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Preliminary Study to Assess the Antioxidant and Antibacterial Activity of Extracts from the Leaves of *Morelia Senegalensis* (A Rich)

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Abstract

African populations rely heavily on traditional medicine, which uses plants to treat human and animal diseases. However, scientific validation of this empirical knowledge concerning the efficacy, toxicity and dosage of the proposed treatments is urgently needed. The aim of this study was to evaluate the antioxidant and antibacterial activity of *Morelia senegalensis* leaf extracts on the 2,2-diphenyl-1-picrylhydrawyl (DPPH) radical and the (2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic) acid) ABTS cation radical and on four reference bacterial strains (E. coli 25922, E. coli 35218, E. faecalis 29212 and S. aureus 29213) using protocols already described in the literature. The phytochemical screening test revealed the presence of flavonoids, alkaloids, tannins, saponins, reducing compounds and sterols in *Morelia senegalensis* leaves. All the extracts showed antioxidant activity to varying degrees. The most active ethyl acetate (AE) extract had half-maximum inhibition concentration (IC_{50})of 0.33 and 0.62 mg/mL for the ABTS and DPPH tests respectively, followed by DCM and EB extracts with IC_{50} of 0.86 and 0.88 mg/mL for the ABTS test respectively, while the vitamin C used as a reference was more active with IC_{50} of 0.04 and 0.06 mg/mL. Antibacterial activity showed inhibition diameters ranging from 7 to 16 mm depending on the concentration and strain considered. The ethyl acetate fraction was the most active on *S aureus* 29213 with an inhibition diameter of 16 mm at 50 mg/mL. These interesting results were confirmed by the Minimal Inhibitory Concentration inhibitory (MIC) and Minimal Bacterial Concentration (MBC) studies, which showed that all the extracts had a bactericidal effect on all the studied strains. *Morelia senegalensis* plant has shown promising preliminary results in the treatment of bacterial infections. Further studies are needed to isolate and characterize the secondary metabolites contained in the leaves of this plant.

Keywords: Bacterial infection; *Morelia senegalensis*; Secondary metabolites; Therapy.

1. Introduction

Infectious diseases remain one of the world's major health concerns. According to Farkhod et al, in 2019, (Reference is in the end of the sentence n° 1) 13.66 million people died worldwide from infection-related causes [1]. Most of these deaths (4.95 million) are associated with antibiotic-resistant pathogens [2]. Microbial resistance has been fueled mainly by the overuse and inappropriate use of antibiotics, coupled with a lack of new drug development by the pharmaceutical industry. Although deaths from bacterial infections have declined in developed countries, they remain a major cause of death in developing countries [3] The spread of resistant strains in pathogens of medical

interest, such as *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, has been associated with almost 5 million deaths worldwide in 2019 [2]. According to the WHO, 1.9 million children die worldwide from respiratory infections, with 70% of these deaths occurring in Africa and Asia in 2000 [4]. Although synthetic antibiotics have revolutionized the treatment of infectious diseases thanks to easier and more plentiful access and production, they are now less effective due to the side-effects and resistance they cause [5].

At present, many researchers are turning to medicinal plants, which have gained particular attention as a source for the production of new drugs. Between 2012 and 2022, the number of scientific articles published on research into antibacterial biomolecules increased by 43% [5].

The report by the Food and Agriculture Organization of the United Nations states that at least 25% of the pharmaceutical drugs in the modern pharmacopoeia are of plant origin, with many others being synthetic analogues derived from bioactive plant compounds [6]. Another study has highlighted the fact that around 60% of anti-tumor and anti-infectious agents now commercially available or at an advanced stage of clinical trials are of natural origin [7]. As far back as 1993, O'Neill estimated that more than 20.000 plant species are used medicinally, and that phytotherapy provides healthcare for more than 80% of the world's population, a large proportion of whom live in developing countries [8, 9].

