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Antibacterial Activity of Three Validated Antisickling Plant Species from the Great Apes Pharmacopoeia in Congo-Kinshasa

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Abstract

Sickle cell disease (SCD) is a hemoglobinopathy characterized by the presence in the blood of erythrocytes containing hemoglobin S. Surprisingly, the geographic distribution of SCD is superposed with that of the malaria which is also a haemolytic disease (like SCD) controlled by the great apes through their diet essentially vegetarian. Recent findings revealed that the plant species like *Carapa procera, Garcinia punctata* and *Pentaclethra macrophylla* belonging to non-human pharmacopoeia possess anti-sickle cell anaemia. In addition, many studies in sickle cell endemic areas indicate that the frequency of bacterial infections in SCD is high, especially in children under five years of age. The aim of the present study was to assess the antibacterial activity of the barks of *C. procera, G. punctata* and *P. macrophylla* in order to later use the bioactive fractions as an adjuvant of penicillin and use it as a starting point for a galenic formulation. This study shows that bacteriostatic activity was observed with all extracts on *Staphylococcus aureus resistant to methicillin* (*G. punctata*: MIC ≤ 62.5 µg/mL; *C. procera* and *P. macrophylla*: MIC ≤ 125 µg/mL) while several fractions and extracts revealed bactericidal activity on *Escherichia coli* (*C. procera* and *G. punctata:* R = MBC/MIC ≤ 2). The total phenolic acid extracts of *G. punctata* showed better bacterial activity (MIC=31.25 µg/mL; MBC= 62.5 µg/mL; R=2). It is therefore desirable to test the combination of extracts from these different plants with conventional antibiotics including penicillin using salmonella and streptococci as models of microbes closely associated to sickle cell disease.

Keywords

Sickle Cell Disease, Bacterial Infection, Adjuvant, Medicinal Plants, Bioactivity, Antibiotics

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1. Introduction

Sickle cell disease is a hemoglobinopathy characterized by the presence in the blood of erythrocytes containing haemoglobin S [1-5]. In the Democratic Republic of Congo (DRC), 2% of the population is ill and the majority of them die before the age of five when they are not medically cared for and those who survive, however, have a damage to certain vital organs, which significantly reduces their life expectancy [6-8]. In children, the majority of deaths remain infectious (50%). Indeed, in Africa, outside urban areas where access to care is facilitated, mortality before the age of five years can reach 95% among children who are homozygotes for the mutation and who live in disadvantaged areas. In fact, sickle cell disease is characterized by extreme susceptibility to encapsulated bacteria [6, 9]. The increased susceptibility of sickle cell patients to bacteria is explained by the presence of the DQB1*03 allele of the major Class II histocompatibility complex in patients (allele of genetic predisposition to microbial infections) while healthy individuals instead possess the allele DQB1*15 [3]. These infections are the basis for the sudden worsening of anaemia and the increased risk of vaso-occlusive seizures. For this purpose, a suitable (appropriate) treatment is the guarantee of a favourable outcome because mortality from these infections remains high [10].

Preventive treatment (prophylaxis) involves the administration of penicillin every day, usually until the age of 15 years old, in order to avoid serious infections and hospitalizations as much as possible. It should be noted, however, that the cases of chemo-resistance of bacteria to penicillin are reported in sickle cell patients [11, 12]. Furthermore, sickle cell disease is a microenvironment that offers a selective advantage to certain infectious agents such as encapsulated bacteria to escape prevention and adapt to the disease and therefore to the host [9].

Identifying compounds that are both capable of potentiating the action of penicillin and endowed with antisickling properties is a new challenge that allows for the development of a novel therapeutic combination approach involving the combination of traditional improved drugs with penicillin.

However, ATB-ATB or ATB-adjuvant combination therapies have been reported in the literature as the best strategy to combat and/or prevent the drug resistance of adaptive sickle cell bacteria [13, 14]. In the last approach, an adjuvant is any compound capable of potentiating the action of ATBs (indirect effect); these include secondary metabolites derived from medicinal plants such as organic acids including ursolic and oleanolic acids [14].

Secondary metabolites are also known for their antibacterial properties (direct effect) [15]. *C. procera, G. punctata* and *P. macrophylla* are three plants from the pharmacopoeia of great apes in the DRC and of which the antisickling activity has been scientifically validated *in vitro*. The barks of these plants contain anthocyanins, quinones, tannins, alkaloids, triterpenoids, saponins, etc. which would confer them the biological activities scientifically validated such as the anti-scavening and antisickling activity [16-18].

