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Evaluation of the superficial antistaphylococcic power of extracts of *combretum racemosum* P. beauv. (Combretaceae)

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Abstract
Staphylococcus Aureus and *Staphylococcus epidermidis* are commensal bacteria of humans and farm animals. They are identified as opportunistic, multi-resistant pathogens, and agents of fatal infections and severe food poisoning in farm animals and humans. In Côte d'Ivoire, little information is available concerning these bacteria of human origin.
The objective of this study was to develop a new natural antistaphylococcal and food preserva- tive, through the *in-vitro* determination of the antistaphylococcal power of *C. racemosum* leaves. For this purpose, a total of 5 crude aqueous and hydro-organic extracts of the leaves of *C. racemosum* were tested on 4 clinical isolates of *S. aureus* and *S. epidermidis* resistant to methicillin, bacterial species often encountered in infections of farm animals and humans.
All isolates such as *S. aureus* (ATTC 25923), *S. aureus* (Méti-R 2062C/15), *S. aureus* (Méti-R 2063/15) and *S. epidermidis* (Méti-R 2070C/15) demonstrated sensitivity to each plant extract according to a dose-response relationship at concentrations ranging from 3.125 to 100 mg/mL. Sensitivity was most observed towards Eeth, which constitutes the most effective antibacterial extract on all the staphylococci studied. This partly justifies the use of *C. racemosum* in Ivorian traditional therapy against superficial infections of the skin, cutaneous appendages and mucous membranes.
For all the extracts tested, the recorded MICs ranged between 3.125 ± 0.00 mg/mL and 100 ± 0.00 mg/mL, while the CMBs ranged between 6.25 ± 0.00 mg/mL and 100 ± 0, 00 mg/mL. Five (5) groups of biomolecules including alkaloids, saponins, steroids, terpenoids and tannins (catechic and gallic) were highlighted at various degrees of concentration by triphytochemistry. The most active extract in this study (Eeth) contains in average concentrations the same biomole- cules mentioned above.
This study made it possible to retain that the macerated crude hydro-ethanolic extract (Eeth) constitutes the most active fraction of all the crude extracts tested in this work. Consequently, it could, subject to toxicological study, be used for the preservation of food against staphylococci and the treatment of staphylococci of the skin, mucous membranes and cutaneous appendages in farm animals and humans.

Keywords: *Combretum racemosum*, superficial antistaphylococcal potency

1. Introduction
Staphylococcus Aureus and S*taphylococcus epidermidis* occupy a key place in veterinary and human medicine because of their multiresistance [1, 2] and their pathogenicity in farm animals and humans. Throughout the world, such as in Côte d'Ivoire, these bacteria are one of the main causes of food poisoning, resulting from the ingestion of food contaminated with enterotoxins [3]. They are also one of the most common causes of nosocomial infections and fatal staphylococci in various locations including the skin [4], skin appendages and mucous membranes. However, the skin, the cutaneous appendages and the mucous membranes constitute the first immun e defense of any living organism against exogenous physico-chemical and biological attacks, even microbial [5]. Food poisoning and animal and human staphylococci are mainly due to Staphylo- coccus aureus [6]. These are common infections, often recurrent, incurable in some cases and can be a source of serious illnesses, including HIV infection. Moreover, these infections, generalized in severely immunocompromised subjects, have experienced a clear expansion in recent years and often lead to death. Despite the considerable progress made in microbial control, microbial resistance has compromised the achievements of modern medicine. They are a reminder that the fight against infections is not over yet [7]. Thus, the need to search for new natural phyto- medicines is essential. In Côte

d'Ivoire, several medicinal plants including *Terminalia laxifolia*, *Parquetina nigrescens*, *Combretum racemosum*, *Bersama abyssinica*, have been studied in this fight, for their antimicrobial activities [8, 9, 10]. In this context, *C. racemosum* caught our attention. It is a pan-African medicinal plant. It is used in traditional therapy in certain African regions against genitourinary and gastrointestinal infections, convulsive cough, diarrhea, male sterility, hemorrhoids, dental pain, tuberculosis culosis [11, 12]. Its potential could then play an important role in the fight against staphylococcal infections. The present study aimed to develop a new natural antistaphylococcal, based on *C. racemosum* P. Beauv. and affordable, for the preservation of foods against staphylococci and the treatment of staphylococcal infections of the skin, skin appendages and mucous membranes. It consisted of characterizing essential biomolecules and testing the antistaphylococcal power of 5 crude aqueous and hydroorganic extracts of *C. racemo- sum*.

2. Materials and Methods

2.1 Plant Material

The plant material used consisted of leaves of *Combretum racemosum*. The authentication was made at the Center National de Floristique of the Félix Houphouët Boigny University of Abidjan-Cocody, by comparison with specimen No 16949 deposited on July 17, 1985.

2.2 Staphylococcal material

The staphylococcal carrier was composed of clinical isolates of *Staphylococcus Aureus* (Méti-R 2062C/15), *Staphylococcus Aureus* (Méti-R 2063C/15) and *Staphylococcus epidermidis* (Méti-R 2070C/15). Some isolates were provided by the CeDReS Bacteriology Service. Others came from the Antibiotics, Natural Substances and Anti-Infectious Microorganisms Surveillance Unit (ASSURMI) of the Department of Bacteriology and Virology of the IPCI. Quality control was ensured by the use of a *Staphylococcus Aureus* reference strain (ATCC 25923). The latter was provided by the Service de Microbiology of the National Public Health Laboratory (LNSP) of Côte d'Ivoire.

