WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 13, Issue 17, 20-38. Research Article ISSN 2277–7105

EVALUATION OF THE ANTISTREPTOCOCCAL POWER OF PHYTOEXTRACTS OF COMBRETUM RACEMOSUM P. BEAUV. (COMBRETACEAE)

Kamou Kamou Richard[1]*, Dibi Emmanuel Aya Diane Boudouin[1], Tra-Bi Ottis[2], Ouattara Karamoko[1], Nanga Yessé Zinzendorf[3], Gnahoué Gouéh[2] and Coulibaly Adama[4]

[1]*Polytechnic University of San-Pedro, UFR Agriculture, Fisheries Resources and Agro-Industry, 01 BP 1800 San-Pedro 01, Ivory Coast.*
[2]*Higer Normal School (ENS) of Cocody, Laboratory of Biochemistry-Microbiology (Section SVT), 08 BP 10 Abidjan 08, Ivory Cost.*
[3]*University Félix Houphouët-Boigny of Cocody, UFR of Pharmaceutical and Biological Sciences, Laboratory of Bacteriology-Virology, 22 BP 582 Abidjan 22, Ivory Cost.*
[4]*University Félix Houphouët-Boigny of Cocody, UFR Biosciences, Biology and Health Laboratory, 22 BP 582 Abidjan 22, Ivory Cost.*

Article Received on
10 July 2024,
Revised on 31 July 2024,
Accepted on 20 August 2024
DOI: 10.20959/wjpr202417-33472

***Corresponding Author**
Kamou Kamou Richard
Polytechnic University of San-Pedro, UFR Agriculture, Fisheries Resources and Agro-Industry, 01 BP 1800 San-Pedro 01, Ivory Coast.

ABSTRACT

Streptococcus pyogenes is becoming increasingly worrying due to its pathogenicity in live-stock and human populations, its resistance to first-line antibiotics, its involvement in cross-contamination of food and food production chains in agro-industry. In addition, it is the third deadliest bacteria in the world, and is the cause of various infections causing approximately 517,000 deaths per year worldwide. The aim of this study was to develop, through experimentation, an innovative, effective and affordable phytomolecule for the microbiological control of bacterial species of the Streptococcus genus in livestock and humans. To do this, a preliminary phytochemical analysis was carried out on five (5) crude aqueous and hydro-organic extracts of the leaves of *C. racemosum*, an ivorian medicinal plant. The in-vitro antistreptococcal power of these extracts was tested on *S. pyogenes* (ATCC® 19615™) and on *S. pyogenes* (20751), two (2) multi-resistant clinical isolates of S*treptococcus pyogenes*, a bacterial species often identified in animal and animal infections. human. The antibacterial test carried out on *S. pyogenes* (ATCC® 19615™) and on *S. pyogenes* (20751) showed that these bacteria are sensitive to each plant extract according to a dose-response relationship at

concentrations ranging from 3.125 to 100 mg/mL, and that Eeth constitutes the most effective antibacterial extract on the streptococci studied. This partly justifies the use of *C. racemosum* in Ivorian traditional therapy against superficial infections of the skin, skin appendages and mucous membranes. For these extracts, the recorded MIC varied between 3.125 ± 0.00 mg/mL and 25 ± 0.00 mg/mL, while the MBC varied between 6.25 ± 0.00 mg/mL and 100 ± 0.00 mg /mL. Preliminary triphytochemistry of the five crude extracts revealed the presence of alkaloids, saponins, steroids, terpenoids and tannins (catechic and gallic) at varying degrees of concentrations in this study. Eeth, the most active extract in this study, contained the bioactive molecules mentioned above in varying concentrations. This study shows that the macerated hydroethanolic crude extract (Eeth) constitutes the most active fraction of all the five crude extracts tested in this work. Consequently, it could, subject to toxicological study, be used for the fight against streptococci and the treatment of superficial streptococcal infections of the skin, mucous membranes and skin appendages in livestock and humans.

KEYWORDS: *Combretum racemosum*, antistreptococcal power, phytoextracts.

1. INTRODUCTION

Streptococcus pyogenes, a parasitic bacteria of the streptococcus genus, is becoming increasingly pathogenic in animal populations on livestock farms and in humans, resistant to first-line antimicrobials, aforementioned of foods and food production chains in agro-industry.[1,2] Throughout the world and in Côte d'Ivoire, various strains of *S. pyogenes* involved in these afore- mentioned cases have been identified.[3]

These strains of *S. pyogenes* are the majority of the cause of the various fatal streptococcal infections identified in animals and humans.[4] Among these infections, we note streptococcal pharyngitis which is the most common, rheumatic heart disease and necrotizing fasciitis and streptococcal toxic shock syndrome.[5,6,7,8] These infections have various locations, including those of the skin, skin appendages and mucous membranes and are responsible for approximately 517,000 deaths per year worldwide.[9] However, the skin, skin appendages and mucous membranes constitute the first immune barrier of any living body against exogenous attacks, particularly microbial ones.[10] In addition, these infections are commonplace, often recurrent, difficult to cure in certain cases and can be the cause of serious illnesses, including HIV infection. Likewise, widespread among severely immuno-depressed subjects, these infections have experienced a marked expansion in recent years.