The aim of this study was to assess the antibacterial activity of the barks of *C. procera, G. punctata* and *P. macrophylla* in order to use later the active fractions as penicillin adjuvant to serve as a starting point for an original galenic formulation. The specific objectives highlighted in the current report were: (i) to split the studied plants using solvents with increasing polarity (n-hexane, dichloromethane, ethyl acetate, methanol); (ii) to extract anthocyanins as well total phenolic acids, and (iii) to assess the antibacterial activity of different extracts along with fractions.

2. Material and Methods

2.1. Biological Material

The stem barks of three plants namely *P. macrophylla* Benth., *C. procera* Decne. and *G. punctata* Oliv were used. The barks of the first two plants were harvested in the lower Congo area in February 2013, in the surrounding area of the Congo River (in the Lower River) and those of *Garcinia punctata* in the surrounding area of Mbandaka (Equateur Province) in June 2013 by Professor Ngbolua Koto-te-Nyiwa. These plants have been identified at the herbarium of the National Institute of Agricultural Studies and Research (NIASR/INERA) located in the Faculty of Sciences at the University of Kinshasa and the Eala Botanical Garden in Mbandaka (*G. punctata*) respectively.

The bacteria strains used were provided by Noguchi Memorial Institute for Medical Research at the University of Legon from Ghana and by the Microbiology Laboratory of Pharmaceutical Sciences Faculty at the University of Kinshasa and by the National Institute of Biomedical Research (DRC) respectively.

2.2. Sample Preparation and Phytochemical Analyses

2.2.1. Plant Material Packaging

Samples of different species were dried in the laboratory temperature (approximately 27°C) two weeks at the Laboratory of Natural Products and Medicinal Chemistry (LASCHIMED). Having been dried, samples were grounded with the help of MOULINEX-branded grinder and sieved using a 1mm-diameter sieve “USA Standard testing Steve” to obtain a fine powder of particle size equal to ± 1 mm. The obtained powder was stored in a glass container.

2.2.2. Increasing Polarity Splitting

Each powder was extracted successively in different organic solvents with increasing polarity (n-hexane, dichloromethane, ethyl acetate and methanol).

Various extracts obtained were evaporated to dryness under reduced pressure using a Buchi-branded rotary evaporator and then preserved. The operation was repeated thrice in order to maximize the extraction yield of different extracts.

2.2.3. Extraction of Anthocyanins

The anthocyanins were extracted according to Bruneton's modified protocol [19]. Briefly, in a beaker, 30 g of the powder of each plant were macerated in 250 mL of the acidified methanol (HCl 1%) for 24 hours. After filtering with a filter paper (Whatman No. 1), the resulting filtered extract was concentrated at the low-pressure using a rotavapor at 40 °C. The resulting concentrate was washed several times with diethyl ether and the precipitate was dried in the oven for 48 hours at 40 °C. The residue obtained was the total extracts of anthocyanins. This extraction was performed in triplicate.

2.2.4. Extraction of Total Phenolic Acids

Organic acids were extracted from different samples used according to the protocol described by [20]. Thirty grams of the powder for each sample were macerated in the methanol/water mixture (70:30) for 72 hours (1:10; v/v). After filtration, the pH of each filtrate was measured using a pHmeter. The filtrates of *P. macrophylla* and *C. procera* had a pH of 4.5 while that of *G. punctata* was 4.0. To the filtrate was added the sodium carbonate (Na_2CO_3) in order to increase the pH to 8. Then, the alkaline filtrate was washed several times with petroleum ether.

The resulting hydro-alcoholic phase (lower phase in the decanting balloon) was concentrated at rotavapor until 1/4 of the initial volume and was then acidified in order to bring up the pH to 3 with acetic acid (concentrated). The acidified fraction was extracted three times by its volume in the ether di-ethyl. The operation was repeated three times and the three ether diethyl soluble fractions (upper phase in the decanting ball) were grouped and then evaporated to dryness. The organic extract obtained constituted the total phenolic acids.

2.3. Antibacterial Activity

The antibacterial activity of different plant extracts was assessed using the disk diffusion method for the sensitivity test and the liquid micro-dilution method for determining inhibition parameters [21-23].

