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Comparative Study of Antimicrobial and Toxic Activities of Seven Medicinal Plants of the Beninese Flora

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Abstract Medicinal plants are endowed important pharmacological properties. Through their broad spectrum of biological activities, people use them for their primary health needs. Uncontrolled use can cause complications for these users. Thus, a study of the antimicrobial activities of pathogenic bacteria in humans would help to elucidate in part the field of action of the plants studied. Our works aims to make a comparative study of antimicrobial and toxic activities of essential oils and dichloromethane extracts. The Results of phytochemical screening tests of dichloromethane extracts show that they are rich in secondary metabolites. Evaluation of antimicrobial activities of essential oils and dichloromethane extracts showed activity against eleven strains tested, with MIC values ranging from 0.312 to 5.00 mg/mL. Dichloromethane extracts have a more interesting inhibitory activity than their essential oils except for essential oils of *C. citratus* and *C. aurantifolia* which exhibit a better inhibitory activity on all the bacteria tested compared to their dichloromethane extracts, except *C. aurantifolia*, Which has better inhibitory activity on *M. luteus* (20 mm) for its dichloromethane extract than its essential oil. Similarly for essential oils from *E. camaldulensis* on *P. mirabilus* (20.5 mm), *C. schoenantus* on *P. aeruginosa* (20.5 mm) and *S. aureus* (16.75 mm) and *P. guajava* on *S. aureus* (19.75 mm) which exhibit better inhibitory activity compared to dichloromethane extracts. Evaluation of larval toxicity, it follows that the essential oils studied are less toxic than campthotecin, the reference compound. Dichloromethane extracts are less toxic than their corresponding essential oils.

Keywords Essential Oils, Dichloromethane Extracts, Antimicrobial Activity, *Artemia Salina*, Pathogenic Bacteria

Introduction

Medicinal plants are an important source of bioactive molecules [1] with important properties that allow the plant to adapt to its environment. Secondary metabolites within the plant have a protective role against heat stress, bacterial or viral [2]. This explains their use by the population for its primary health needs. In Benin, the demand for treatment with medicinal plants by the population remains high [3-4]. The population uses these medicinal plants, but the vast majority ignore the data relating to their phytochemical and pharmaco-toxicological properties. This may be beneficial or increase the risks of treatment for these users [3].

Cymbopogon citratus, *Cymbopogon giganteus*, *Cymbopogon nardus*, *Cymbopogon schoenantus*, *Psidium guajava*, *Eucalyptus camaldulensis* and *Citrus aurantifolia*, are medicinal plants from the Beninese flora used by the population for antimicrobial control [5].

In literature, several authors have indicated that these plants are used in antimicrobial control [5-8]. In order to enhance these medicinal plants acclimatized in Benin, the objective of this work is to assess the antimicrobial activity of eleven pathogenic bacteria and the toxicity of dichloromethane extracts (DCM) and essential oils and then to make a comparative study.

Materials and Methods

Plant material

Cymbopogon citratus **(**DC) Stapf, *Cymbopogon giganteus* (Chiov**),** *Cymbopogon nardus* (L.) Rendle, *Cymbopogon schoenantus* (L.) Spreng, *Eucalyptus camaldulensis* Dehnh (Myrtaceaes), *Psidium guajava* Linn (Myrtaceaes) and *Citrus aurantifolia* (Christm.) Swingle (Lime) were collected, from the Botanical Garden of the Abomey-Calavi University and identify at the University of Abomey-Calavi Herbarium.

Microorganism's cultures

Eleven bacterial strains including six Gram negative bacteria (*Escherichia coli* ATCC 25922, *Escherichia. coli* O157H7, *Pseudomonas aeruginosa* ATCC 27853, *Proteus mirabilus* A24974, *Proteus vulgaris* A25015 and *Salmonella typhi* R309514021) and five Gram positive bacteria (*Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* T22695, *streptococcus oralis, Micrococcus luteus* ATCC 10240 and *Enterococcus faecalis* ATCC 29212) were used in this study.