Furthermore, despite the considerable progress made, microbial resistance has compromised made use of the achievements of modern medicine. They remind us that the fight against these infections is not yet over.[11] Thus, the need to search for new natural phyto-medicines, effective, of low toxicity and easily accessible to populations of all social strata, is essential. In Côte d'Ivoire, various medicinal plants including *Terminalia laxifolia*, *Parquetina nigrescens*, *Combretum racemosum*, *Bersama abyssinica*, have been the subject of studies for their antimicrobial activities.[12,13,14] 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.[15,16] Its potential could then play an important role in the fight against streptococcal infections.

The aim of this work was to develop, through experimentation, an innovative, effective and affordable phytomolecule for the microbiological control of bacterial species of the streptococcus genus and their superficial infections linked to the skin, skin appendages and mucous membranes. in livestock and humans. It consisted of characterizing essential biomolecules from *C. racemosum* leaves and testing their antistreptococcal power in five crude aqueous and hydro-organic extracts.

II. MATERIAL AND METHODS

2-1. Plant material

The plant material used consisted of leaves of *Combretum racemosum*. The authentication was made at the National Floristic Center of the Félix Houphouët Boigny University of Abidjan-Cocody, by comparison with specimen N^{o} 16949 deposited on July 17, 1985.

2-2. Streptococcal material

The streptococcal support consisted of clinical isolate of *Streptococcus pyogenes* (20751). This isolate was provided by the CeDReS Bacteriology Department. Quality control was ensured by the use of a reference strain *Streptococcus pyogenes* (ATCC® 19615™). The latter was provided by the Microbiology Department of the National Public Health Laboratory (LNSP) of Ivory Cost.

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 streptococcal germ used. Oxacillin is a spectrum bactericide narrow member of the Beta-lactam family, of the penicillin group M.

2-4. Plant treatments and spraying

After authentication, the *C. racemosum* leaves were carefully sorted and removed from foreign bodies. They were cut, washed with distilled water, dried away from the sun and in the open air for one (1) week.

At the end of 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 ^{o}C for the preparation of the different extracts.

2-5. Preparation of aqueous crude extracts

The crude macerated aqueous 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 through hydrophilic cotton and once through 3 mm Wattman paper. The filtrate obtained was concentrated in an oven at 50 °C for one (1) week to give the macerated aqueous crude extract (Eaq).[17] The decocted aqueous crude extract (Edec) was obtained according to the method of.[18]

Thus, one hundred grams (100 g) of the dried leaf powder was first dissolved in one liter (1 L) of cold distilled water in a container at 37 ^{o}C. Everything was boiled for 15 min on a hot plate at 100 °C. After cooling and homogenization in a blender at room temperature (37 ^{o}C), the mixture obtained was drained in a square of white cloth, then filtered twice through hydrophilic cotton, and once through Wattman 3 mm paper. The filtrate obtained was concentrated in an oven at 50 °C for one (1) week.

2-6. Preparation of macerated hydro-organic crude extracts

The crude ethyl hydroacetate extract (Eace) was prepared according to the method described by.[19] To do this, one hundred grams (100 g) of leaf powder were 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 ^{o}G.L), then homogenized in a blender at 37 ^{o}C. Each homogenate

obtained was first drained through a square of white cloth, then filtered twice through hydrophilic cotton and once through 3 mm Wattman paper. The filtrate obtained was concentered in the oven at 50 °C for one (1) week.

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

2-7. Characterization of the biomolecules of the different crude extracts

The phytochemical study carried out according to the methods described by[20, 21] 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 colored indicators based on the reduction in a medium (alkaline or basic) of the reagent mixture by the oxidizable groups of the secondary metabolites, leading to the formation of reduction products of color which depends on the environment. For each extract, 10 identification tests by color reactions were carried out. Solutions with indicators have a positive reaction, this indicates the presence of bioactive compounds in the extracts of *C. racemosum* leaves.

2-7. Evaluation of the antistreptococcal effect of different extracts of *C. racemosum*

2-7.1. Sterility test of different extracts

The aim of this test was to verify that the extract does not contain any bacterial or fungal germs. To do this, 0.1 g of the extract to be tested was first diluted in 10 mL of thioglycholate broth then incubated at 37°C for 24 hours. After this period, the turbidity of the broth is assessed by eye. This broth was then inoculated on a Petri dish containing the nutrient agar and, on another dish, containing the Sabouraud agar, finally incubated under the same conditions, for three days with an observation every 24 hours to check if germs grew in Petri dishes. The substance is declared sterile if no colonies are visible on the agar plate.[22]

2-7.2. Preparation of the concentration range 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. Everything was brought to a boil for 1 minute, distributed into clean, dry vials and sterilized by autoclave at 121 °C for 15 minutes.

The range of concentrations for each extract was prepared in test tubes according to the double dilution method with the geometric progression of reason ½.[23] 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 distributed 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 contents of T_1 were transferred into the T_2 tube. This operation was repeated to prepare the next tube and so on until tube T_{10}. Thus, a range of extract concentrations was obtained ranging from 200 to 0.3906 mg/mL. All culture tubes thus obtained were retained for inoculation.

2-7.3. Sensitivity test of streptococcal strains to different extracts

The bacterial inoculum was prepared from a 24-hour young colony. To do this, two 24-hour bacterial colonies were taken 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 bacte- ria/mL.