2.3.1. Sensitivity Test

This duplicate test was performed on three clinical strains notably *Staphylococcus aureus,* Methicillin Resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa* for a well-defined concentration of 200 µg/mL of plant extracts. The principle of this test is to place a disc paper impregnated with plant extract on the surface of a pre-cultured gel medium with bacterial suspension. The diameter of the growth inhibition zone of bacteria formed around the disk was measured. Briefly, drugs were prepared by dissolving 20 mg of dry extracts in 1 mL of DMSO for a concentration of 20 000 µg/mL. This solution has been diluted 100 times in order to obtain the 200 µg/mL concentration parent solution.

For each strain, bacterial suspension was prepared in aseptic conditions by homogenizing 3 to 5 well-isolated colonies from bacterial culture (transplanted on the enriching medium Blood agar) in Mueller Hinton broth. The turbidity of the broth was adjusted in order to have a density equivalent to that of Mac Farland's standard 0.5 (1.5×10^8 UFC.mL^{-1}).

The bacterial strains were then seeded on Mueller Hinton agar according to the swab technique [24]. The 6 mm-diameter sterile disc paper soaked by 20 µL of each plant extract at the concentration of 200 µg/mL were deposited on the surface of each petri dish containing Mueller Hinton's agar previously inoculated with bacterial suspension. The DMSO-soaked sterile disc paper was used as a negative control.

A disc of antibiotics (Gentamicin, Cefoxitine, Tobramycin) chosen according to the recommendations of the Antibiogram Committee of the French Society of Microbiology, served as a positive control. The boxes are left for an hour at room temperature, and then incubated at 37 °C between 18 and 24 hours.

After incubation, the inhibition diameter was measured in mm using a graduated ruler (disk diameter included). According to interpretive standards of inhibition zone diameters, microorganisms are reported as sensitive, intermediate or drug resistant [21].

2.3.2. Micro-dilution Method in Liquid Medium

It is a quantitative method much more used in research laboratories and allows determining with some accuracy the inhibitory and bactericidal concentrations of a given drug compared to a specific microorganism. The test was conducted on methicillin resistant *S. aureus* strains ATCC 33591 and *E. coli* ATCC 27195. This method helps to prepare decreasing concentrations of the test substance under the same volume. Subsequently, the same volume of bacterial suspension was added to each well in the exponential growth phase and then incubated at 37 °C for 18 to 24 hours. The minimum inhibitory concentration (MIC) is the smallest concentration of the drug, which completely inhibits any

visible growth of a microorganism in the microplate after incubation at 37 °C after the indicated time for incubation. Its determination is based on the assessment of the sensitivity of a microorganism to an antimicrobial product.

On the other hand, the minimum bactericidal concentration (MBC) is the lowest concentration of substance capable of killing more than 99.9% of bacterial inoculum (less than 0.1% of survivors) after 18 to 24 hours of incubation at 37 °C. Their determination is based on subculture from the MIC on an agar medium. Briefly, two culture media namely Tryptone Soya Broth (TSB) and Tryptone Soya Agar (TSA) were tested for validation of the method. The stock solutions for plant extracts were prepared by dissolving 20 mg of dry extracts in 1 mL of DMSO for a concentration of 20,000 µg/mL. This solution was diluted 20 times in order to obtain the 1000 µg/mL of concentration from the stock solution.

(i). Preparing for Bacterial Suspension

Each germ contained in the storage agar was cultured in a selective medium. Using a sterile platinum hand, an aliquote of each germ was collected and seeded in Chapman's agar (for *S. aureus)* and in Mac Conkey's agar or EMB (for *E. coli*). After a-day-incubation, opaque, whitish colonies appeared and they are characteristic of *S. aureus* on Chapman's agar, while the red colonies, large with a murky halo characterize *E. coli* on Mac Conkey's agar.

Bacterial suspension was prepared by removing 3 isolated colonies from the strain to be tested on a selective medium using a sterile handles and placing them in sterile saline solution contained in a tube. The turbidity of the suspension was adjusted to obtain a density equivalent to that of the standard 0.5 MacFarland (1.5×10^{8} UFC.mL^{-1}).