On the other hand, free radicals are known to be the major cause of various chronic and degenerative diseases. Oxidation is a natural process in organisms for the production of energy to fuel biological cycles. Conversely, the production of oxygen-derived free radicals is implicated in the onset of numerous diseases such as arthritis, atherosclerosis, rheumatoid arthritis and cancer, as well as many degenerative diseases associated with ageing [10]. This situation has prompted a continuous search for various plant sources containing medicinal substances to help overcome these pathologies. Medicinal plants contain a wide range of chemical compounds, such as alkaloids, flavonoids, tannins, terpenoids and phenolic compounds, which have a variety of known biological activities, including antioxidant, anti-inflammatory, antidiarrheal, antibacterial and antidiabetic properties. [11, 12]. Plants in the Rubiaceae family have been studied for their antimicrobial activities against certain drug-resistant pathogens [10, 13, 14]. In the light of this, updated information on the properties and uses of any medicinal plants belonging to this group needs to be studied. Our team therefore set out to find secondary metabolites extracted from the *Morelia senegalensis* plant. *Morelia senegalensis* is a Senegalese plant belonging to the Rubiaceae family. It is an often-bushy shrub that can reach 3 to 12 m in height. It is found in marginal forests and on the banks of savannah rivers, throughout Senegal, north and south of Nigeria, and as far as Congo (Brazzaville) and Sudan [15]. Outside from the taxonomic descriptions available online, the only publications found on *Morelia senegalensis* concern inventories of plant species specific to certain regions [16, 17]. To our knowledge, no biological activity studies have been carried out on the organs of this plant. Consequently, the present study would constitute a new step towards new scientific discoveries on the phytotherapy of *Morelia senegalensis*. The objectives of this study are therefore to extract secondary metabolites from *Morelia senegalensis* leaves and then evaluate their antioxidant and antibacterial activities.

2. Materials and Methods

2.1. Materials

2.1.1. Plant material

The plant material consisted of the leaves of *Morelia Senegalensis*, a plant of the Senegalese flora. The leaves were collected in south-eastern Senegal in the Department of Tambacounda with GPS coordinates: 13.35252, -13.369598; in February 2023.

2.1.2. Bacterial strains

The bacterial strains used in this study were obtained from the National Public Health Laboratory in Thiès. These were reference strains comprising two Gram-positive bacteria: *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212 and two Gram-negative bacteria: *Escherichia coli* ATCC 35218, *Escherichia coli* ATCC 25922. These reference strains were stored at -20°C in Brain Heart Broth (BCC) medium until use.

2.2. Methods

2.2.1. Preparation of plant extracts

The harvested leaves were washed, dried in the shade for two weeks and ground to a powder using an electric grinder before being used for the extraction.

a. Extraction

- **Delipidation with petroleum ether**
- Extraction was carried out by introducing 100 g of Morelia senegalensis leaf powder into an Erlenmeyer flask containing 200 mL of petroleum ether. The resulting mixture was macerated for 48 h using a magnetic stirrer and then filtered through filter (Isolab MN 617, 110 mm) paper. The filtrate obtained was reduced to dryness using a rotary evaporator. The operation was repeated three times in succession.
- **Hydroalcoholic extraction**

Hydroalcoholic extraction was carried out by introducing the Marc obtained after ether extraction into an Erlenmeyer flask containing 300 mL of hydroalcoholic solvent (70/30). The mixture obtained was stirred and then

filtered using filter (Isolab MN 617, 110 mm) paper. The filtrate obtained was evaporated using a rotary evaporator. The operation was repeated three times in succession.

b. Fractionation

Seventeen grams of the crude extract (hydroalcoholic) was introduced into a separatory funnel before being washed successively with solvents of increasing polarity, namely dichloromethane (3 times) then ethyl acetate (3 times). The solvents from each fraction were combined and then evaporated to dryness using a rotary evaporator. The resulting factions were kept at 4°C in a refrigerator until use.

- **Determining the yield of extracts and fractions**

The yield is the quantity of extract or fraction obtained from a plant material or crude extract. The yield was obtained using the following formula:

$$\mathbf{R} = \frac{m_i}{m_0} x100$$

R= percentage yield (%) ; m_0 *= mass of plant material powder;* m_i *= mass of extract or fraction.*

2.2.2. Phytochemical Screening

Research into the groups of bioactive chemical families (tannins, alkaloids, saponins, flavonoids, steroids, terpenoids and reducing compounds) contained in *Morelia Senegalensis* leaves was carried out using standard characterization methods? Not need reference is given [18, 19].