The test of bacterial sensitivity to extracts characterized by inoculation of the range of extract concentrations was carried out according to the method of[24] and of.[23] 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”. Into 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 done so that 1 mL of extract of 200 mg/mL was transferred into tube H_1, that of 100 mg/mL into tube H_2, and so on until tube H_{10} which was received 1 mL of extract of 0.1953 mg/mL. The H_C tube which serves as a bacterial growth control control received, instead of extract, 1 mL of sterile distilled water. Finally, 1 mL of sterile Müeller-Hinton Broth, concentrated twice 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 extract medium to half. Thus, the concentration of tube H_1 increased 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) hemolysis tubes of cultures with

concentrations ranging from 100 to 0.1953 mg/mL were obtained. After a first measurement of the initial turbidity value of each culture using "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.

2-7.4. Determination of the survival percentage of streptococcal germs

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

The percentage of streptococcal survival was obtained stepwise. First, the growth of streptococci was carried out by making the difference between the density value measured before incubation and that after 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 of calculating the percentage of survival of streptococcal germs in the test tubes was done according to the following formula.

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

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

2.7.5. Determination of antibacterial parameters

The MIC is the lowest concentration of *C. racemosum* extract for which there is absence turbidity. Its determination was made from the measurement of the turbidity induced by the growth of the streptococci studied. It therefore corresponds to the concentration of the first tube from which no disorder was observed with the naked eye. Therefore, this is the first tube where the di value is equal to df (di = df). This operation was repeated 3 times in a row.[25] All tubes in which there was an absence of streptococcal germs are kept for the determination of the Minimum Bactericidal Concentration (MBC).

The CMB is the lowest concentration of extract in the tube that leaves at most 0.01 % streptoco-viable compared to the initial inoculum. After reading the CMI, the contents of the tubes of tests where there was an absence of visible growth of germs was sown in lines 5 cm long, on a Müeller-Hinton agar plate, starting with the CMI tube. This series of Petri dishes is named B. Next, 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 of 5 cm long, on another Müeller-Hinton agar plate, at the using a calibrated 2 µL loop, then incubated for 24 hours at 37 °C.[22] 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 MBC was determined by comparing the bacterial growth of dishes A and B.[12] Thus, the smallest tube concentration that has less than 0.01 % viable bacteria relative to the initial inoculum is the CMB.

2-7.6. Determination of antistreptococcal 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/MIC ratio is less than or equal to 2, on the other hand it is said to be bacteriostatic if the CMB/MIC is greater than 2.[25]

2-7.7. Statistical analyzes

The antibacterial parameter values 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 varying degrees of concentrations of four (4) main groups of active ingredients in this work. The first group represented by free quinones was completely absent from all the extracts analyzed. 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 biologically active molecules highlighted is the same in the crude

extracts Eaq, Edec and Emet. The raw extracts Eace and Eeth have practically the same profile (Table I).

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-type polyphenols.[26]

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.[27] Eaq, Edec and Emet therefore best extract the active ingredients sought in the leaves of *C. racemosum* in this work. These biomolecules are already known for their antimicrobial activities.[28,29,26] This result is consistent with those obtained by[30,31,32] and by[33] during their work. The various chemical groups revealed in the leaves of *C. racemosum* give the plant its numerous therapeutic indications in a traditional environment, and confirms its activity against infections.

The third analysis of phytochemical results indicates that alkaloids, steroids, terpenoids, as well as saponin and tannin polyphenols are more abundant in Eeth. This result would be due to the fact that polyphenols are generally more soluble in hydro-alcoholic mixtures.[27]

The latest phytochemical analysis noted that alkaloids, steroids, terpenoids and saponin-like polyphenols are more abundant in Eace. The presence of terpenoids would be 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 phytochemical compounds.[33]

Additional analysis of the phytochemistry results led to the conclusion that extracts aqueous raw materials (Eaq and Edec) analyzed, the aqueous decoction (Edec) best extracts the active ingredients compared to the aqueous macerated (Eaq). The reason would lie in the extraction method and the effect of temperature. In the present study, the extraction by decoction at high temperature (100 ^{o}C) as well as the exhaustion of water at reduced pressure made it possible to obtain the maximum of biomolecules while preventing denaturation or probable modification of these substances.[34] This result validates the traditional form of use (decocted) of *C. racemosum* leaves in certain regions of Ivory Cost.

According to another additional analysis of the phytochemistry results, the crude extracts with water (Eaq and Edec) best extract the identified biomolecules, compared to the crude extracts with water-organic solvents (Eace, Eeth and Emet). This result could be explained by the fact that water is more polar than the hydro-organic 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 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 bioactive compounds		Crude aqueous extracts		Crude hydro-organic extracts		
		Eaq	Edec	Eace	Eeth	Emet
Alkaloids	B	+	+	+	++	+++
Flavonoids		+	++	-	-	+
Total polyphenols		+	++	-	-	+
Free quinones		-	-	-	-	-
Saponins		+	++	+	+	+++
Steroids		+	++	+++	++	+
Terpenoids		+	++	+++	++	+
Tannins	Cat	+	++	-	+	+
	Gal	+	+	-	+	+

Eaq: Macerated aqueous crude extract; Edec: Decocted aqueous crude extract; Eace: Macerated ethyl hydroacetate crude extract;

Eeth: Crude hydro-ethanolic extract macerated; Emet: Crude hydro-methanolic extract macerated.

