	-	-	-	-	-	-	9.00±0.01	-	-
	RME	-	-	-	-	-	9.00±0.01	-	-	-	9.00±1.41
PC	Amx	45.00	-	-	23.00	10.00	27.00	25.00	-	10.00	-
	Chl	38.00	25.00	25.00	30.00	15.00	32.00	30.00	-	38.00	-
	Pen	40.00	-	-	-	-	-	-	-	-	25.00
NC	Hex	-	-	-	-	-	-	-	-	-	-
	EtOAc	-	-	-	-	-	-	-	-	-	-
	Sdw	-	-	-	-	-	-	-	-	-	-

Cj: *C. jejuni;* Ea: *E. aerogenes;* Ec: *E. cloacae;* Esc: *E. coli;* Pa: *P. aeruginosa;* Se: *S. enteridis;* Sf: *S. flexneri;* Vp: *V. parahaemolyticus;* Ye: *Y. enterocolitica;* Pm: *P. mirabilis* PC: Positive control (Amx: Amoxicillin 25µg; Chlor: Chloramphenicol 30µg; Pen: Penicillin 6µg); NC: Negative control (Hex: Hexane; EtOAc: Ethyl acetate; Sdw: sterile distilled water); −: No activity.

displayed a specific activity spectrum that could be due to difference between the chemical nature and concentration of bioactive compounds in extracts. The results obtained with microdilution method were more reliable than those with disc diffusion. That might be due to the fact that bioactive compounds were in direct contact with germs in liquid medium whereas they diffused little or not at all in solid medium.

There was no consensus on the acceptable level of inhibition for natural products (Benko and Crovella, 2010). For Dalmarco et al. (2010), for crude extracts and fractions, a MIC lower than 100 µg/mL was considered as an excellent effect, from 100 to 500 µg/ml as moderate, from 500 to 1000 µg/mL as weak, and over 1000 µg/ml as inactive. According to Kouitcheu et al. (2013), when a crude extract was used, the MIC values of 8 mg/mL or below against any microorganism tested was considered as active.

If the scale adopted by Dalmarco et al. (2010) was used as a reference, 10 extracts displayed an excellent effect, 8 a moderate effect, 5 a weak effect then the remaining extracts were inactive. Excellent effects were observed on *P. mirabilis* (RME), *S. enteridis* (PME), *B. cereus* (LME, PEA, REA), *S. pneumoniae* (LME), *S. pyogenes* (REA), *C. albicans* (LME) and *C. guilliermondii* (LME, SEA). Moderate effects, were found against *E. aerogenes* (SEA), *P. mirabilis* (LME), *P. aeruginosa* (LME), *C. perfringens* (LME), *S. aureus* (LME), *S. pyogenes* (RME, PEA, LME) and *C. guilliermondii* (SME). Weak effects were observed on *E. aerogenes* (SEA), *P. aeruginosa* (LME), *S. aureus* (REA) and *S. pneumoniae* (REA, RME).

The most efficient extracts were RME (MIC=MBC= 0.097 mg/ml) against *Y. enterolytica* in Gram (-) bacteria, REA (MIC=MBC=0.048 mg/ml) against *S. pyogenes* in Gram (+) bacteria and LME (MIC=MFC=0.048 mg/ml) against *C. guilliermondii.* Some of the extracts were very effective against some organisms (LME against *B. cereus, S. pneumoniae, C. albicans* and *C. guilliermondii,* REA against *B. cereus* and *S. pyogenes*) while others were totally inactive (SME against *S. pneumoniae* and *S. pyogenes*).

However, if the interpretation of Kouitcheu et al., (2013) was taken into account, only nine extracts had MIC higher than 8 mg/mL on some germs, which means that all the other extracts of *C. bernieri* used showed

Table 6. *In vitro* Antimicrobial Activity (inhibition zone diameter in mm) of extracts (1 mg/disc) on Gram (+) bacteria.

Plant parts/controls	Extracts	*Bc*	*Cp*	*Ef*	*Lm*	*Sa*	*Spn*	*Spy*
Leaf	LHE	-	-	-	-	7.00±0.01	-	-
	LEA	-	-	-	-	-	-	-
	LME	9.00±0.01	8.00±0.01	-	-	10.00±0.01	12.67±1.25	12.33±1.70
Seed	SHE	-	-	-	-	7.00±0.01	-	-
	SEA	-	-	-	-	9.00±0.82	11.33±0.47	9.00±0.01
	SME	-	-	-	-	-	8.33±1.25	8.00±0.01
Pod	PHE	-	9.67±1.25	-	-	7.00±0.01	-	-
	PEA	11.33±0.47	8.33±0.47	-	-	10.33±0.47	13.00±0.82	12.00±1.41
	PME	-	-	-	-	7.00±0.01	8.00±0.01	7.00±0.01
Root	RHE	-	-	-	-	-	-	8.00±0.82
	REA	11.33±0.94	7.00±0.01	-	-	11.00±0.01	13.00±0.82	**15**.00**±0.01**
	RME	9.00±1.41	10.00±0.01	-	-	8.00±0.01	13.00±0.83	12.00±1.41
PC	Amx	15.00	27.00	-	-	37.00	26.00	32.00
	Chl	38.00	30.00	-	30.00	30.00	25.00	22.00
	Pen	15.00	-	-	-	35.00	23.00	25.00
NC	Hex	-	-	-	-	-	-	-
	EtOAc	-	-	-	-	-	-	-
	Sdw	-	-	-	-	-	-	-