(ii). Dilution of Extracts and Incubation of Microplate

We used a polystyrene steric microplate of 96 wells of 8 lines (A-H) and 12 columns (1-12). Using a 100 µL, 50 µL multi-channel micropipette, and TSB broth were placed in all wells of the microplate from A_1-A_{12} to H_1-H_{12}. Then, using a micropipette of 100 µL, 50 µL of the stock solution of each extract were introduced into the wells of the first column (from A_1 to H_1) and were well mixed with TSB broths by successive aspiration and release into microplates. From this arrangement, two series of dilutions were made in the TSB broth in order to obtain a concentration range between 500 µg/mL and 0.957 µg/ mL (i.e. 50 µL of the first well were collected and transferred to the second well and so on to the tenth well and this last well was eliminated. Then, using a multi-channel micropipette, 10 µL of the bacterial suspension was transferred to all wells of the microplate except wells No. 11 (negative control).

Wells No. 12 served as a positive control of microbial growth (inoculum mixed in the growing medium without the extract). Finally, the microplates were covered and incubated in the oven at 37 °C for 24 hours.

(iii). Determining Minimum Concentration (MIC)

After 24 hours of incubation, the MIC was read after adding 5 µL of Resazurine dye (7-Hydroxy-3*H*-phenoxazin-3-one 10-oxide) 0.1 mg/mL of concentration in all wells; a lightly fluorescent blue dye. The principle is based on the ability of living bacterial cells to reduce blue resazurine in fluorescent resofurine coloured pink by mitochondrial dehydrogenase succinate in the presence of NADH or NADPH as a reductive agent. Dead cells devoid of this enzyme remain blue. The MIC is then read in the first wells with no bacterial growth. The growth in the well containing the extracts was compared to that of wells No. 12 that has been used to control the bacterial growth. The test is only valid if there is acceptable growth in these control wells. If growth is insufficient in these wells, the microplate is reincubated and the MIC is read after 48 hours. It should be noted that when the MIC is ≤ 250 µg/mL, the drug is considered active on the bacterial strain [25].

(iv). Determining Minimum Bactericidal Concentration (MBC)

MBC was determined by streaking on TSA agar 10 µL of the contents of the microplate wells that gave no visible bacterial growth from the MIC. The seeded dishes were incubated for 24 hours at 37 °C. The lowest concentration at which there is no growth in subculture was considered as MBC.

(v). Determining the Bactericidal or Bacteriostatic Character of the Extract

According to the liquid micro-dilution method, it was possible to specify whether extracts inhibit (bacteriostatic effect) or kill pathogenic bacteria (bactericidal effect). It is the MBC/MIC ratio that confirms the bactericidal or bacteriostatic character of the substance concerned. When this ratio is greater than 4 but less than 16, the drug is called bacteriostatic; if this ratio is less than or equal to 4, the drug is considered bactericidal. On the other hand, when it equals 1, the drug is called absolute bactericide. However, when this ratio is 32 or higher, bacteria are tolerant to the extract [25].

3. Results and Discussion

3.1. Sensitivity Test

The average values of the growth inhibition diameters of *S. aureus,* MRSA and *P. aeruginosa* by different extracts (200 µg/mL) are presented in the table below.

Table 1. Inhibition Diameters (mm) of extracts on different selected strains.

Plant species	n-hexane	Dichloromethane	Ethyl acetate	Ethanol
Staphylococcus aureus				
P. macrophylla	-	1.825 ± 0.041	1.55 ± 0.025	1.2 ± 0.035
C. procera	-	1.7 ± 0.035	1.475 ± 0.012	1.3 ± 0.063
G. punctata	0.925 ± 0.000	1.9 ± 0.035	1.225 ± 0.041	1.675 ± 0.041
Positive control 1	2.15±0.056 mm			
Methicillin Resistant *S. aureus* (MRSA)				
P. macrophylla	-	1.35 ± 0.025	1.375 ± 0.0414	1.3 ± 0.035
C. procera	-	1.2 ± 0.061	1 ± 0.035	0.75 ± 0.025
G. punctata	0.9 ± 0.376	1.575 ± 0.407	1.2 ± 0.035	1.65 ± 0.056
Positive control 2	1.525±0. 019 mm			
Pseudomonas aeruginosa				
P. macrophylla	-	-	-	-
C. procera	-	-	-	-
G. punctata	-	1.3 ± 0.035	0.925 ± 0.04145	0.925 ± 0.041
Positive control 3	2.325±0.041 mm			

Legend: (Positive control 1: Gentamycin; Positive control 2: Cefoxitine and Positive control 3: Tobramycin both charged at 10 µg).