B: Bouchardat; Cat: Catechics; Gal: Gallics; - : Absence of bioactive compounds; +: Presence of bioactive compounds.

3-2. Antistreptococcal power of different extracts

The preliminary test which was used to authenticate the resistant profile of streptococcal germs to oxacillin (Oxa), the reference antibiotic, showed that all the streptococci studied are resistant to Oxa, therefore to methicillin (Méti). Indeed, Oxa is inactive on *S. pyogenes*

(ATCC® 19615™) at the MIC > 0.125 ± 0.00 µg/mL, and on *S. pyogenes* (20751) at the MIC > 2 ± 0.00 µg/mL.

Furthermore, Figures 1 and 2 illustrate the antistreptococcal power of the different extracts tested. They show a dose-response relationship between the survival rate of the streptococci studied and the concentration of extract from the culture media. Indeed, as the extract concentration (mg/mL) increases in the culture medium, the turbidity intensity of the streptococci decreases. This result made it possible to conclude that all the extracts tested have antibacterial power against the streptococci studied. This result is comparable to that obtained by[35] and by[11] during their work with *C. micranthum*. According to[36], the synergistic action of alkaloids, steroids, terpenoids and polyphenols (phenol acids and simple phenols, flavonoids, saponins, tannins) would be the cause of the observed antistreptococcal power. Indeed, phenolic substances such as flavonoids, P-coumaric acid, caffeic acid, saponins, tannins are quite widespread in plants and have a broad spec- trum of antibacterial activity.[37,38,39] Alkaloids like Sanguinarine and Coraline, as well as steroids and terpenoids are active against pathogenic bacteria.[40,41] These substances act in synergy to varying degrees on the 50S subunit of the bacterial ribosome by slowing down the growth of the bacteria or destroying them. This is demonstrated by the differences recorded at the level of MIC, IC_{50} and CMB (Table I). This result justifies, in part, the use and effect of *C. racemosum* in the treatment of microbial conditions in a traditional environment.

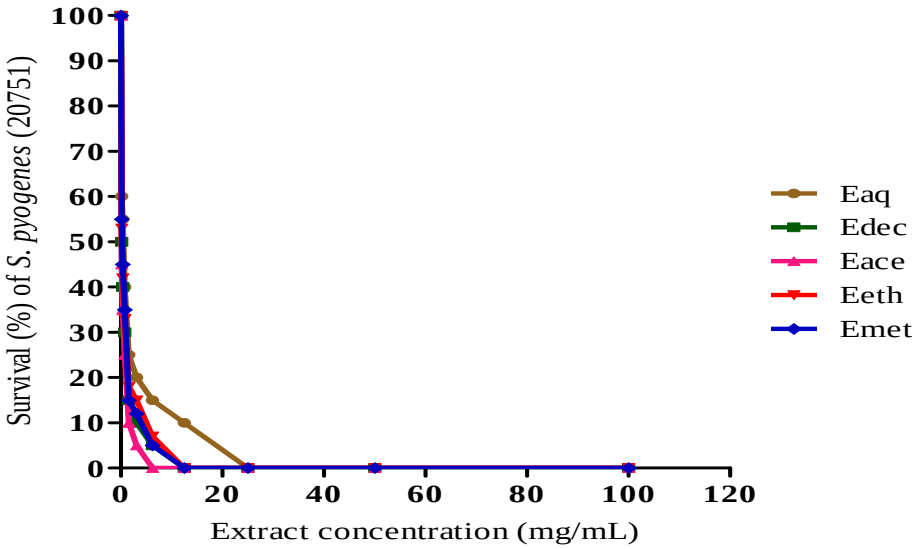

Figure 1: Survival rate (%) of *Streptococcus pyogenes* (20751) depending on the extract concentration (mg/mL) of *C. racemosum* leaves.

Eaq: Macerated aqueous crude extract; Edec: Decocted aqueous crude extract; Eace: Macerated ethyl hydroacetate crude extract;

Eeth: Crude hydro-ethanolic extract macerated; Emet: Crude hydro-methanolic extract macerated.