2.3 Reference antibacterial

The reference antibacterial used as a control was oxacillin capsule. It was used to test the authenticity of the profile of the staphylococcal germs used. Oxacillin is a bactericide narrow spectrum of the Beta-lactam family, of the group of penicillins M. It is very active on staphylo- cocci.

2.4 Plant treatments and spraying

After authentication, the leaves of *C. racemosum* were carefully sorted and freed of foreign bodies. They were cut, washed with distilled water, dried away from the sun and in the open air for one (1) week. After drying, they were reduced to powder using an electric grinder. The fine powder obtained was stored in sterile, clean, dry bottles and kept in the laboratory away from humidity, and at a temperature of 20 °C for the preparation of the various extracts.

2.5 Preparation of crude aqueous extracts

The macerated aqueous crude extract (Eaq) was obtained by homogenizing one hundred grams (100 g) of the powder the dried leaves in 1 liter (1 L) of distilled water in a blender at 37 °C for 10 min. The homogenate obtained was drained through a square of white cloth, then filtered twice on absorbent cotton and once on 3 mm Wattman paper. The filtrate obtained was concen- trated in an oven at 50 °C for one (1) week to give the macerated aqueous crude extract (Eaq) [13].

The decocted aqueous crude extract (Edec) was obtained according to the method of [14]. Thus, one hundred grams (100 g) of the powder of the dried leaves was first dissolved in one liter (1 L) of cold distilled water in a container at 37 °C. The whole was boiled for 15 min on a hot plate at 100 °C. After cooling and homogenization in a blender (mixer) at room temperature (37 °C), the mixture obtained was wrung out in a square of white cloth, then filtered twice on absorbent cotton, and once on 3 mm Wattman paper. The filtrate obtained was concentrated in an oven at 50 °C for one (1) week.

2.6 Preparation of raw macerated hydro-organic extracts

The crude ethyl hydro-acetic extract (Eace) was prepared according to the method described by [15]. To do this, one hundred grams (100 g) of leaf powder was dissolved in one liter (1 L) of a solution of cold water and pure ethyl acetate (300 mL of cold distilled water for 700 mL of pure ethyl acetate 99.5 ºG.L), then homogenized in a blender at 37 °C. Each homogenate obtained was first wrung out in a square of white cloth, then filtered twice on absorbent cotton and once on 3 mm wattman paper. The filtrate obtained was concentrated in an oven at 50 °C for one (1) week.

The macerated hydro-ethanolic (Eeth) and macerated hydro-methanolic (Emet) crude extracts were obtained in the same way as the hydro-acetic extract, except that the ethyl acetate was replaced by ethanol. and methanol.

2.7 Characterization of the biomolecules of the different crude extracts

A phytochemical study carried out according to the methods described by [16, 17] on the extracts Eaq, Edec, Eace, Eeth and Emet made it possible to highlight the main essential chemical groups contained in the leaves of *Combretum racemosum*. It is a set of identification reactions and color indicators based on the reduction in medium (alkaline or basic) of the reagent mixture by the oxidizable groups of the secondary metabolites, leading to the formation of color reduction products which is a function of the environment. For each extract, 10 identification tests by color reactions were carried out. Solutions with indicators react positively, indicating the presence of bioactive compounds in *C. racemosum* leaf extracts.

2.8 Evaluation of the antistaphylococcal effect of different extracts of *C. racemosum*

2.8.1 Sterility test of the different extracts

The purpose of this test was to verify that the extract does not contain any bacterial or fungal germs. For this, 0.1 g of the extract to be tested was first diluted in 10 mL of thioglycholate broth and then incubated at 37 °C for 24 hours. After this time, the turbidity of the broth is assessed with the naked eye. This broth was then inoculated onto a Petri dish containing nutrient agar and onto another dish containing Sabouraud agar, finally incubated under the same conditions for three days with observation every 24 hours to check whether germs have pushed into Petri dishes. The substance is declared sterile, if no colonies are visible on the agar plate [18].

2.8.2 Preparation of the range of concentrations of the different extracts

Mueller-Hinton broth was used for the preparation of the concentration range of each extract. This broth was obtained by homogenizing twenty-four grams (24 g) powder in 1 liter

(1 L) of distilled water. The whole was brought to a boil for 1 minute, dispensed into clean, dry vials and sterilized in autoclave at 121 °C for 15 minutes. The range of concentrations for each extract was prepared in test tubes according to the method of double dilution with the geometric progression of ratio 1/2 [19]. For each extract, each series consists of 10 test tubes numbered from T_1 to T_{10}. Ten (10) mL of distilled water are first dispensed into tube T_1 and 5 mL into the other 8 tubes. Two grams (2 g) of sterile extract were then diluted in the T_1 tube to obtain a concentration of 200 mg/mL. After homogenization, 5 mL of the content of T_1 was transferred to tube T_2. This operation was repeated to prepare the next tube and so on until tube T_{10}. Thus, a range of extract concentrations was obtained from 200 to 0.3906 mg/mL. All the culture tubes thus obtained were selected for inoculation.