Essential oils and Dichloromethane extracts

500 g of each fresh plants material were extracted in steam distillated for 3 h [5] and the oils obtained were stored at - 4°C.

25 g of powders from the dried leaves of plants studied was extracted in soxhlet with 400 mL of dichloromethane for 2 hours at a boiling point of 40°C. The extracts are then reduced in a rotary evaporator at 40°C and dried in oven. The extracts obtained were stored at - 4°C.

Phytochemical Screening

The phytochemical screening of dichloromethane extract of plants was carried following the method of Houghton and Raman [9]**.** It is based on differential precipitation and colouration reactions of the main groups of chemical compounds contained in extract.

Larval toxicity test

The larval toxicity test was carried out on *Artemia Salina* larvae according to the method described by Michael et *al.* [10]. This test consists of determining the 50% lethal concentration (LC_{50}) which is the concentration from which at least fifty percent of the larvae contained in the culture medium were killed. It was determined from the graph showing the number of dead larvae as a function of different concentrations.

Antimicrobial activity

Essential oils and Dichloromethane extracts emulsions

40 µL of essential oil and 20µL of Tween 80 are added to 2 mL of Mueller Hinton medium, all introduced into a hemolysis test tube and homogenized. The mixture thus obtained has a concentration of 20 mg/mL.

200mg of each dichloromethane extract was weighed into a sterile flask to which 2 mL of acétone and 8 mL of sterilized distilled water were added to obtain a concentration of 20 mg/mL.

Sensitivity test

The agar perforation method inspired from those of Dah-Nouvlessounon et *al*. [11] was used to screen for antimicrobial activity. Fifty microliter of essential oils or dichloromethane extract solution (20 mg/mL) was aseptically lodged in the hole. These dishes were incubated at 37 °C for 24 and 48 h. Each sample was used in triplicate for the determination of antibacterial activity.

Determination of Minimum Inhibitory Concentrations (MIC) and Bactericidal Concentration (MBC)

The Minimum Inhibitory Concentrations (MIC) of essential oils or dichloromethane extract was performed by macrodilution method. Nine (9) dilutions of 1 mL of emulsion were performed to obtain the concentrations of 10 mg/mL, 5 mg/mL, 2.5 mg/mL, 1.25 mg/mL, 0.625 mg/mL, 0.312 mg/mL, 0.156 mg/mL, 0.078 mg/mL and 0.039 mg/mL in screw caped. To 1 mL of the above concentrations was added 1 mL of the bacteria inoculum (10^6 UFC/mL) to obtain 2 mL as a final volume. Culture medium without samples and others without microorganisms were used in the tests as control. Tubes were incubated at 37°C for 18-24 hours and growth was indicated by turbidity. The MIC is the lowest concentration of the compound at which the microorganism tested does not demonstrate visible growth (turbidity) [11].

The MBC was determined by solid medium culture of all of the tubes from the MIC to high concentrations. These dishes were incubated at 37 ° C for 24 h. The highest dilution that yielded no bacterial growth on solid medium was taken as MBC [11].

Statistical analysis

The Excel spreadsheet was used to calculate the means and standard deviations. The Student's *t*-test was used to determine if the difference between results obtained for different samples, and that between results of samples and controls were significant. The statistically significant difference was set at $p < 0.05$.

Results and Discussion

Phytochemical Analysis

The qualitative analysis of the powder and the dichloromethane extract (DCM) of the plants studied was carried by color or precipitation reactions. The results are shown in Table 1.