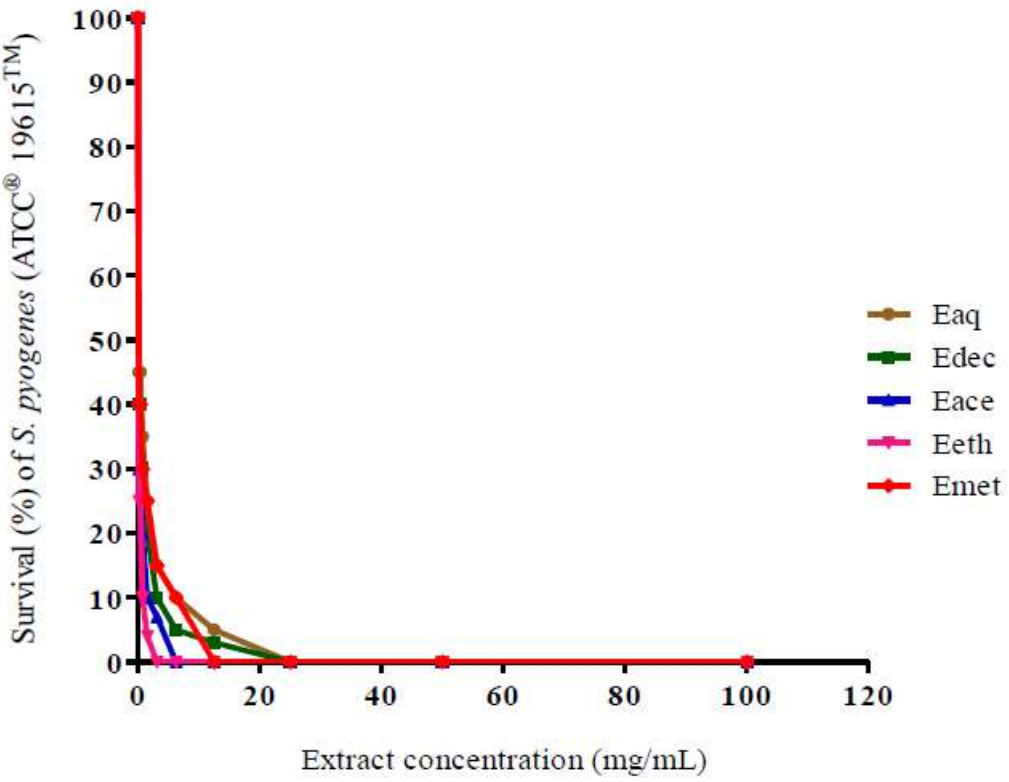

Figure 2: Survival rate (%) of *Streptococcus pyogenes* (ATCC® 19615TM) as a function extract concentration (mg/mL) of *C. racemosum* leaves.

Eaq: Macerated aqueous crude extract; Edec: Decocted aqueous crude extract; Eace: Macerated ethyl hydroacetate crude extract; Eeth: Crude hydro-ethanolic extract macerated; Emet: Crude hydro-methanolic extract macerated.

The antibacterial parameters (MIC, CMB, IC_{50}), the effectiveness ratio (CMB/MIC) and especially the type of antistreptococcal power of the different extracts tested are presented in Table I.

For *S. pyogenes* (ATCC® 19615™) and *S. pyogenes* (20751) studied, the extracts tested present an MIC which is 25 ± 0.00 mg/mL for Eaq, 6.25 ± 0.00 mg /mL for Eace, 12.5 ± 0.00 mg/mL for Emet, which varies between 12.5 ± 0.00 and 25 ± 0.00 mg/mL for Edec, between

3.125 ± 0 .00 and 12.5 ± 0.00 mg/mL for Eeth. Eeth presents the lowest MIC of the streptococci studied.

The CMB is 100 ± 0.00 mg/mL for Eaq, 12.5 ± 0.00 mg/mL for Eace and 25 ± 0.00 mg/mL for Emet, it oscillates between 25 ± 0.00 and 50 ± 0.00 mg/mL for Edec, and between 6.25 ± 0.00 and 25 ± 0.00 mg/mL for Eeth. Eeth shows the lowest minimum concentration which kills 99.99 % of the streptococci studied.

The IC_{50} recorded varies between 0.1100 ± 0.0140 and 2930 ± 0.0976 mg/mL for Eaq, between 0.1028 ± 0.0142 and 0.1280 ± 0.00 mg/mL for Edec, between 0.0171 ± 0.0241 and 0.1062 ± 0.0150 mg/mL for Eace, between 0.0061 ± 0.0326 and 0.1128 ± 0.0151 mg/mL for Eeth, and between 0.0215 ± 0.0162 and 0.1161 ± 0.0336 mg/mL for Emet. Eeth shows the lowest inhibitory concentration of 50 % (IC_{50}) of the streptococci studied (table I).

The MICs > 625 pg/mL in this study showed that the antistreptococcal potency of the *S. pyogenes* (ATCC® 19615™) and *S. pyogenes* (20751) extracts studied is low according to.[42] For[42], in fact, the power 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. Likewise, the statistical analysis indicated that the different observations at the level of MIC and CMB were not significant ($p > 0.05$) for the streptococcus studied. Consequently, the activity of the extracts is not statistically influenced by the sensitive phenotype of the Streptococcus subjected to the experiment.

According to the CMB/MIC ratio ≤ 2 for certain extracts and CMB/MIC > 2 for others, Edec, Eace, Eeth and Emet are bactericidal on the streptococci studied, Eaq is bacteriostatic on this same bacterium according to.[25] According to this author, in fact, an anti-microbial substance is said to be bactericidal if its CMB/MIC ratio ≤ 2, and bacteriostatic when its CMB/MIC ratio > 2. Edec, Eace, Eeth and Emet would have acted on *S. pyogenes* (ATCC® 19615™) and *S. pyogenes* (20751) by attacking the peptidoglycan of the wall, which would have caused destabilization of the bacteria and led to their death.[1] As for Eaq, it would have acted on the protein system of *S. pyogenes* (ATCC® 19615™) and *S. pyogenes* (20751), by attaching to the 50S subunit of the ribosome, thus leading to an inhibition of protein synthesis. *S. pyogenes* (ATCC® 19615™) and *S. pyogenes* (20751) would not have died, but they were unable to develop or multiply. Eaq would therefore have inhibited the multiplication of these streptococci without killing them.