2.8.3 Sensitivity test of staphylococcal strains to different extracts

The bacterial inoculum was prepared from a 24 hours young colony. To do this, two 24 hours bacterial colonies were picked using a Pasteur pipette and emulsified in a test tube containing 10 mL of sterile Muller-Hinton broth. The mixture was incubated at 37 °C for 3 hours. After this incubation, a suspension of 0.3 mL of this pre-culture was taken and diluted in 10 mL of sterile Muller-Hinton broth, then homogenized, thus constituting the bacterial inoculum estimated at 10^6 bacteria/mL.

The test of bacterial sensitivity to extracts characterized by an inoculation of the range of extract concentrations was carried out according to the method of [20] and [19]. Thus, a series of eleven (11) sterile hemolysis tubes numbered from H_1 to H_{11} was first produced. The first ten tubes (from H_1 to H_{10}) are called «test tubes» and the last tube (H_{11}) is denoted «growth control tube or H_C». In this series of tubes, 1 mL of extract of well-known concentration was then introduced according to the range of concentrations previously prepared. This distribution of extract was made so that 1 mL of 200 mg/mL extract was transferred to tube H_1, that of 100 mg/mL to tube H_2, and so on up to tube H_{10} which was received 1 mL of 0.1953 mg/mL extract. The H_C tube which serves as a bacterial growth control witness received, instead of extract, 1 mL of sterile distilled water. Finally, 1 mL of sterile Müeller-Hinton Broth, twice concentrated and already contaminated with the bacterial germ to be tested, was added to all the tubes. This distribution of extract of well-known concentration in each of the hemolysis tubes already containing 1 mL of inoculum, reduced the concentration of the medium in extract to half. Thus, the concentration of tube H_1 went from 200 mg/mL to 100 mg/mL. That of tube H_2 from 100 mg/mL to 50 mg/mL up to tube H_{10} with a new concentration of 0.1953 mg/mL. Thus, ten (10) culture hemolysis tubes of concentrations ranging from 100 to 0.1953 mg/mL were obtained. After a first measurement of the value of the initial turbidity of each culture with the "Densimat", all the loaded tubes were incubated at 37 °C for 24 hours. They were used to determine the survival percentage of each bacterial germ. The experiment was carried out in triplicate.

2.8.4 Determination of the survival percentage of staphylococcal germs

After 24 hours of incubation at 37 °C, the turbidity value of each culture was directly measured a second time with the "Densimat".

The percentage of staphylococcal survival was obtained in stages. First, the growth of staphylo- cocci was carried out by making the difference between the value of the density measured before the incubation and that after the incubation for each tube. The value obtained for tube N° 11 (growth control tube) represents 100% survival. Then, the values obtained for the ten (10) other tubes (test tubes) were subsequently expressed as a percentage of survival compared to that of the control tube. Finally, the method for calculating the percentage of germ survival in the test tubes was made according to the following formula:

$$S = \frac{(d_f - d_i)}{(D_f - D_i)} \times 100$$

Where S is the percentage of staphylococcus survival, D_i the density value of the control tube before incubation, D_f the density value of the control tube after incubation, di the density value of the experimental tube before incubation and d_f the density value of the experimental tube after incubation.

2.8.5 Determination of antibacterial parameters

The MIC is the lowest concentration of *C. racemosum* extract for which there is no of turbidity. Its determination was made from the measurement of the turbidity induced by the growth of the staphylococci studied. It therefore corresponds to the concentration of the first tube from which no disturbance was observed with the naked eye. Therefore, this is the first tube where the di value equals d_f ($d_i = d_f$). This operation was repeated 3 times in a row. All the tubes in which there was an absence of staphylococcal germs are kept for the determination of the Minimum Bactericidal Concentration (MBC). The CMB is the lowest concentration of extract from the tube that leaves no more than 0.01% viable staphylococci compared to the initial inoculum. After reading the MIC, the contents of the test tubes where there was no visible growth of germs were inoculated in lines 5 cm long, on a Müeller-Hinton agar dish, starting with the test tube. the CMI. This series of Petri dishes is named B. Then, the starting inoculum (tube H_{11} or H_C) was diluted from 10^{-1} to 10^{-4} (count). The five dilutions obtained (10^0, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}) were also inoculated in 5 lines 5 cm long, on another plate of Müeller-Hinton agar, at the using a calibrated 2 µL loop, then incubated for 24 hours at 37 °C [18]. This Petri dish was named A. The 10^{-4} dilution represents 0.01% survival and constitutes the bactericidal control. After incubation, the number of bacterial colonies on each line of Petri dish B was compared to that of Petri dish A. The CMB was determined by comparing the bacterial growth of dishes A and B (8). Thus, the smallest concentration of the tube that has less than 0.01% viable bacteria compared to the initial inoculum is the CMB.

2.8.6 Determination of antistaphylococcal potency

The CMB/CMI report made it possible to specify the mode of action of the substance. The

extract is said to be bactericidal if the CMB/CMI ratio is less than or equal to 2, on the other hand it is said to be bacteriostatic if the CMB/CMI is greater than 2 [21].

2.8.7 Statistical analyzes

The values of the antibacterial parameters of each extract were determined using Graphpad Prism 5.01 software. Results are given as mean ± SE (n = 3), using Column Statistics.