Bc: *B. cereus;* Cp: *C. perfringens;* Ef: *E. faecalis;* Lm: *L. monocytogenes;* Sa: *S. aureus;* Spn: *S. pneumoniae;* Spy: *S. pyogenes* Amx: Amoxicillin 25 µg; PC: Positive control (Amx: Amoxicillin 25 µg; Chlor: Chloramphenicol 30 µg; Pen: Penicillin 6 µg; NC: Negative control (Hex: Hexane; EtOAc: Ethyl acetate; Sdw: sterile distilled water); −: No activity.

Table 7. *In vitro* Antimicrobial Activity (inhibition zone diameter in mm) of extracts (1mg/disc) on yeasts and molds

Plant part	Extract	Yeast			Mold	
		Ca	*Cg*	*Ck*	*Af*	*An*
Leaf	LHE	-	7.00±0.01	-	-	-
	LEA	-	7.00±0.01	7.00±0.01	-	-
	LME	8.00±0.01	8,67± 0.94	7.00±0.01	-	-
Seed	SHE	-	7.00±0.01	-	-	-
	SEA	-	13.00±0.82	-	-	-
	SME	-	11.00±0.01	-	-	-
Pod	PHE	-	7.00±0.01	-	-	-
	PEA	-	7.00±0.01	-	-	-
	PME	-	7.00±0.01	-	-	-
Root	RHE	-	8.00±0.01	-	-	-
	REA	-	7.00±0.01	-	-	-
	RME	-	8.00±0.82	-	-	-
PC	Mic	18.00	30.00	33.00	28.00	23.00
NC	Hex	-	-	-	-	-
	EtOAc	-	-	-	-	-
	Sdw	-	-	-	-	-

Ca: *C. albicans;* Cg: *C. guilliermondii;* Ck: *C. krusei;* Af: *A. fumigatus;* An: *A. niger* PC: Positive control (Mic: Miconazole 50µg); NC: Negative control (Hex: Hexane; EtOAc: Ethyl acetate; Sdw: sterile distilled water); −: No activity.

Table 8. MIC and MBC values (mg/mL) of *C. bernieri* extracts (1mg/disc) on Gram(-) bacteria

Gram(-) Bacteria	Extracts	MIC (mg/ml)	MBC (mg/ml)	MBC/MIC
Enterobacter aerogenes	LME	0.195	25	128.21
Enterobacter cloacae	SEA	0.781	25	32.01
	PEA	6.25	25	4.00
Pseudomonas aeruginosa	LME	0.781	25	32.01
	RHE	0.195	25	128.21
Salmonella enteridis	PME	0.097	12.5	128.87
	RHE	12.5	12.5	1.00
	RME	12.5	25	2.00
Shigella flexneri	PME	3.125	25	8.00
Vibrio parahaemolyticus	SEA	1.562	6.25	4.00
	PEA	1.562	25	16.01
	PME	3.125	6.25	2.00
	REA	1.562	1.562	1.00
Yersinia enterolitica	SME	1.562	12.5	8.00
	PME	1.562	12.5	8.00
Proteus mirabilis	LME	0.195	0.781	4.01
	SEA	1.562	3.125	2.00
	PME	1.562	0.781	0.50
	RME	0.097	0.097	1.00

antimicrobial activities.

All the extracts had bactericidal action (MBC/MIC ≤ 4) in certain bacteria and bacteriostatic action (MBC/MIC >) 4) in other ones. For example LME was bactericidal against *B. cereus* and *C. perfringens* but bacteriostatic against *S. aureus* and *S. pneumoniae*. The comparison of *A. bernieri* extract activities to foreign *Crotalaria* species was not easy because antimicrobial activity was assessed under different conditions (other microorganism strains and extract doses used).

Compared to available data, the IZD of *C. bernieri* extracts were generally of the same order of magnitude as those of leaf ethyl acetate extract from *C. madurensis* against *B. subtilis* and *S. aureus* (IZD=14 mm), *M. luteus* (IZD=12 mm), *E. coli* and *C. albicans* (IZD=10 mm (Bhakshu et al., 2008) and leaf ethanol extract from *C. pallida* against *X. axanopodis* (IZD=16 mm), *E. coli*(IZD=14 mm) and *C. michiganensis* (IZD=13 mm) (Govindappa et al., 2011). Root methanol extract from *C. burhia* was more efficient with an IZD of 18 mm against *B. subtilis* and *P. aeruginosa* (Sandeep et al., 2010).