Of all the extracts tested in this study, Eeth showed the lowest IC_{50}. It acted more quickly on *S. pyogenes* (ATCC® 19615™) and *S. pyogenes* (20751), compared to the other extracts. The lowest IC_{50} at which Eeth acted on streptococci is 0.0061 ± 0.0326 mg/mL. This allows us to confirm that the Eeth extract has a more effective action against *S. pyogenes* (ATCC® 19615™). This result allows us to conclude that Eeth constitutes the best antistreptococcal extract tested. Hence, it could be useful to treat streptococci of the skin, skin appendages, mucous membranes, and to fight against streptococci causing 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. Among these molecules, it could have P-coumaric acid which acts by preventing the growth of *S. pyogenes*[43], as well as caffeic acid.

Of the two aqueous extracts (Eaq and Edec) tested on streptococci, the aqueous decoction (Edec) presented the lowest speed of action, compared to the aqueous macerated (Eaq). This result allows us to conclude that Edec from the leaves of *C. racemosum* is more active on bacteria than Eaq. Alkaloids, flavonoids, catechic and gallic tannins are mainly responsible for this interesting activity obtained at Edec. This result is consistent with that of the work of[44].[44] 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 consti- tuents previously isolated from the leaves of *C. micranthum* are alkaloids, flavonoids, catechic and galic 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 noted that overall, the decoctions are more active than the macerated ones, except for *G. senegalensis*. [44] attributed this activity to the presence of tannins.

Table II: Antibacterial parameters and type of antistreptococcal power (type of activity) of leaf extracts *C. racemosum*.

Souche	Antibacterial parameters				Effectiveness report (CMB/CMI)	Type of activity
	Crude extracts	CMI (mg/mL)	CMB (mg/mL)	CI_{50} (mg/mL)		
Streptococcus pyogenes (20751)	Eaq	25 ± 0,00[a]	100 ± 0,00[a]	0,2930 ± 0,0976[b]	4	Bacteriostatic
	Edec	12,5 ± 0,00[a]	25 ± 0,00[a]	0,1280 ± 0,00[b]	2	Bactericidal
	Eace	6,25 ± 0,00[a]	12,5 ± 0,00[a]	0,1062 ± 0,0150[b]	2	Bactericidal
	Eeth	12,5 ± 0,00[a]	25 ± 0,00[a]	0,1128 ± 0,0151[b]	2	Bactericidal
	Emet	12,5 ± 0,00[a]	25 ± 0,00[a]	0,1161 ± 0,0336[b]	2	Bactericidal
Streptococcus	Eaq	25 ± 0,00[a]	100 ± 0,00[a]	0,1100 ± 0,014[b]	4	Bacteriostatic
	Edec	25 ± 0,00[a]	50 ± 0,00[a]	0,1028 ± 0,0142[b]	2	Bactericidal

pyogenes (ATCC® 19615™)	Eace	6,25 ± 0,00[a]	12,5 ± 0,00[a]	0,0171 ± 0,0241[b]	2	Bactericidal
	Eeth	3,125 ± 0,00[a]	6,25 ± 0,00[a]	0,0061 ± 0,0326[b]	2	Bactericidal
	Emet	12,5 ± 0,00[a]	25 ± 0,00[a]	0,0215 ± 0,0162[b]	2	Bactericidal

Eaq: Macerated aqueous crude extract; Edec: Decocted aqueous crude extract; Eace: Macerated ethyl hydroacetate crude extract; Eeth: Crude hydro-ethanolic extract macerated; Emet: Crude hydro-methanolic extract macerated; CMI: Minimum Inhibitory concentration; CMB: Minimum Bactericidal concentration;

CI_{50}: Concentration for fifty percent inhibition.

On the same line, the MICs, CMFs and IC_{50s} assigned the same letter are not significantly different at the 5 % threshold ($p > 0.05$).

3. CONCLUSION

This study has set itself the general objective of developing through experimentation, an innovative antibacterial phyto-molecule from a natural source, effective and affordable, for the microbiological control of bacterial species of the Streptococcus genus and their superficial infections, through phytochemical screening and evaluation of the antistreptococcal power of five crude extracts of *Combretum racemosum* leaves. This study set itself the general objective of developing a new antibacterial from a natural source through the evaluation of the antistreptococcal power of five crude extracts of *C. racemosum* leaves.

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 antistreptococcal test showed that all the extracts tested have anti-streptococcal power on the streptococci studied.

However, Eeth constitutes the most effective extract against streptococci, activity attributable to alkaloids, saponins, sterols, terpenes and especially tannins. Of the aqueous crude extracts (Eaq and Edec) tested, it would be advisable to favor the aqueous decoction (Edec).

Given its potential antistreptococcal power revealed in this study, Eeth could, after a toxico-logical study, serve as a phytomedicine to fight against streptococci and streptococcal infections of the skin, mucous membranes and skin appendages in farm animals and humans.