2.2.3. Antioxidant Activity of Extracts and Fractions

Scavenging DPPH radical·

- The scavenging activity of the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was measured using the Molyneux method?not need reference is given [20], slightly modified as described in our previous work ? not need reference is given [21].
- **Trapping of the ABTS.+ cation radical**

The ABTS.+ radical cation scavenging capacity of the extracts was determined by the method used by Dieng [22] with a slight modification.

Briefly, a volume of 1.5 mL of ABTS.+ solution was mixed with 50 µL of extract at different concentrations (0.0625; 0.125; 0.25; 0.5; 1mg/mL). Absorbance was measured with a spectrophotometer at 734 nm after 10 min incubation in the dark at room temperature. Three tests were carried out for each concentration of product tested and the results are expressed as percentage inhibition (PI).

2.2.4. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

- Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined using the method described by Moroh [23], with a slight modification. The MIC is the lowest concentration of a substance that can inhibit the growth of bacteria for 18 to 24 h at 37°C.
- **Determination of the Minimum Inhibitory Concentration (MIC)**

Each extract or fraction was prepared with a range of concentrations from 3.125 to 50 mg/mL in sterile distilled water containing 5% DMSO using a geometric dilution series at the rate of 2. For each bacterial strain, an inoculum was prepared with turbidity adjusted to 0.5 Mc Farland (approximately 108 CFU/mL) and then diluted to 106 CFU/mL in brain heart broth (BCC). In hemolysis tubes, 1 mL of each concentration of extract was mixed with 1 mL of bacterial inoculum. The concentration range prepared was then diluted by half to obtain a final concentration of 1.56 to 25 mg/mL. Growth (positive) and sterility (negative) controls were also prepared. The tubes were incubated at 37°C for 24 hours, after which absorbances were measured using a spectrophotometer at 625 nm to assess bacterial growth. The MIC for each extract against a given bacterial strain was determined as the lowest concentration where no visible growth of bacteria was observed.

- **Determining the minimum bactericidal concentration (MBC)**

To determine the MIC, a bactericidal test was carried out by streaking dilutions of 100, 10-1, 10-2, 10-3 and 10-4 of the growth control, corresponding respectively to 100%, 10%, 1%, 0.1% and 0.01% survivors on new agar in a Petri dish. After the MIC reading, tubes with no visible bacterial growth were streaked onto new agar and incubated at 37°C for 24 hours. The streaks were then compared with the bactericidal control. The MBC was defined as the lowest concentration where the subculture showed bacterial growth less than or equal to 0.01% survivors.

2.2.5. Determination of Antibacterial Activity by Standard Antibiogram

The antibacterial activity of the different extracts was assessed using the disk diffusion method. Petri dishes containing Mueller-Hinton agar were inoculated with a 108 CFU inoculum of the bacteria tested. Blotting paper discs 6mm in diameter, impregnated with 10 µL of solution at different concentrations of each extract (50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml and 3.125 mg/mL), were placed on the surface of the agar medium. The plates were incubated at 37°C for 24 h in the oven according to the protocol described by Salvat, *et al.* [24]. Antimicrobial activity was assessed by measuring the diameter of the zones of inhibition around the discs. Aqueous solutions of

10% DMSO were used as the negative control and commercial antibiotic discs as the positive control. Each experiment was repeated three times for each extract tested.

3. Results

3.1. Extraction and Fractionation Efficiency

Secondary metabolites were extracted from *Morelia Senegalensis* leaves by maceration with a hydroalcoholic solvent. According to Dah-Nouvlessounon [25] the ability to extract bioactive compounds from plants is strongly linked to the affinity of the solvent on the one hand and the chemical composition of the plant on the other. The yields obtained for petroleum ether and hydroalcoholic extracts are 4.44% and 20.24% respectively.