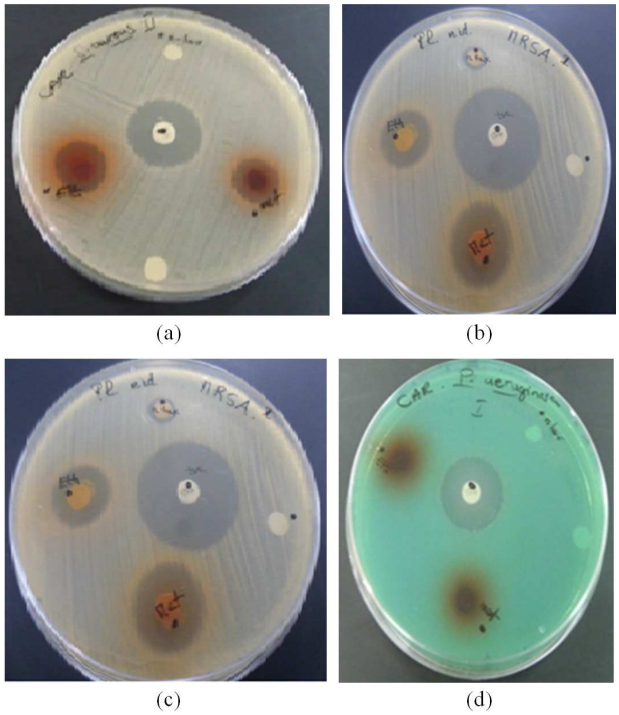


Figure 1. Effect of *C. procera* on *S. aureus*; (b) Effect of *G. punctata* on MRSA; (c) Effect of *G. punctata* on MRSA; (d) Effect of *C. procera* on *P. aeruginosa* [Mueller Hinton medium].

According to NCCLS [21], *S. aureus* strains are resistant to the soluble fraction in n-hexane and methanol extracts but sensitive to dichloromethane and ethyl acetate of *P. macrophylla*. For *C. procera,* however, these strains were resistant to n-hexane and ethyl acetate but sensitive to dichloromethane and methanol extracts.

Finally, for *G. punctata*, these strains only resist the n-hexane extract, while were intermediate to the soluble fraction in ethyl acetate, and sensitive to the dichloromethane and methanol extracts. As to *Pentaclethra macrophylla,* MRSA strains were resistant to n-hexane, and were intermediate to dichloromethane, ethyl acetate and methanol extracts.

On the other hand, these strains were resistant to n-hexane, ethyl acetate and methanol extracts but intermediate to dichloromethane extract for *C. procera*. Regarding *G. punctata,* the selected strains were resistant to n-hexane extract, intermediate to ethyl acetate extract and sensitive dichloromethane and methanol extracts. Meanwhile, *P. aeruginosa* was resistant to all extracts and fractions of plants except dichloromethane extract of *G. punctata* that showed a slight bacterial inhibition. In light of these results, *G. punctata* would be more active followed by *P. macrophylla* and *C. procera.* Thus, the antibacterial activity of these plants can be improved by combining them with conventional ATBs in order to combat and/or prevent the drug-resistance (pharmaco-resistance) of bacteria.

3.2. Micro-dilution in Liquid Medium Test

Values of minimal inhibitory concentrations (MICs), minimum bactericidal concentrations (MBC) and MBC/MIC ratios of fractions and plant extracts on reference strains of MRSA (ATCC 33591) and *E. coli* (ATCC 27195) are presented below.

Table 2. Values of MIC, MBC and MBC/MIC ratio of plant extracts on MRSA (ATCC 33591).

Fractions	*P. macrophylla*			*C. procera*		*G. punctata*	
	MIC (µg/mL)	MBC (µg/mL)	R	MIC (µg/mL)	MBC (µg/mL)	MIC (µg/mL)	MBC (µg/mL)
n-hexane	62.5	>500	-	31.25	>500	31.25	>500
Dichloromethane	31.25	>500	-	15.625	>500	7.81	>500
Ethyl acetate	125	>500	-	31.25	>500	15.63	>500
Methanol	62.5	500	8	62.5	>500	62.5	>500
Anthocyanins	125	>500	-	125	>500	62.5	>500