REFERENCES

1. Battraud P. (2017). La résistance aux antibiotiques, un mythe ou une réalité ? Thèse de Doctorat, Université de Lille 2, France, 119p.
2. CDC (Centers for Disease Control and Prevention) (2017). About Antimicrobial Resistance. Antibiotic/Antimicrobial Resistance (AR/AMR). Saving Lives, Protecting People, 7-24. https://www.cdc.gov/drugresistance/about.html. Consulté le 24/05/2019.
3. Vela A.L., Villalòn P., Sàez-Nieto J.A., Chacòn G., Domingez L. et Fernàndez-Garayzabal J.F. (2017). Caractérisation de *Streptococcus pyogenes* à partir d'échantillons cliniques animaux, Espagne. *Urgence Infectious Diseases*, 23(12): 2011-2016.
4. Carapetis J. R., Steer A.C., Mulholland E.K. et Weber M. (2005). The global burden of group A streptococcal diseases. *The Lancet Infectious Diseases*, 5(11): 685-694.
5. Walker M.J., Barnett T.C., McArthur J.D., Cole J.N., Gillen C.M., Henningham A. et Al. (2014). Disease manifestations and pathogenic mechanisms of Group A Streptococcus. Clinical microbiology reviews, 27(2): 264-301.
6. Franklinos, L. H., Efstratiou A., Macgregor S. K., John S. K., Hopkins T., Cunningham A. A. et Lawson B. (2015). *Streptococcus pyogenes* Infection in a Free-Living European Hedgehog (Erinaceus europaeus). Ecohealth, 12: 689-692.
7. Samir A., Abdel-Moein K. A. et Zaher H. M. (2020). Emergence of penicillin-macrolide-resistant *Streptococcus pyogenes* among pet animals: An ongoing public health threat. Comp. Immunol. Microbiol. Infect. Dis, 68: 101390.
8. Bryant A. E. et Stevens D. L., J. E. Bennett, R. Dolin, et M. J. Blaser (eds.), Mandell, Douglas, et Bennett's (2020). *Streptococcus pyogenes* Principles and Practice of Infectious Diseases, 9th ed., Elsevier, p. 2446.
9. World Health Organization (2005). The current evidence for the burden of Group A Strepto-coccal diseases.
10. Chambeaud F. (2012). Les staphylocoques en pathologie cutanée chez le chien : connaissances actuelles. Thèse d'Etat de Doctorat Vétérinaire**,** Université Claude-Bernard-Lyon I (Médecine -Pharmacie), 65-67.
11. Abreu A.C., McBain A.J. et Simoes M. (2012). Plants as sources of new antimicrobials and resistance modifying agents. *Natural Product Reports*, 29(9): 1007-1021.
12. Konan K.F., Guessennd K.N., Oussou K.R., Bahi C., Coulibaly A., Djaman A.J. et Dosso M. (2013). Effet antibactérien de l'extrait aqueux de l'écorce de *Terminalia glaucescens* Planch ex Benth (Combretaceae) sur la croissance *in vitro* des entérobactéries

productrices de bêta- lactamases à spectre élargi (EBLSE). *International Journal of Biological and Chemical Science*, 8(3): 1192-1201.

13. Béné K. (2014). Etude ethnobotanique des plantes médicinales utilisées dans le Département de Transua (Côte d'Ivoire). Mémoire de Master d'Ecologie Tropicale (Option Végétale), UFR Bio- sciences, Université Félix Houphouët-Boigny, Abidjan, Côte d'Ivoire, 50p.
14. Tiebre M.S., Ouattara D., Vroh B.T.A. et Zirihi G.N. (2015). Diversité des plantes médicinales utilisées dans le traitement traditionnel de la diarrhée sur les marchés d'Abidjan (Côte d'Ivoire). *Journal of Animal and Plant Sciences*, 26(2): 4081-4096.
15. Burkill H.M. (1985). The useful plants of west tropical Africa. Families A-D. 2nd Edition. The flora of west tropical Africa. Kew, Richmond, United Kingdom. *Royal Botanic gardens*, 960p.
16. Onocha P. A., Audu E.O., Ekundayo O. et Dosumu O.O. (2005). Phytochemical and anti-microbial properties of extracts of *Combretum racemosum*. *Acta Horticulturae, 675*, 1: 97-101.
17. Béné K., Camara D., Fofié N.B.Y. et Zirihi G.N. (2015). Etude ethnobothanique, activité antifongique *in-vitro* sur *Candida albicans* et toxicité sur les cellules HFF de *Hirrizonia-abissinica* Oliv. (*Simaroubaceae*), une plante de la pharmacopée ivoirienne. *Journal of Applied Biosciences*, 94(2015): 8815-8824.
18. Zirihi G. N. et Kra A. K. M. (2003). Evaluation de l'activité antifongique de *Microglossa Pirifolia* (*Lamarck*) O. Kuntze (*Asteraceae*) «Pymi» sur la croissance *in-vitro* de *Candida albicans*. *Revue de Médecine et Pharmacie Afrique*, 17: 11-19.
19. Ackah J.A.A.B, Kra A.M., Zirihi G.N. et Guédé-Guina F. (2008). Evaluation de l'activité antifongique de tekam, un extrait de plante, sur la croissance *in vitro* de *Candida albicans*. *Revue Ivoirienne Sciences Technologies*, 11: 119-129.
20. Harborne J.B. (1998). Phytochemical methods: A Guide to modern techniques of plants Analysis. 3rd Ed., Chapman and Hall, London, United Kingdom, 17-103.
21. Trease G. et Evans S.M. (2002). Pharmacognosy. 15th ed. English Language Book Society, London. *Bailliere Tindall*, 23-67.
22. Diaha K. H. (1999). Epidemiosurveillance de la résistance aux antibiotiques de l'Ecosystème Bactérien à Abidjan en 1997. Thèse en Médecine n°2259/99. UFR des Sciences Médicales, Université de Cocody Abidjan, 196p.