Liquid-liquid fractionation of the hydroalcoholic extract yielded dichloromethane (F. DCM) and ethyl acetate (F. AcOEt) fractions of 2.74% and 3.14% respectively. However, most of the secondary metabolites remained soluble in the residual aqueous fraction (91.85%).

3.2. Phytochemical Screening

The results of the phytochemical screening carried out using qualitative staining and precipitation tests on all these extracts and fractions are presented in Table 1. The crude extract and aqueous fraction contain all the metabolites of interest except sterols and terpenes. The DCM fraction contained alkaloids and flavonoids, while the AcOEt fraction tested positive for tannins, flavonoids, reducing compounds and saponins. The petroleum ether extract contains mainly sterols and terpenoids.

Table-1. Results of phytochemical screening tests

	E. EP	F. DCM	F. AE	F. A	E. B
Tannins	-	-	+	+	+
Alkaloids	-	+	-	+	+
Flavonoids	-	+	+	+	+
Reducing compounds	-	-	+	+	+
Sterols et terpenes	+	-	-	-	-
Saponins	-	-	+	+	+

E. EP : Petrolum extract ; **F. DCM** : Dichlorométhane fraction; **F. AE** : Ethyl acétate fraction ; **F. A** : Aqueous fraction ; **E. B** : Raw extrait

3.3. Antioxidant activity assessed with DPPH$^{\cdot}$ and ABTS$^{\cdot+}$

The *in vitro* antioxidant activity tests show that all the extracts studied exhibit antioxidant properties to varying degrees and depending on the nature of the extract. The results of the ABTS$^{\cdot+}$ cation radical reduction test, reveals that the ethyl acetate fraction is the most active (IC_{50}= 0.33±0,01 mg/mL) compared with the dichloromethane fraction and the crude extract, which have IC_{50} of 0.86 mg/mL and 0.88±0,00 mg/mL respectively. As for the DPPH radical reduction test shows that the ethyl acetate fraction (IC_{50}= 0.62±0,01 mg/mL) and the crude extract (IC_{50}=1.82±0,05 mg/mL) are the most active (Table 2).

Table-2. Results of antioxidant activity tests on extracts and fractions

	E. B	E. A	F. AE	F. DCM	Vitamine C
ABTS CI_{50} (mg/mL)	0.88 ± 0.00	0.96 ± 0.00	0.33 ± 0.01	0.86 ± 0.03	0.04 ± 0.02
DPPH	1.82 ± 0.05	3.858 ± 0.06	0.62 ± 0.01	3.47 ± 0.08	0.06 ± 0.00

3.4. Minimum Inhibitory and Bactericidal Concentration (MIC, MBC)

The most significant results were recorded with the dichloromethane and ethyl acetate factions against Gram-positive bacteria *Enterococcus faecalis* ATCC 29212 and *Staphylococcus aureus* ATCC 29213, followed by slightly lower to absent activity on Gram-negative bacteria with MICs ranging from 6.25 to 12.5 mg/ml. The MBC/MIC ratio was used to determine the mode of action of the substance studied. Table 3 below shows the results of the macro-dilution.

Table-3. Results of the macro-dilution and calculation of the CMB/CMI ratio

		E. Coli 25922	*E. Coli* 35218	*S. aureus* 29213	*E. faecalis* 29212
F. DCM	CMI	6.25	6.25	6.25	3.12
	CMB	12.5	12.5	6.25	6.25
	CMB/CMI	**2**	**2**	**1**	**2**
F. AE	CMI	6.25	6.25	6.25	3.12
	CMB	12.5	12.5	6.25	3.12
	CMB/CMI	**2**	**2**	**1**	**1**
F. A	CMI	12.5	12.5	6.25	12.5
	CMB	25	25	12.5	25
	CMB/CMI	**2**	**2**	**2**	**2**
E.B	CMI	12.5	12.5	6.25	12.5
	CMB	25	25	12.5	25
	CMB/CMI	**2**	**2**	**2**	**2**

Légend. **E. Brut** : Hydroalcoholic extract ; **F. A** : Aqueous fraction ; **F. DCM** : Dichlorométhane fraction ; **F. AE** : Ethyl acetate fraction.