3. Results and Discussion

3.1 Biomolecules contained in the different extracts analyzed

The result of characterization of the essential biomolecules of the different extracts revealed the presence at various degrees of concentrations of four (4) main groups of active principles in this work. The first group represented by free quinones was totally absent from all the analyzed extracts. The second group represented by alkaloids, flavonoids, total polyphenols, saponins, steroids, terpenoids and tannins was found in Eaq, Edec and Emet. The third represented by alkaloids, saponins, steroids, terpenoids and tannins was present only in Eeth. The last group represented by alkaloids, saponins, steroids and terpenoids was present in Eace. The profile of the biologically active molecules highlighted is the same in the crude extracts Eaq, Edec and Emet. The crude extracts Eace and Eeth have practically the same profile (Table 1).

Analysis of the first group of chemical compounds indicates that water, methanol, ethyl acetate and ethanol used as extraction solvents in this study have no affinity for free quinone polyphenols [22].

The analysis of the second group of chemical compounds indicates that water (polar solvent) and the water-methanol mixture have a greater affinity for alkaloids, steroids, terpenoids, total polyphenols, and polyphenols of types flavonoids, saponins and tannins [23]. Eaq, Edec and Emet therefore best extract the active principles sought in the leaves of *C. racemosum* in this work. These biomolecules are already known for their antimicrobial activities [24, 25, 22]. This result is in line with those obtained by [26, 27, 28] and by [29] during their work. The various chemical groups revealed in the leaves of *C. racemosum* give the plant its many therapeutic indications in traditional environments, and confirm its activity against infections.

The third analysis of the phytochemical results indicates that alkaloids, steroids, terpenoids, as well as polyphenols such as saponins and tannins are more abundant in Eeth. This result would be due to the fact that polyphenols are generally more soluble in hydro-alcoholic mixtures [23].

The latest phytochemical analysis denoted that alkaloids, steroids, terpenoids and saponin-like polyphenols are more abundant in Eace. The presence of terpenoids is due to the addition of water to ethyl acetate which would have increased the solubility and polarity of the water-ethyl acetate mixture, thus ensuring the extraction of a large number of these phytochemicals [30].

Further analysis of the phytochemistry results concluded that extracts aqueous crudes (Eaq and Edec) analysed, the aqueous decoction (Edec) extracts the active ingredients better than the aqueous macerated (Eaq). The reason would lie in the extraction method and the effect of temperature. In the present study, the course of the extraction by decoction at high temperature (100 °C) as well as the exhaustion of water at reduced pressure made it possible to obtain the maximum of the biomolecules by preventing the denaturation or the probable modification of these substances [31]. This result validates the traditional form of use (decoction) of *C. racemosum* leaves in certain regions of Côte d'Ivoire.

According to another additional analysis of the phytochemistry results, the water-based crude extracts (Eaq and Edec) extract the identified biomolecules the best, compared to the water-organic solvent crude extracts (Eace, Eeth and Emet). This result could be explained by the fact that water is more polar than the hydroorganic solutions (water-ethyl acetate, water-ethanol and water-methanol) used in this work. In addition, extraction by water is quantitative, while that by organic solvents is qualitative.

A final additional analysis of the phytochemistry results obtained with the hydro-organic extracts indicated that Emet is richer in biomolecules, compared to Eace and Eeth. The reason would be that methanol is more miscible with water than ethyl acetate and ethanol. In order of increasing miscibility, ethyl acetate is less miscible with water, then comes ethanol, and finally methanol. In this classification, the water-methanol solution therefore has plus the property of dissolving biomolecules, or extracting them from the leaves of *C. racemosum*.

Table I: Profile of the main essential biomolecules revealed in the different extracts from the leaves of *C. racemosum*

Main essential bioactive compounds		Aqueous raw extracts		Raw hydro-organic extracts		
		Eaq	Edec	Eace	Eeth	Emet
Alkaloids	B	+	++	+	++	+++
Flavonoids		+	++	-	-	-
Total polyphenols		+	++	-	-	+
Free quinones		-	-	-	-	-
Saponins		+	++	+	+	+++
Steroids		+	++	+++	++	+
Terpenoids		+	++	+++	++	+
Tannins	Cat	+	++	-	+	+
	Gal	+	+	-	+	+