Table 1: Results of phytochemical screening of aqueous and dichloromethane extracts

				Aqueous extracts							Dichloromsthane extracts						
Chemical groups	**Differents compounds searched**			***Cc.***	***Cg.***	***Cn.***	***Cs.***	***Ec.***	***Pg.***	***Ca.***	***Cc.***	***Cg.***	***Cn.***	***Cs.***	***Ec.***	***Pg.***	***Ca.***
Phenolic compounds	Tannins	T- catechics		+	+	+	+	+	+	+	-	+	+	+	+	+	+
		T- Gallics		-	+	+	+	+	+	-	-	+	-	-	+	+	-
	Flavonoids	Flavones		+	+	+	+	+	+	+	+	+	+	+	-	+	+
		Leuco anthocyanins		+	+	+	+	+	+	-	+	+	+	+	+	+	-
	Anthocyanins			+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Anthracenic derivatives	Libres		-	+	-	-	+	+	-	-	+	-	-	+	+	-
		Combineds	Reducing compounds	+	+	+	+	+	+	-	-	+	-	-	+	+	-
			O-Heterosides	-	+	-	-	-	+	-	-	+	-	-	+	-	-
			reduced O-H genin	+	+	+	+	+	+	+	+	+	+	+	-	+	+
			C- Heterosides	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nitrogen compounds	Alkaloids			+	-	-	+	+	-	-	+	-	-	-	+	-	-
Steroids and Triterpenoids	Steroids			+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Triterpenes			+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Saponosides			+	-	-	-	+	+	+	-	-	-	-	+	+	+
	Cardiotonic glycosides			+	+	+	+	+	+	-	+	+	+	+	+	+	-
Quinone derivatives				-	+	+	-	+	+	+	-	+	+	-	+	+	-
Cyanogenic derivatives				-	-	-	-	-	-	-	-	-	-	-	-	-	-

Cc. = Cymbopogon citratus, Cg. = Cymbopogon giganteus, Cn. = Cymbopogon nardus, Cs. = Cymbopogon schoenantus, Ec. = Eucalyptus camaldulensis, Pg. = Psidium guajava, Ca. = Citrus Aurantifolia, (+) = Present, (-) = Absent

Analysis of this table revealed in the powder and DCM extract of the plants studied, the presence of polyphenolic compound (tannins, flavonoids, anthocyanins). Anthracene derivatives, steroids and triterpenes are also present there. The presence of alkaloids is only noted in powder of *E. camaldulensis, C. citratus and C. schoenantus and in DCM extracts of E. camaldulensis and C. citratus. Quinone derivatives are only absent in samples of C. aurantifolia, C. citratus and C. schoenantus.*

In literature, to our knowledge, we have no results on the phytochmic screening of DCM extracts from the plants studied. But similar works on other organic extracts has been done and our results corroborate those reported by Umar et *al*. [6] and Ekpenyong et al. [12] for acetone, chloroform and ethanolic extracts of *C. citratus*; Haddouchi et *al*. [13] for the methanolic extracts of *C. schoenantus*; Ibrahim et *al*. [14] for the methanolic and aqueous extracts of *E. camaldulensis*; Rajkumar et *al*. [15] and Ouattara-Soro et *al*. [16] for the ethanolic, methanolic, aqueous and hexane extracts of *C. aurantifolia*; Livingston and Sundar [17] and Mishra et *al*. [18] for the aqueous and ethanolic extracts of *P. guajava*.

Antimicrobial activities of dichloromethane extracts by determining of diameter of zone inhibition

Diameter of zones inhibition was determined by solid medium diffusion method and the results obtained are collated in Table 2. According to [19], the diameter of the zones of inhibition is depend on four levels activity: (D <8 mm); medium (9 mm ≤ D ≤ 14 mm); strong (15mm ≤ D ≤ 19mm); very strong (D> 20 mm).

Analysis in Table 2, it appears that the DCM extracts inhibited at least four of strains tested. Two of extracts, namely *P. guajava* and *E. camaldulensis*, inhibited all the strains tested. *P. guajava* extract exhibits strong activity against *E. coli* O157:H7 (17mm), *P. aeruginosa* (17.50mm), *S. oralis* (19mm) and *M. luteus* (17.75mm) then a very strong activity on *E. faecalis* (21.5mm). On the other hand, the extract from *E. camaldulensis* exhibits strong activity on *E. faecalis* (18.5mm) and *S. aureus* (15mm) then very strong activity on *S. epidermidis* (20.25mm).