23. Koné W.M., Kamanzi A.K., Terreaux C., Hostettmann K., Traore D. et Dosso M. (2004). Traditional medicine in North Côte d'Ivoire: screening of 50 medicinal plants for antibacterial activity. *Journal of Ethnopharmacology*, 93: 43-49.
24. Dosso M. et Faye-Kette H. (2000). Contrôle de qualité de l'antibiogramme en pratique courante: Expérience du laboratoire de bactériologie de l'Institut Pasteur de Côte d'Ivoire, *Le Bactériologiste Internationale,* nº spécial, 53p.
25. Fauchere J.L. et Avril J.L. (2002). Bactériologie Générale et Médicale. *Ellipses*, 365p.
26. Cowan M.M. (1999). Plants products as anti-microbial activity. *Clinical Microbiology Reviews*, 12(4): 564-582.
27. Mahmoudi S., Khali M. et Mahmoudi N. (2012). Etude de l'extraction des composés phénoli- ques de différentes parties de la fleur d'artichaut: *Cynara scolymus* L. *Nature et Technologie*, 9(2013): 35-40.
28. Rizvi S.H, Shoeb A., Kapil R.S. et Popli P.S. (1980). Two diuretic triterpenoids from anti-dermamenasu. *Phytochemistry*, 19(11): 2409-2410.
29. Chodera A., Dabrowska A., Sloderbach A., Skrzypczak L. et Budzianowski J. (1991). Effect of flavonoid fractions of *Solidago virgaurea* L. on dieresis and levels of electrolytes. *Acta Polyclinic Pharmaceutical*, 48(5-6): 35-37.
30. Harbone J.P. (1991). Méthodes phytochimiques", 2nd Ed., Dakota du Nord. Londres. *Oxford Université Presse*, 4p.
31. Mcgaw L. J., Rabe T., Sarg S.G., Jager A.K, Eloff J.N. et Van S.J. (2001). An investigation on the biological activity of Combretum species, *Journal of Ethnopharmacology*, 75(1): 45-50.
32. Okwuosa O.M.T.B., Chukwura E.L., Chukwuma G.O., Enweani I.B., Agbakoba N.R., Chukwuma C.M., Manafa P.O. et Umedum C.U. (2012). Phytochemical and antifungal activities of the leaves and roots of *Uvaria chamae*, the leaves and bark of *Spondias mombi* and the leaves of *Combretum racemosum*. *African Journal of Medical Sciences*, 41: 99-103.
33. Green R.J. (2004). Antioxidant activity of Peanut Plant Tissues. [M.S. thesis], North Carolina State University, Raleigh, NC, USA. *Journal of Ethnopharmacology*, 60(1): 1-8.
34. Djahra A. B. (2014). Etude phytochimique et activité antimicrobienne, antioxydante, anti-hépatotoxique du Marrube blanc ou *Marrubium vulgare L*. *Researchgate.* Thèse de Doctorat, Université Badji Mocktar-Annaba, Algérie, 115p.
35. Malcolm S.A. et Sofowora E.A. (1969). Activité antimicrobienne de certains remèdes populaires nigérians et leurs plantes constituantes. *Lloydia*, 32: 512-517.

36. Burt S. (2004). Essential oils: their antibacterial properties and potential applications in food a review. *International Journal of Food Microbiology*, 94(3): 223-253.
37. Xiao Z.T., Zhu Q. et Zhang H.Y. (2014). Identifying antibacterial targets of flavonoids by comparative genomics and molecular modeling. *Open Journal of Genomics*, 3(1): 1-8.
38. Stojković D., Petrović J., Soković M., Glamočlija J., Kukić M. J. et Petrović S. (2013). *In-situ* antioxidant and antimicrobial activities of naturally occurring caffeic acid, p-coumaric acid and rutin, using food systems. *Journal of the Science of Food and Agriculture*, 93(13): 3205-3208.
39. Colak S.M., Yapici B.M. et Yapici A.N. (2010). Determination of antimicrobial activity of tannic acid in pickling process. *Romanian Biotechnological Letters*, 15(3): 5325-5330.
40. Giri P. et Kumar G.S. (2010). Molecular recognition of poly (A) targeting by protoberberine alkaloids: *in-vitro* biophysical studies and biological perspectives. *Molecular biosystems*, 6: 81-88.
41. Singla D., Sharma A., Kaur J.; Panwar B., Gajendra P.S. et Raghava. (2010). BIAdb: A curated database of benzylisoquinoline alkaloids. *Biomedical Center of Pharmacology*, 10(4): 1-8.
42. Kuete V., Tabopda T. K., Ngameni B., Nana F., Tshikalange T. E et Ngadjui B.T. (2010). Anti-mycobacterial, antibacterial and antifungal activities of *Terminalia superba* (*Combretaceae*). *South African Journal of Botany*, 76: 125-131.
43. Khatkar A., Nanda A., Kumar P et Narasimhan B. (2014). Synthesis, antimicrobial evaluation and QSAR studies of p-coumaric acid derivatives. *Arabian Journal of Chemistry*, 10(2): 3804-3815.
44. Bassene E., Mahamat B., Lo M., Boye C.S. et Faye B. (1995). Comparaison de l'activité antibactérienne de trois Combretaceae: *C. micranthum, Guiera senegalensis* et *Terminalia avicennioides*. *Fitoterapia*, 66(1): 86-87.