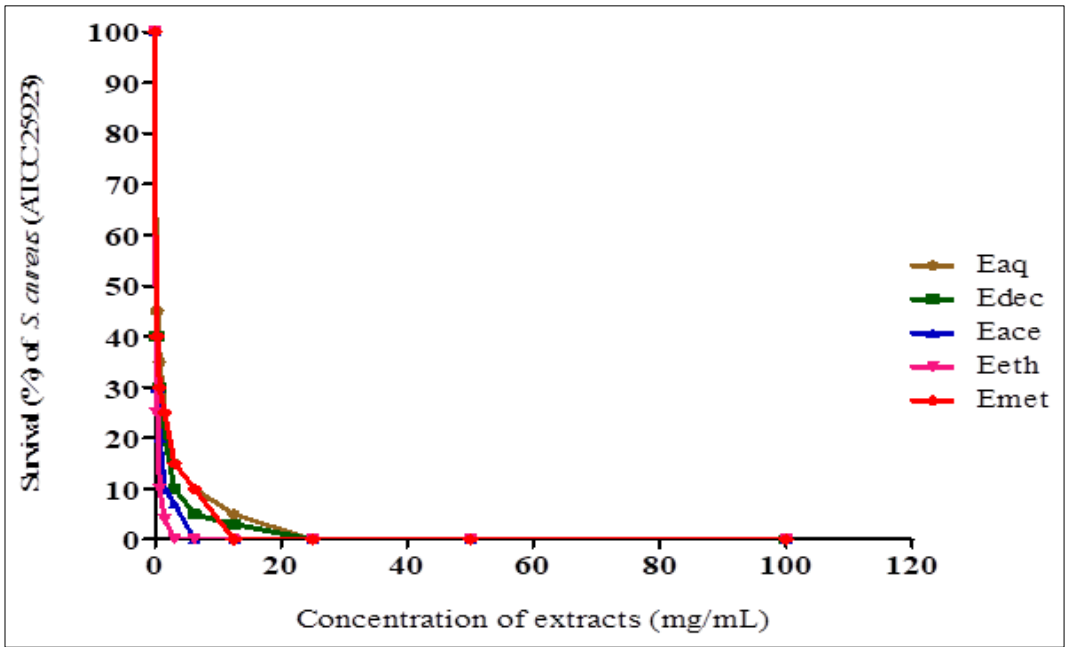
Eaq: Raw macerated aqueous extract; Edec: decocted aqueous raw extract; Eace: Crude hydro-acetic extract of macerated ethyl; Eeth: Crude hydro-ethanolic macerated extract; Emet: Crude hydro-methanolic macerated extract.
B: Bouchardat; Cat: Catechics; Gal: Gallics; - : Absence of bioactive compounds; +: Presence of bioactive compounds.

3.2 Antistaphylococcal power of the different extracts

The preliminary test used to authenticate the resistant profile of staphylococcal germs to oxa- cillin (Oxa), the reference antibiotic, showed that all the staphylococci studied are resistant to Oxa, therefore to methicillin (Méti). Indeed, Oxa is inactive on *S. aureus* (ATTC 25923) at MIC > 0.125 ± 0.00 µg/mL, on S. aureus (Méti-R 2062C/15) at MIC > 2 ± 0.00 µg/mL, on *S. aureus* (Méti-R 2063 C/15) at MIC > 0.5 ± 0.00 µg/mL and on *S. epidermidis* (Méti-R 2070C/15) at MIC > 4 ± 0.00 µg/mL.

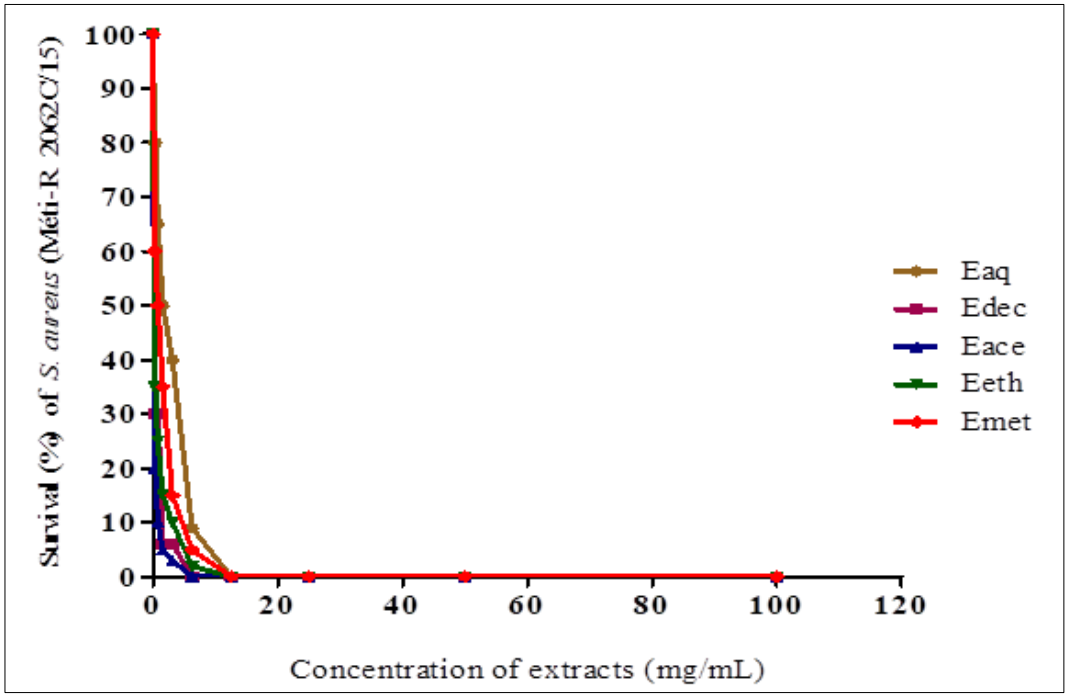
Furthermore, Figures 1 to 4 illustrate the antistaphylococcal power of the various extracts tested. They show a dose-response relationship between the survival rate of the staphylococci studied and the concentration of extract from the culture media. Indeed, as the concentration of extract (mg/mL) increases in the culture medium, the turbidity intensity of the staphylococci decreases. This result made it