3.5. Standard Antibiotic Susceptibility Test

Standard antibiogram tests showed significant zones of inhibition mainly with the dichloromethane and ethyl acetate fractions against Gram-positive bacteria, *Enterococcus faecalis* ATCC 29212 and *Staphylococcus aureus* ATCC 29213. Figure 1 and Tables 4a and 4b illustrate these results, showing inhibition diameters of 7 to 16 mm. These results are dependent on the strain and concentration used.

Table-4a. Antibiogram of reference antibiotics

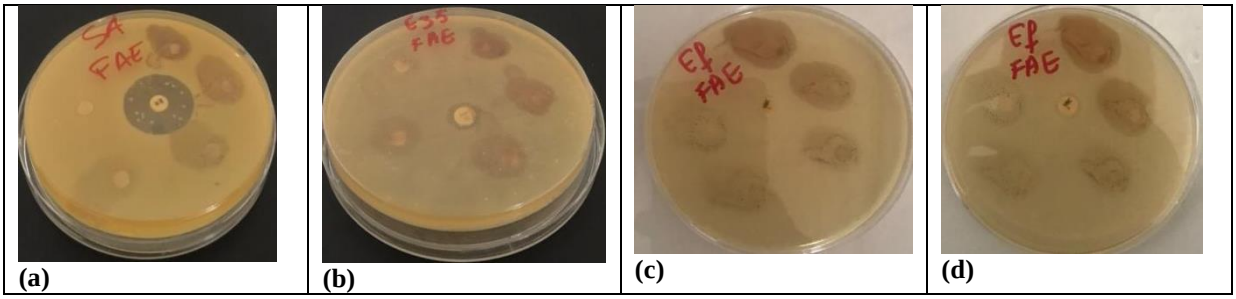
	E. Coli 25922	*E. Coli* 35218	*S. aureus* 29213	*E. faecalis* 29212
Amikacine	**21**			
Vancomycine		**21**		
Tikacilline			**22**	
Pipéracilline				**11**

Tableau-4b. Result of the antibiogram test of extracts and fractions

	Concentration mg/ml	S. aureus 29213	E. faecalis 29212	E. Coli 25922	E. Coli 35218
		Inhibition diameter (mm)			
F. DCM	50	15	12	13	14
	25	13	10	11	12
	12.5	11	8	9	9
	6.25	10	-	7	8
	3.12	8	-	-	-
F. AE	50	16	10	12	14
	25	14	8	10	12
	12.5	11	7	8	10
	6.25	9	-	-	9
	3.12	7	-	-	8
F. A	50	12	10	10	10
	25	10	8	8	9
	12.5	8	-	7	8
	6.25	-	-	-	-
	3.12	-	-	-	-
E. B	50	11	10	11	11
	25	10	8	10	10
	12.5	8	-	8	8
	6.25	-	-	-	-
	3.12	-	-	-	-

Légend. **E.B:** Raw extract ; **F. A** : Aquous fraction ; **F. DCM** : Dichlorométhane fraction ; **F. AE** : Ethyl acetate fraction