If comparison was based on antimicrobial indexes, LME (MIC=0.781 mg/ml, MBC=25 mg/ml) and REA (MIC=0.195 mg/ml, MBC=25 mg/ml) were more efficient against *P. aeruginosa* than the leaf methanol extract from *C. quartiniana* (MIC=MBC=37.5 mg/ml) (Omori et al., 2011). The leaf hexane extract from *C. retusa* (MIC=0.125 mg/ml, MBC=37.5 mg/ml) (Maregesi et al., 2008) was less active against *B. cereus* than LME (MIC=0.097 mg/ml, MBC=0.195 mg/ml), PEA and REA (MIC=0.048 mg/ml, MBC=0.195 mg/ml). By contrast, *C. bernieri* extracts were less active on *P. mirabilis* (MIC between 0.097 and 1.56 mg/ml) than a peptide isolated from *C. pallida* seeds (MIC=0.030 mg/ml) (Pelegrini et al., 2009).

Compared to the antibacterial activities from other plant extracts, several *C. bernieri* extracts were more efficient than methanolic aerial part extracts of *Inula viscosa* against *B. subtilis* (MIC=25 mg/ml, MBC=50 mg/ml) and *S. aureus* (MIC=12.5 mg/ml, MBC=50 mg/ml) (Larbi et al., 2016). By contrast, tuber ethyl acetate extract of *Tropaeolum pentaphyllum* against *E. coli* (MIC=0.02 mg/ml, MBC=0.64 mg/ml), *P. aeruginosa* (MIC=0.04 mg/ml, MBC=0.64 mg/ml) (da Cruz et al., 2016) and organic extract (aerial parts) of *Rapanea parvifolia* against *E. faecalis* (MIC=0.03 mg/ml, MBC=0.06 mg/ml) (Suffredini et al., 2006) were more efficient.

Table 9. MIC and MBC values (mg/ml) of *C. bernieri* extracts (1mg/disc) on Gram(+) bacteria.

Gram (+) Bacteria	Extract	MIC (mg/ml)	MBC (mg/mL)	MBC/MIC
Bacillus cereus	LME	0.097	0.195	2.01
	PEA	0.048	0.195	4.06
	REA	0.048	0.195	4.06
	RME	1.562	1.562	1.00
Clostridium perfringens	LME	0.195	0.390	2.00
	PHE	12.5	25	2.00
	PEA	6.25	12.5	2.00
	RME	6.25	>25	-
Staphylococcus aureus	LME	0.195	6.25	32.05
	SEA	6.25	12.5	2.00
	PEA	3.125	25	8.00
	REA	0.781	25	32.01
	RME	12.5	25	2.00
Streptococcus pneumoniae	LME	0.097	3.125	32.22
	SEA	3.125	12.5	4.00
	SME	12.5	25	2.00
	PME	1.562	6.25	4.00
	REA	0.781	>25	-
	RME	0.781	>25	-
Streptococcus pyogenes	LME	0.195	1.562	8.01
	SEA	1.562	12.5	8.00
	SME	12.5	25	2.00
	PEA	0.195	0.781	4.01
	RHE	25	>25	-
	REA	0.048	0.048	1.00
	RME	0.195	12.5	64.10

Table 10. MIC and MBC values (mg/ml) of *C. bernieri* extracts (1mg/disc) on yeasts.

Yeasts	Extraits	MIC (mg/ml)	MFC (mg/ml)	MFC/MIC
Candida albicans	LME	0.097	0.195	2.01
Candida guilliermondii	LME	0.048	0.048	1.00
	SEA	0.048	1.562	32.54
	SME	0.195	25	128.21
	RHE	12.5	>25	-
	RME	12.5	>25	-

On fungi, LME (MIC=0.097 mg/ml, MFC=0.195 mg/ml) was more efficient than leaf methanolic extract of *Myrtus nivellei* against *C. albicans* (MIC=4.5 mg/ml) (Touaibia and Chaouch, 2015) whereas LME, SEA and SME against *C. guilliermondii* (MIC=0.08 mg/ml, MFC=0.32 mg/ml) were less efficient than ethyl acetate extract of *T. pentaphyllum* (da Cruz et al., 2016).

Conclusion

This study clearly demonstrates the potential of *C. bernieri* as a source of interesting natural wide spectrum antimicrobial molecules. All its parts were efficient and could be easily found in significant amounts for the plant grows in fields, in the vicinity of homes, on roadsides and can be cultivated. Furthermore, according to our survey of local populations, *C. bernieri* is consumed by zebus but no cases of poisoning have yet been reported. At present, our works are concerned with the isolation of pure compounds from different extracts of *C. bernieri* and the elucidation of their structures in order to better evaluate their pharmacological activity.

In view of later therapeutic use of *C. bernieri*, study on various experimental models of animals is also on going to assess the harmful effects it might have.

Conflict of Interests

The authors have not declared any conflict of interests.

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