possible to conclude that all the extracts tested have an antibacterial power on the staphylococci studied. This result is comparable to that obtained by [32] and by [7] during their work with *C. micranthum*. According to [33], the synergistic action of alkaloids, steroids, terpenoids and polyphenols (acid phenols and simple phenols, flavonoids, saponins, tannins) would be the cause of the observed antistaphylococcal power. Indeed, phenolic substances such as flavonoids, P-coumaric acid, caffeic acid, saponins, tannins are quite widespread in plants and have a wide spectrum of antibacterial activity [34, 35, 36]. Alkaloids such as sanguinarine and coraline, as well as steroids and terpenoids are active against pathogenic bacteria [37, 38]. These substances would act in synergy to varying degrees on the 50S subunit of the bacterial ribosome by slowing down the growth of the bacteria or by destroying them. This is demonstrated by the differences recorded at the level of the MICs, IC50s and CMBs (Table II). This result justifies, in part, the use and the effect of *C. racemosum* in the treatment of microbial diseases in a traditional environment.



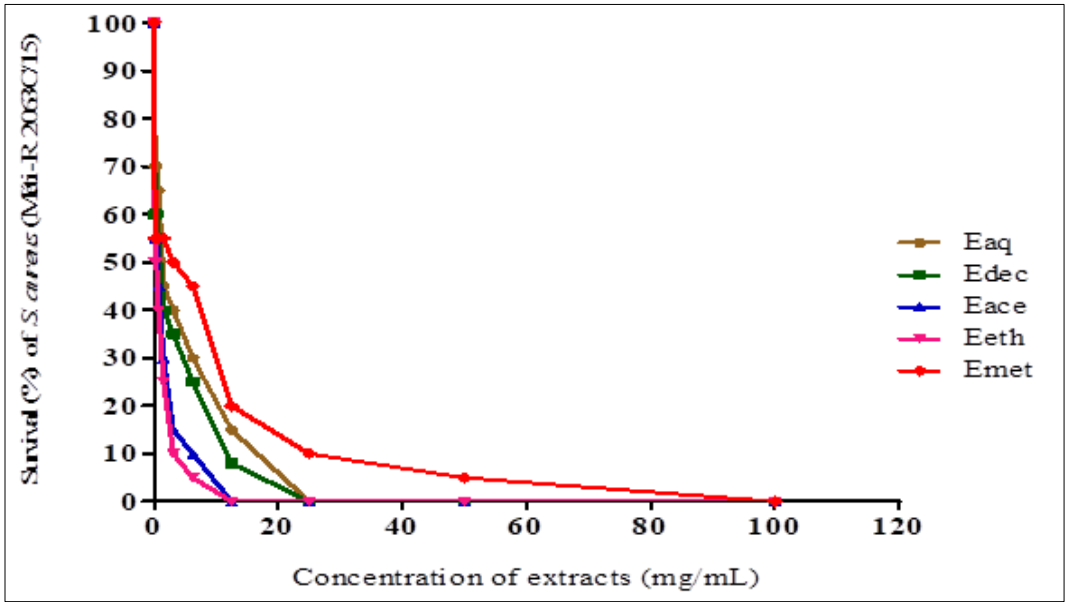
Eaq: Raw macerated aqueous extract; Edec: decocted aqueous raw extract; Eace: Crude hydro-acetic extract of macerated ethyl; Eeth: Crude hydro-ethanolic macerated extract; Emet: Crude hydro-methanolic macerated extract.

Fig 1: Survival rate (%) of *Staphylococcus Aureus* (ATTC 25923) as a function of the concentration of extract (mg/mL) of the leaves of *C. racemosum*



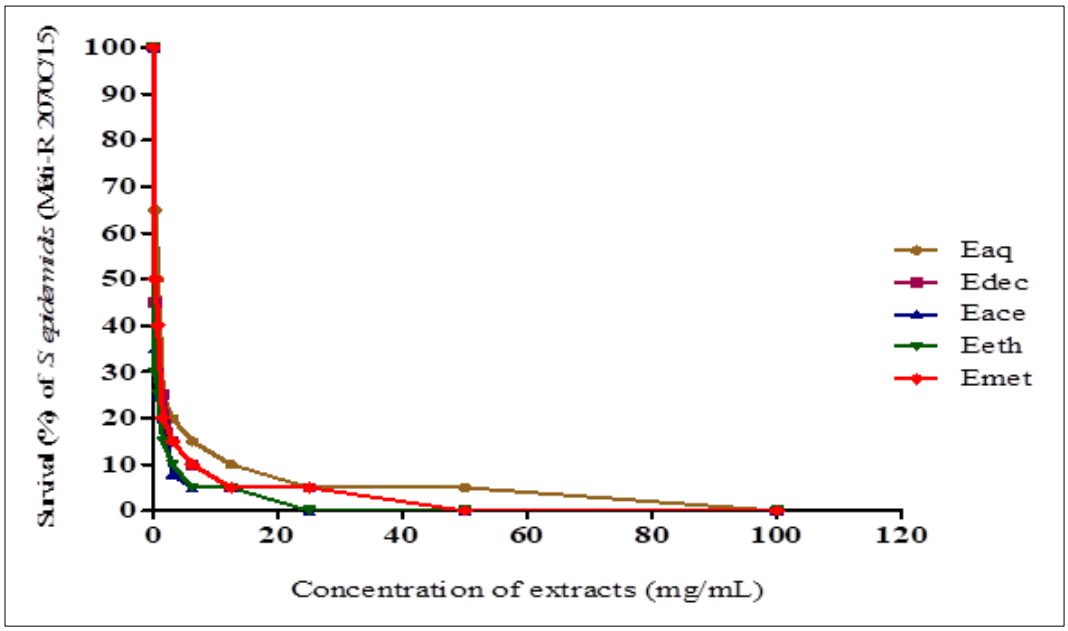
Eaq: Raw macerated aqueous extract; Edec: decocted aqueous raw extract; Eace: Crude hydro-acetic extract of macerated ethyl; Eeth: Crude hydro-ethanolic macerated extract; Emet: Crude hydro-methanolic macerated extract.