Table 2: Inhibition diameter zone of essential oils and DCM extract (mean ± sd. n = 3)

Bacteria		**Gram negative**							**Gram positive**			
Plants extracts		**Inhibition Diameter (mm)**										
		E. co.	***E. co.* O157:H7**	***P. mi.***	***P. vu.***	***P. ae.***	***S. ty.***	***S. au.***	***S. ep.***	***S. or.***	***E. fa.***	***M. lu.***
C. citratus	DCM	16±1.41	10.5±0.71	13.5±0.58	10±0.00	-	-	10.25±1.25	13.5±2.12	-	-	-
	#EO	17,25±3.20	20±0.00	19.5±0.71	43.75±0.5	30 ± 0.00	17.5±3.51	21±0.81	25±3.20	40.5±0.71	22.5±0.71	32.25±2.06
C. giganteus	DCM	12.5±0.71	16.5± 0.71	18±1.41	-	-	-	13±0.00	-	-	-	-
	#EO	-	11±1.41	9.5±0.71	-	17.5±0.71	-	12.5±0.71	14.5±0.5	12±1.41	-	-
C. nardus	DCM	9.5±0.71	15±1.41	14±1.41	11±0.70	-	-	-	-	-	-	-
	#EO	-	-	9.25±0.95	9±1.41	16.25±1.5	-	18±1.41	17.25±2.62	-	-	10.5±0.71
C. schoenantus	DCM	-	20±0.00	-	15.5±0.35	11.5±0.71	-	12.5±1.30	21 ± 1.41	-	-	-
	#EO	9.75±0.5	10.75±0.5	-	9±1.73	20.5±0.71	14.5±0.71	16.75±1.5	20 ± 0.00	10±2.45	15.5±0.71	19.5±0.71
P. guajava	DCM	14,00±1.73	17±0.00	14.25±1.5	12.75±1.23	17.5±2.35	10.25±1.21	12±1.15	14.75±2.21	19±2.08	21.5±1.5	17.75±2.19
	#EO	-	16.5±2.06	10.5±0.57	11±1.15	10±0.00	-	19.75±0.57	-	-	10±1.41	9.5±0.57
E. camaldulensis	DCM	14.75±0.5	14.75±0.96	14.50±2.88	11.75±0.52	12.75±1.63	13.75±2.12	15±0.82	20.25±3.77	13.25±1.27	18.5±1.5	14.75±1.46
	#EO	-	-	20.5±0.71	11.75±1.53	10.5±0.71	-	-	-	-	-	-
C. aurantifolia	DCM	-	-	21.5±2.12	-	14.75±0.5	-	-	7.5±0.5	-	21.25±0.71	20±0.00
	#EO	31.5±0.71	17.5±2.06	27±2.94	31.5 ± 2.94	23±2.94	21±1.41	17±1.83	16.75±1.20	13.25±1.70	21.25±0.71	11.75±0.71
Acetone	NA											

E. co. : Escherichia coli, P. mi. : Proteus mirabilus, P. vu. : Proteus vulgaris, P. ae. : Pseudomonas aeruginosa, S. ty. : Salmonella typhi, S. au. : Staphylococcus aureus, S. ep. : Staphylococcus epidermidis, S. or. : streptococcus oralis, E. fa. : Enterococcus faecalis, M. lu. : Micrococcus luteus, P. guajava : Psidium guajava, E. Camaldulensis :

Eucalyptus Camaldulensis, C. aurantifolia : Citrus aurantifolia, C. : Cymbopogon, (-) = not detected, NA : Not Active, (±) = standard deviation of three separate experiments, EO : Essential oils, DCM =Dichloromethane, [#] : [5]

These two extracts exhibit moderate activity on the other bacteria. *C. aurantifolia* extract inhibits five bacteria including three gram positive with very strong activity against *M. luteus* (20 mm) and *E. faecalis* (21.25 mm) then a weak activity against *S. epidermidis* (7.5 mm) and two gram negative bacteria, namely a very strong activity on *P. mirabilus* (21.5 mm) and a strong activity on *P. aeruginosa* (14.75 mm).