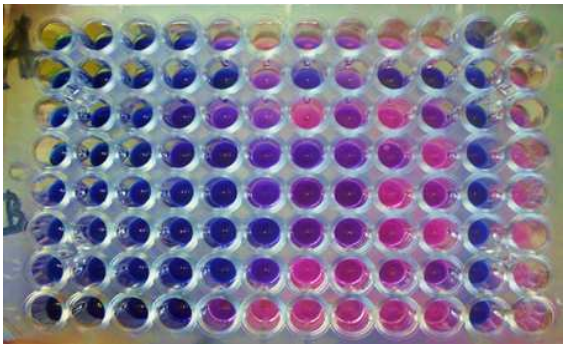
Fractions	*P. macrophylla*			*C. procera*		*G. punctata*	
	MIC (µg/mL)	MBC (µg/mL)	R	MIC (µg/mL)	MBC (µg/mL)	MIC (µg/mL)	MBC (µg/mL)
Phenolic acids	125	>500	-	31.25	>500	7.81	>500

(Legend: MIC: minimal inhibitory concentrations MBC: minimum bactericidal concentrations, R=MBC/MIC)

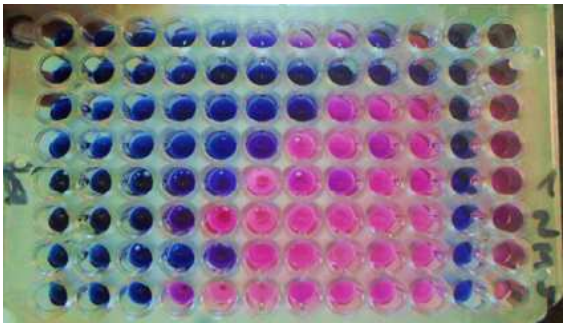
Table 3. Values of MIC, MBC and MBC/MIC ratio of plant extracts on *E. coli* ATCC 27195.

Fractions	*P. macrophylla*			*C. procera*			*G. punctata*		
	MIC (µg/mL)	MBC (µg/mL)	R	MIC (µg/mL)	MBC (µg/mL)	R	MIC (µg/mL)	MBC (µg/mL)	R
n-hexane	3.90625	125	32	125	125	1	125	125	1
Dichloromethane	125	125	1	125	250	2	125	125	1
Ethyl acetate	125	250	2	125	125	1	125	125	1
Methanol	125	250	2	62.5	62.5	1	125	250	2
Anthocyanins	62.5	500	8	62.5	62.5	1	125	>500	
Phenolic acids	125	>500		125	125	1	31.25	62.5	2

(Legend: MIC: minimal inhibitory concentration, MBC: minimum bactericidal concentration, R=MBC/MIC)

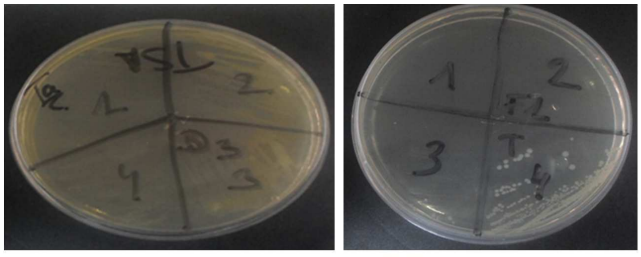


(a)



(b)

Figure 2. (a) Effect of extracts (n-hexane, dichloromethane, ethyl acetate and methanol) from *P. macrophylla* and *C. procera* on *S. aureus*; (b) Effect of plant extract (n-hexane, dichloromethane, ethyl acetate and methanol) and organic acids from *G. punctata* as well as anthocyanins extracts from the three plants on *S. aureus* [TSB medium].



(a) (b)

Figure 3. Subculture from (a) microplate wells of *G. punctata* anthocyanins that gave no visible bacterial growth starting from the MIC; (b) microplate wells of P. macrophylla anthocyanins that did not give any visible bacterial growth starting from the MIC [TSA medium].