Fig 2: Survival rate (%) of *Staphylococcus Aureus* (Méti-R 2062C/15) according to the concentration of extract (mg/mL) of the leaves of *C. racemosum*



Eaq: Raw macerated aqueous extract; Edec: decocted aqueous raw extract; Eace: Crude hydro-acetic extract of macerated ethyl; Eeth: Crude hydro-ethanolic macerated extract; Emet: Crude hydro-methanolic macerated extract.

Fig 3: Survival rate (%) of *Staphylococcus Aureus* (Méti-R 2063 C/15) according to the concentration of extract (mg/mL) of the leaves of *C. racemosum*



Eaq: Raw macerated aqueous extract; Edec: decocted aqueous raw extract; Eace: Crude hydro-acetic extract of macerated ethyl; Eeth: Crude hydro-ethanolic macerated extract; Emet: Crude hydro-methanolic macerated extract.

Fig 4: Survival rate (%) of *Staphylococcus epidermidis* (Méti-R 2070C/15) in function of extract concentration (mg/mL) of *C. racemosum* leaves

The antibacterial parameters (CMI, CMB, IC50), the efficacy ratio (CMB/CMI) and above all the type of antistaphylococcal power of the different extracts tested are presented in Table II. The MICs > 625 pg/mL in this study showed that the antistaphylococcal power of the extracts on all the staphylococci studied is low according to [39]. For [39], in fact, the potency of a plant extract is significant when the MIC < 100 pg/mL, it is said to be moderate if 100 pg/mL < MIC ≤ 625 pg/mL and is low if the MIC > 625 pg/mL. Similarly, the statistical analysis indicated that the different observations at the level of the MICs and the CMBs were not significant ($p > 0.05$) for the staphylococci studied. Therefore, the activity of the extracts is not statistically influenced by the sensitive phenotype of the different staphylococci subjected to experimentation.

According to the CMB/CMI ratio ≤ 2 for some extracts and CMB/CMI > 2 for others, Eace, Eeth and Emet are bactericidal on all the staphylococci studied, Eaq is bactericidal on *S. epidermidis* and bacteriostatic on the rest of the bacteria, Edec is bacteriostatic on *S. aureus* (Méti-R 2062) and bactericidal on the rest of the bacteria according to [21]. According to this author, an antimicrobial substance is said to be bactericidal if its CMB/CMI ratio ≤ 2, and bacteriostatic when its CMB/CMI ratio > 2. The bactericidal extracts would have acted on the bacterium by attacking the peptidoglycan of the cell wall, which would have caused a destabiliza- tion of

the bacterium and led to its death [40]. As for the bacteriostatic extracts, they would have acted on the protein system of the staphylococcus, by binding to the 50S subunit of the ribosome, thus leading to an inhibition of protein synthesis. The staphylococcus would not have died, but it could not grow or multiply. These bacteriostatic extracts would therefore have inhibited the multiplication of these staphylococci without killing them. Of all the extracts tested in this study, Eeth showed the lowest IC_{50}. It acted faster on all the staphylococci studied, compared to the other extracts. The lowest IC_{50} at which this extract acted on staphylococci is 0.0161 ± 0.0336 mg/mL. This value was obtained on *S. aureus* (ATTC 25923), which thus confirms that the Eeth extract has a more effective action against *S. aureus* (ATTC 25923). This result leads to the conclusion that Eeth is the best antistaphylococcal extract tested. Hence, it could be useful to treat staphylococci of the skin, cutaneous appendages, mucous membranes, and to fight against staphylococci responsible for these diseases. The effectiveness of Eeth is due to the presence of alkaloids, saponins, steroids, terpenoids and tannins. These phytomolecules are appropriate, directed against the bacteria studied. These compounds could include P-coumaric acid which works by preventing the growth of *Staphylococcus Aureus* [41], as well as caffeic acid. Of the two aqueous extracts (Eaq and Edec) tested on staphylococci, the aqueous decoction (Edec) presented the slowest speeds of action, compared to the aqueous macerated (Eaq). This result leads to the conclusion that the Edec of the leaves of *C. racemosum* is more active on the bacterial isolates than the Eaq. Alkaloids, flavonoids, catechic and gallic tannins are mainly responsible for this interesting activity obtained in Edec. This result is consistent with that of the work of [42, 42] compared the antibacterial activity of macerated and aqueous decoction of three Combretaceae: *Combretum micranthum* (roots), *Guiera senegalensis* (roots) and *Terminalia avicennioides* (leaves and roots). The constituents previously isolated from the leaves of *C. micranthum* are alkaloids, flavonoids, catechic and gallic tannins. The leaves of *G. senegalensis* have been found to be rich in tannins and also contain alkaloids. In view of the results obtained, it was noticed that overall, the decoctions are more active than the macerated ones, except for *G. senegalensis* [41] attributed this activity to the presence of tannins.