Regarding the genus *Cymbopogon*, DCM extract of *C. citratus* shows an average activity on six bacteria tested except a strong activity on *E. coli* (16 mm).*C. schoenantus* extract shows activity against three gram-negative bacteria: medium on *P. aeruginosa* (11.5 mm), strong on *P. vulgaris* (15.5 mm) and very strong on *E coli* O157:H7 (20 mm) and two gram-positive bacteria: medium on *S. aureus* (12.5 mm) and very strong on *S. epidermidis* (21 mm). *C. giganteus* extract shows an average activity on *S. aureus* (13.0 mm), a gram-positive bacterium, and on three gram-negative bacteria, an average activity on *E. coli* (12.5 mm), strong on *E. coli* O157:H7 (16.5 mm) and *P. mirabilus* (18.0 mm).As for *C. nardus* extract, it shows medium activity on *E. coli* (9.5 mm), *P. mirabilus* (14 mm) and *P. vulgaris* (11 mm) and strong on *E. coli* O157:H7 (15 mm), all gram negative bacteria. On the other hand, it has no activity on gram-positive bacteria.

In literature, few results are available on evaluation of antimicrobial activity of DCM extracts of plants studied. But with regard to DCM extract of *E. camaldulensis*, [20] obtained, at a concentration of 10 mg/mL, an average activity on *S. aureus* (13 mm) and *P. aeruginosa* (14 mm), a strong activity on *S. typhi* (15 mm), which confirms inhibitory activity of our extract against these bacteria. Other authors, on the other hand, evaluated the antimicrobial activity of the plants studied using other organic solvents.

The growth of zone inhibition varies depending on microorganisms tested. Largest diameters of zone inhibition were recorded on Gram-negative bacteria for essential oils and DCM extracts. This confirms results reported by El-Mahmoud [21], showing largest diameters against Gram-negative bacteria.

Minimum Inhibitory Concentrations (MIC) of Dichloromethane extracts

MIC of EO and DCM extracts are shown in Table 3. The table shows that our EOs and DCM extracts inhibits the growth of bacteria studied with MICs values ranging from 0.312 to 5 mg/mL.

The lowest MIC value is obtained at 0.312 mg/mL for DCM extracts of *P. guajava* (on *S. oralis*, *E. faecalis* and *M. luteus*), *E. camaldulensis* (on *S. epidermidis* and *E. faecalis*), *C. aurantifolia* (on *P. mirabilus* and *M. luteus*), *C. citratus* (on *E. coli*) and *C. schoenantus* (on *S. epidermidis* and *E. coli* O157:H7).

The greatest MIC value is 5 mg/mL for DCM extracts of *C. aurantifolia* (on *S. epidermidis*) and *C. nardus* (on *E. coli* and *P. vulgaris*). These results show that at these MIC values, the strains indicated were the most sensitive to the extracts.

Aligiannis et *al*. [22] proposed a classification of plant, on the basis of MIC results, which is as follows: strong inhibition (MIC < 0.5 mg/mL), moderate inhibition (0.6mg/mL ≤ MIC ≤ 1.5mg/mL), and low inhibition (MIC > 1.6 mg/mL).

By referring to this classification, extracts having a MIC = 0.312 mg/mL, strongly inhibit these different strains mentioned above. On other bacteria, extracts showed moderate or weak inhibition. Regarding gram-negative bacteria, the extracts studied inhibited at least one of the bacteria tested.

To our knowledge, are no results available in relation to antimicrobial activity of DCM extracts of this plants, only [20], for the DCM extract of *E. camaldulensis* obtained a MIC on *S. typhi* (10 mg/mL), *P. aeruginosa* (10 mg/mL) and *S. aureus* (0.625 mg/mL). This result confirms antimicrobial activity of this extract compared to these different inhibited strains.