Table 2 shows that all fractions and plant extracts are active on the MRSA ATCC 33591 strains (MIC ≤ 250 µg/mL) but present bacteriostatic effect. In general, whatever the nature of the fractions and extracts, the results obtained prove sufficiently that *G. punctata* is more active than *C. procera* and *P. macrophylla*. However, the dichloromethane fraction and the total phenolic acids extract from *G. punctata* showed the greatest activity. The dichloromethane soluble fraction is thought to be rich in terpenic compounds which are well known for their pleiotropic antibacterial activity including the dismemberment of the microbial membrane, inhibition of protein synthesis at the level of translation, inhibition of glycolysis metabolic pathway and the enzyme AhpC (Alkyl hydroperoxide reductase subunit C) involved in the detoxification of reactive oxygen species [26]. The same is true for the dichloromethane fraction of *C. procera* and that of *P. macrophylla* which were more active than the other fractions. The ethyl acetate fraction of *G. punctata,* being active, would be rich in polyphenols responsible for this activity. While the n-hexane soluble fractions also showed better activity than methanol extracts. This suggests that many apolar compounds such as the terpenoids present in them would act against bacteria and be much more active than many of the phenolic compounds found in methanol extracts.

In addition, the antibacterial activity, expressed in terms of inhibition of the enzyme succinate dehydrogenase (EC 1.3.99.1), bio-marker of cellular viability, of organic acids is higher than that of anthocyanins (G. *punctata* > *C. procera* > *P. macrophylla*).

The bacteriostatic activity obtained with plant extracts demonstrates that plants would act by inhibiting the growth and reproduction of bacteria without being able to kill them and this by interfering with cellular metabolism inhibition of DNA biosynthesis and bacterial proteins [27]. To this end, these extracts would prevent the formation of biofilm to allow the body to activate the humoral and/or cellular-

mediated immunity needed to eliminate bacteria. Moreover, phenolic compounds are well known for their immuno-modulating activity. Many phenolic compounds are endowed with immunosuppressive properties by inhibiting maturation and activating dendritic cells. Still others stimulate cell-mediated immunity. This is particularly the case for macrophages, which play an important role in the control of infections. Their activation by phenolic compounds is induced mainly through the MAPK signal transduction pathway [28]. It should also be noted that in this study, *E. coli* ATCC 27195 is more sensitive to plant extracts than MRSA ATCC 33591. Organic acid extracts from *G. punctata* revealed the greatest antibacterial activity with a MIC of 31.25 µg/mL and a bactericidal effect. In addition to these extracts, *C. procera* anthocyanins also showed a good bacterial activity with a MIC of 62.5 µg/mL. However, with the exception of the n-hexane soluble fraction, extracts of anthocyanins and organic acids from *P. macrophylla* and anthocyanin extracts of *G. punctata* that showed bacteriostatic activity, the other fractions showed a bactericidal effect on *E. coli*. *ATCC* 27195. The antibacterial activity is therefore a function of the plant used and therefore its content on secondary bioactive metabolites. Furthermore, the biosynthesis of secondary metabolites is controlled by environmental and genetic factors of which the climate, the ecology of the harvest site, harvest period, etc. [29, 30].

It is important to note that the resistance developed by some strains to certain drugs is due to their ability to inactivate the bioactive compounds contained in the extracts and/or modify their targets [27]. However, the results indicate that plant extracts may have a broad spectrum of action and serve as sources of new antibacterial agents [15].

As a matter of fact, it is well established that secondary metabolites inhibit the proteins of the superfamily "ABC transporters" involved in the chemo-resistance of bacteria to drugs [31].

Since sickle cell subjects are prone to repeated tissue infarction, usually leading to functional asplenia, they are therefore exposed to frequent infections due to immune deficiency abnormality [1, 32].

Based on the results obtained in this study, the prospect of developing a phytomedicine based on these plants with pleitropic pharmaco-biological activities (antisickling and antibacterial) would be of great added value as it would improve the life expectancy of these sick subjects.

4. Conclusion and Suggestions

The aim of this study was to evaluate the antibacterial activity of the barks of *C. procera, G. punctata* and *P. macrophylla.* Bacteriostatic activity was observed with all extracts on MRSA *(G. punctata:* MIC ≤ 62.5 µg/mL; *C. procera* and *P. macrophylla:* MIC ≤ 125 µg/mL) while several fractions and extracts revealed bactericidal activity on *E. coli* (*C. procera* and *G. punctata:* R-MBC/MIC ≤ 2). Total phenolic acids extracts from *G. punctata* showed better bacterial activity (MIC ≤ 31.25 µg/mL; MBC 62.5 µg/mL; R ≤ 2). It is therefore desirable to test the combination of extracts from these different plants with conventional antibiotics including penicillin using salmonella and streptococci as models of microbes associated with sickle cell disease.

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