Table 1: Antibacterial parameters and type of antistaphylococcal potency (type of activity) of extracts from the leaves of *C. racemosum*

`Strains	Raw extracts	CMI` (mg/mL)	Antibacterial parameters	CMB (mg/mL)	Efficiency report) (CMB/CMI)	Type of activity
Staphylococcus Aureus (ATTC 25923)	Eaq	25 ± 0.00a	100 ± 0.00a	0.1200 ± 0.015b	4	Bacteriostatic
	Edec	25 ± 0.00a	50 ± 0.00a	0.1128 ± 0.0152b	2	Bactericidal
	Eace	6.25 ± 0.00a	12.5 ± 0.00a	0.0271 ± 0.0251"	2	Bactericidal
	Eeth	3.125 ± 0.00a	6.25 ± 0.00a	0.0161 ± 0.0336b	2	Bactericidal
	Emet	12.5 ± 0.00a	25 ± 0.00a	0.0315 ± 0.0172b	2	Bactericidal
Staphylococcus Aureus (Meti-R 2062C/15)	Eaq	12.5 ± 0.00a	50 ± 0.00a	1.5625 ± 0.00b	4	Bacteriostatic
	Edec	6.25 ± 0.00a	25 ± 0.00a	0.1262 ± 0.0150"	4	Bacteriostatic
	Eace	6.25 ± 0.00a	12.5 ± 0,00a	0.1052 ± 0.0150"	2	Bactericidal
	Eeth	12.5 ± 0.00a	12.5 ± 0,00a	0.1128 ± 0.015"	1	Bactericidal
	Emet	12.5 ± 0.0a	25 ± 0.00a	0.3906 ± 0.0b	2	Bactericidal
Staphylococcus Aureus (Meti-R 2063C/15)	Eaq	25 ± 0.00a	100 ± 0.0a	1.900 ± 0.600"	4	Bacteriostatic
	Edec	25 ± 0.00a	25 ± 0.00a	0.9766 ± 0.5859"	1	Bactericidal
	Eace	12.5 ± 0.00a	25 ± 0.00a	0.1930 ± 0.0976"	2	Bactericidal
	Eeth	6.25 ± 0.00a	12.5 ± 0.00a	01617 ± 0.0336b	2	Bactericidal
	Emet	25 ± 0.00a	25 ± 0.00a	1.125 ± 0.22 lb	1	Bactericidal

Table 2 : (suite) Paramètres antibactériens et type de pouvoir (type d'activité) antistaphylococcique des extraits des feuilles de *C. racemosum*

Staphylococcus epidermidis (Méti-R 2070C/15)	Eaq	100 ± 0.00^a	100 ± 0.00^a	0.3906 ± 0.00^b	1	Bactericidal
	Edec	50 ± 0.00^a	50 ± 0.00^a	0.3315 ± 0.0172^b	1	Bactericidal
	Eace	25 ± 0.00^a	25 ± 0.00^a	0.2161 ± 0.0336^b	1	Bactericidal
	Eeth	25 ± 0.00^a	25 ± 0.00^a	0.2161 ± 0.0336^b	1	Bactericidal
	Emet	50 ± 0.00^a	100 ± 0.00^a	0.3262 ± 0,015^b	2	Bactericidal

Eaq: Raw macerated aqueous extract; Edec: decocted aqueous raw extract; Eace: Crude hydro-acetic extract of macerated ethyl; Eeth: Crude hydro-ethanolic macerated extract; Emet: Crude hydro-methanolic macerated extract; MIC: Minimum Inhibitory Concentration; CMB: Minimum Bactericidal Concentration;

IC_{50}: Concentration for Fifty percent Inhibition. On the same line, the MICs, CMFs and CI_{50}s assigned the same letter are not significantly different at the 5% level ($p > 0.05$).

3. Conclusion

The general objective of this study was to develop a new antistaphylococcal agent from a natural source through the evaluation of the antistaphylococcal power of five crude extracts from the leaves of *C. racemosum*.

The preliminary result of the phytochemical screening of the different extracts showed that the leaves of *C. racemosum* contain alkaloids, sterols, terpenes, total polyphenols, and polyphenols of the flavonoid, saponin and tannin types (catechic and gallic) to varying degrees concentrations.

The result of the antistaphylococcal test showed that all the extracts tested have an antistaphylo- coccal power on the staphylococci studied. However, Eeth is the most effective extract on staphylococci, activity attributable to alkaloids, saponins, sterols, terpenes and especially tannins. Of the crude aqueous extracts (Eaq and Edec) tested, preference should be given to the aqueous decoction (Edec).

Considering its potential antistaphylococcal power revealed in this study, Eeth could, after a toxicological study, serve as a food preservative against staphylococci and as an alternative to antibiotics against staphylococcal infections of the skin, mucous membranes and skin appendages in farm animals and humans.

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