Table 3: Minimum Inhibitory (MIC) and Bactericidal (MBC) Concentrations, and Antibiotic power (MBC / MIC)

Plants Extracts		Parameters (mg/mL)	*E. co.*	*E. co.* O157:H7	*P. mi.*	*P. vu.*	*P. ae.*	*S. ty.*	*S. au.*	*S. ep.*	*S. or.*	*E. fa.*	*M. lu.*
C. citratus	DCM	MIC	0.312	2.5	0.625	2.5	–	–	2.5	1.25	–	–	–
		MBC	2.5	5	2.5	2.5	–	–	5	10	–	–	–
		MBC/MIC	8	2*	4*	1*	–	–	2*	8	–	–	–
	#EO	MIC	0.625	0.312	0.625	0.312	0.312	0.625	0.625	0.625	0.625	0.625	0.625
		MBC	2.5	5	5	1.25	2.5	10	1.25	5	5	5	2.5
		MBC/MIC	4*	16	8	4*	8	16	2*	8	8	8	4*
C. giganteus	DCM	MIC	2.5	1.25	2.5	–	–	–	2.5	–	–	–	–
		MBC	5	2.5	10	–	–	–	10	–	–	–	–
		MBC/MIC	2*	2*	4*	–	–	–	4*	–	–	–	–
	#EO	MIC	–	2.5	5	–	0.625	–	2.5	1.25	2.5	–	–
		MBC	–	10	> 10	–	2.5	–	5	5	10	–	–
		MBC/MIC	–	4*	–	–	4*	–	2*	4*	4*	–	–
C. nardus	DCM	MIC	5	1.25	1.25	5	–	–	–	–	–	–	–
		MBC	> 10	5	1.25	> 10	–	–	–	–	–	–	–
		MBC/MIC	–	4*	1*	–	–	–	–	–	–	–	–
	#EO	MIC	–	–	2.5	2.5	0.625	–	0.312	1.25	–	–	2.5
		MBC	–	–	> 10	> 10	2.5	–	5	5	–	–	> 10
		MBC/MIC	–	–	–	–	4*	–	16	4*	–	–	–
C. schoenantus	DCM	MIC	–	0.312	–	0.625	2.5	–	1.25	0.312	–	–	–
		MBC	–	1.25	–	10	> 10	–	2.5	2.5	–	–	–
		MBC/MIC	–	4*	–	16	–	–	2*	8	–	–	–
	#EO	MIC	2.5	2.5	–	2.5	0.312	2.5	0.312	0.625	2.5	1.25	0.312
		MBC	5	5	–	> 10	2.5	10	1.25	2.5	> 10	5	2.5
		MBC/MIC	2*	2*	–	–	8	4*	4*	4*	–	4*	8
P. guajava	DCM	MIC	1.25	0.625	1.25	1.25	0.625	2.5	1.25	1.25	0.312	0.312	0.312
		MBC	2.5	5	2.5	5	2.5	> 10	2.5	1.25	5	1.25	5
		MBC/MIC	2*	8	2*	4*	4*	–	2*	1*	16	4*	16
	#EO	MIC	–	1.25	2.5	2.5	2.5	–	0.625	–	–	2.5	2.5
		MBC	–	2.5	> 10	> 10	5	–	5	–	–	> 10	> 10
		MBC/MIC	–	2*	–	–	2*	–	8	–	–	–	–
E. camaldulensis	DCM	MIC	1.25	1.25	1.25	2.5	1.25	1.25	0.625	0.312	1.25	0.312	0.625
		MBC	5	1.25	1.25	5	2.5	5	2.5	1.25	5	1.25	> 10
		MBC/MIC	4*	1*	1*	2*	2*	4*	4*	4*	4*	4*	–
	#EO	MIC	–	–	0.625	5	2.5	–	–	–	–	–	–
		MBC	–	–	5	> 10	> 10	–	–	–	–	–	–
		MBC/MIC	–	–	8	–	–	–	–	–	–	–	–
C. aurantifolia	DCM	MIC	–	–	0.312	–	1.25	–	–	5	–	–	0.312
		MBC	–	–	2.5	–	1.25	–	–	> 10	–	–	1.25
		MBC/MIC	–	–	8	–	1*	–	–	–	–	–	4*
	#EO	MIC	0.312	0.625	0.312	0.312	0.625	0.312	0.625	0.625	2.5	0.625	0.625
		MBC	1.25	10	2.5	2.5	5	5	2.5	2.5	> 10	2.5	5
		MBC/MIC	4*	16	8	8	8	16	4*	4*	–	4*	8

MBC/MIC with * (Bactericidal effects) and without * (Bacteriostatical effects), *E. co. : Escherichia coli, P. mi. : Proteus mirabilus, P. vu. : Proteus vulgaris, P. ae. : Pseudomonas aeruginosa, S. ty. : Salmonella typhi, S. au. : Staphylococcus aureus, S. ep. : Staphylococcus epidermidis, S. or. : streptococcus oralis, E. fa. : Enterococcus faecalis, M. lu. : Micrococcus luteus, P. guajava : Psidium guajava, E. Camaldulensis : Eucalyptus Camaldulensis, C. aurantifolia : Citrus aurantifolia, C. : Cymbopogon,* (-) = not detected, EO : Essential oils, DCM =Dichloromethane, # : [5]

Minimum Bactericidal Concentrations (MBC) of Dichloromethane extracts

It is this parameter that makes it possible to determine the bactericidal effect of extracts studied. The minimum bactericidal concentrations (MBC) of DCM extract are given in table 3. Analysis of this table shows that our extracts have a concentration that ranges from 1.25 mg/mL to 10 mg/mL. The lowest value is obtained at 1.25 mg/mL for extracts of: *P. guajava* (on *S. epidermidis*, *E. faecalis*), *E. camaldulensis* (on *S. epidermidis*, *P. mirabilus E. faecalis*), *C aurantifolia* (on *M. luteus* and *P. aeruginosa*), *C. nardus* (on *P. mirabilus*), *C. schoenantus* (on *S. epidermidis*). Strains that were sensitive to the different extracts and whose CMBs could not be determined showed concentrations > 10 mg/mL.

Antibiotic power (MBC / MIC) of Dichloromethane extracts

According to [23], the MBC/MIC ratio allows us to better appreciate the antibiotic power of extracts studies. When this ratio MBC/MIC ≤ 4, extract is said to be bactericidal and when MBC/MIC > 4, extract is qualified as bacteriostatic. This ratio was calculated and recorded in Table 3.

Analysis of this table, it appears that our extracts exert more bactericidal than bacteriostatic effects with the exception of the extract of *C. schoenantus*. The extract from *E. camaldulensis* is most bactericidal followed by *P. guajava*, *C. citratus* and *C. giganteus* on different strains tested. The extracts inhibit gram negative bacteria more than gram positive bacteria. These different bactericidal activities observed for our extracts could be explained by presence of different groups of secondary metabolites identified.

In literature, authors have demonstrated that polyphenols are the main antimicrobial compounds of plants, having various modes of action and exhibiting inhibitory and lethal activities against a large category of microorganisms [24-26] indicated that the antibacterial activity of polyphenols can be explained by mechanism of toxicity of flavonoids against microorganisms. Likewise, authors have shown that tannins have the capacity to exert an inhibitory and lethal effect on various strains and in particular on *E. coli*, *S. aureus*, *P. aeruginosa*, *P. mirabilis*, *M. luteus*, *S epidermidis*, *S. oralis*, *P. vulgaris* and *E. faecalis* [27-28], therefore have the capacity to prevent the development of bacterial colonies by destroying their cell walls [28]. Analysis of our works and those from literature, we can conclude that a synergistic action of the various polyphenolic compounds present in our extracts would explain the antimicrobial activity.

Comparison of the antimicrobial activities of essential oils and DCM extracts.

Analysis of results of biological activities shows that:

- DCM extracts have a more interesting inhibitory activity than essential oils with except of EOs of *C. citratus* and *C. aurantifolia* which exhibit better inhibitory activity on all the bacteria tested compared to their DCM extracts, except *C. aurantifolia* which exhibits better inhibitory activity on *M. luteus* (20 mm) for its DCM extract than its essential oil. The same goes for essential oils from *E. camaldulensis* on *P. mirabilus* (20.5 mm), *C. schoenantus* on *P. aeruginosa* (20.5 mm) and *S. aureus* (16.75 mm) and *P. guajava* on *S. aureus* (19.75 mm) which exhibit better inhibitory activity compared to DCM extracts.
- DCM extracts are more bactericidal than their corresponding essential oils except of essential oils of *C. giganteus*, *C. schoenantus* and *C. aurantifolia*.

Toxicity of essential oils and dichloromethane extracts

The extracts studied are tested for their toxicity on shrimp larvae (*Artemia salina* L.). The results of the tests are given in Table 4 and are expressed in LC_{50}.

Analysis of the table shows that all of our oils have an LC_{50} value > 24 µg/mL well above value (LC_{50} = 13 µg/mL) of camptothecin, the reference compound. They are therefore less toxic.

For DCM extracts, the lethal half-concentrations range from 1114.46 µg/mL to 2315.59 µg/mL and are at least 80 times less toxic than camptothecin reference compound.

Table 4: Toxic power of essential oils and DCM extracts

Plants	Toxicity (LC_{50}, µg/mL)	
	EOs	**DCM**
C. citratus	^{e}64.29 ± 0.01	^{c}1560.02 ± 0.02
C. giganteus	^{g}133.39 ± 0.03	^{c}1559.99 ± 0.01
C. nardus	^{c}27.5 ± 0.01	^{b}1114.46 ± 0.05
C. schoenantus	^{c}30.19 ± 0.03	^{d}1798.57 ± 0.04
P. guajava	^{d}50.50 ± 0.02	^{e}2191.23 ± 0.03
E. camaldulensis	^{f}85.75 ± 0.01	^{f}2495.86 ± 0.24
C. aurantifolia	^{b}24.56 ± 0.01	^{e}2280.50 ± 0.02
Campthotecin	^{a}13.27 ± 0.02	

These LC_{50} values make it possible to confirm that the extracts studied do not exhibit toxicity.
According to the scale of [29], an extract is classified as non-toxic when an $LC_{50} \geq 100$ µg/mL. Using this scale, we can conclude that our DCM extracts are not toxic. But when it comes to EOs, except of *C. giganteus*, other EOs show moderate toxicity. The EOs of our plants are more toxic than their corresponding DCM extracts. This would explain the use of these plants without great risk of poisoning in traditional medicine by the population for fight against infections.

Conclusion

Plants are important natural sources of bioactive substances. This explains their uses by the population for fight against infections.
Qualitative analysis of DCM extracts from the plants studied reveals that they are rich in secondary metabolites. From the evaluation of antimicrobial activities of DCM extracts, it appears that extracts of *E. camaldulensis* and *P. guajava* are most bactericidal. Our DCM extracts are more antimicrobial than their corresponding essential oils (Eos) with the exception of EOs of *C. giganteus*, *C. schoenantus* and *C. aurantifolia*. The evaluation of larval toxicity, it follows that DCM extracts are less toxic than their corresponding EOs. The EOs studied are less toxic than campthotecin, a reference compound. These results confirm the use of these plants by the population for the treatment of infections caused by such pathogenic bacteria.
In perspective, it would be interesting to quantify the levels of polyphenolic compounds in DCM extracts in order to better explain the correlation between antimicrobial activity and the levels of polyphenolic compounds.

Conflict of interest

The authors have not declared any conflict of interest.

Contributions from authors

Each author contributed equitably.

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