Figure-1. Antibacterial activity of extracts and fractions



(a) **(b)** **(c)** **(d)**

4. Discussion

The chemical compounds were obtained by extraction and fractionation with yields ranging from 2.74 to 20% depending on the type of extraction solvent used. The hydroalcoholic residue showed a yield of 91.8%, demonstrating the high affinity of polar compounds for this solvent. This difference in polarity explains the differences observed in the extracts. These were 2.7 and 3.14% for the dichloromethane and ethyl acetate fractions respectively. Phytochemical screening of these extracts revealed the presence of alkaloids, tannins, flavonoids, saponins and sterols (Table 1). These results are in line with those of Sharif, *et al.* [26], who revealed the presence of these chemical families in species of the Rubiaceae family. These secondary metabolites act through specific mechanisms on the biological activity of organisms and play an important role in the therapy of several diseases. In recent years, the antibacterial activity of alkaloids has been widely evaluated in the biomedical field. Studies on the antibacterial mechanism of natural alkaloids show that they can disrupt the bacterial cell membrane Li [27], affect DNA function and inhibit protein synthesis [28]. Natural alkaloids are therefore potentially active against a range of bacteria, including methicillin-resistant *Staphylococcus aureus* [29], a common species and causative agent of clinical infections. Alkaloids are often used as basic compounds in the development of new antimicrobial drugs. Several *in vivo* and clinical studies have reported that alkaloids have various pharmacological effects, including anticancer, antiviral, anti-inflammatory and antibacterial activities [29]. Flavonoids, for their part, have reported good antibacterial potential with bacteriostatic activities against various Gram-negative bacteria such as *E. coli* and *P. aeruginosa*. They also have an inhibitory effect on *S. aureus* and on the growth of spore-forming *B. subtilis* [30]. Evaluation of the antibacterial activity of *Morelia senegalensis* leaf extracts using the standard antibiogram test (table 4b) shows interesting results with inhibition diameters ranging from 7 to 16 mm depending on the concentration and bacterial strain considered. For example, the ethyl acetate fraction most active on *S. aureus* had a diameter of 16mm at 50mg/mL, while for the same concentration the dichloromethane fraction most active on *E. faecalis*, *E. coli* 25922 and *E. coli* 35218 gave inhibition diameters of 12, 13 and 14mm respectively. It was also found that the nature of the strains (gram+ or gram-) did not significantly influence the activity of the extracts. The antibiotics used as positive controls (table 4a) showed inhibition diameters ranging from 11 to 22 mm depending on their specificity to the strain in question. Tikacillin had a diameter of 22mm on *S. aureus*, a gram-positive strain, while amikacin and vancomycin had the same diameter of 21mm on gram-negative strains of *Escherichia coli*.

According to the classification of antibacterial activity proposed by Ponce, *et al.* [31], a compound is considered very sensitive if its inhibition diameter is between 15 and 19 mm, and sensitive if the inhibition diameter is between 8 and 14 mm.

Based on this classification, we can say that fractions AE and DCM are very sensitive on *S aureus* at 50mg/mL, while all the other extracts and fractions: F. A, E. B, F. AE, F. DCM are sensitive from 25 mg/mL on all the strains studied. These results are confirmed by the data obtained from the test to determine the minimum inhibitory and bactericidal concentrations and the MBC/MIC ratio (table 3). According to Sanogo [32], an extract is bactericidal when its MBC is equal to its MIC or if the MBC/MIC ratio is less than or equal to 4. It is said to be bacteriostatic if the MBC/MIC ratio is greater than 4. On the basis of this assertion, we can conclude that all the *Morelia senegalensis* leaf extracts tested are bactericidal, since the MBC/MIC ratios are between 1 and 2, with MICs ranging from 6.25 to 12.5 depending on the extract and strain considered. These results are comparable to those of other studies carried out on the species *Mitracarpus scaber* belonging to the same family (Rubiaceae) where the MIC found was 6.25 for a concentration of 50mg/mL, as attested by the work of Ouadja, *et al.* [33].

In this study, a global analysis led to the conclusion that Gram- bacteria (*Escherichia coli* 25922 and *Escherichia coli* 35218) are less sensitive to extracts than Gram+ bacteria (*Staphylococcus aureus* 29213 and *Enterococcus faecalis* ATCC 29212). In fact, most antibacterial agents are more active against Gram-positive bacteria than Gram-positive bacteria. The low sensitivity of Gram-negative bacteria could be attributed to the hydrophilic nature of their membrane, which blocks the penetration of hydrophobic molecules such as polyphenols [34]. The bacterial wall of *Escherichia. Coli* is very rich in lipopolysaccharides, which may be partly responsible for the difficulty hydrophobic molecules have in penetrating the membrane [26].

All the extracts tested in this study showed varying degrees of activity in inhibiting the DPPH and ABTS radicals (Table 2). For both methods, the ethyl acetate fraction was the most active with IC_{50} of 0.33 and 0.62 mg/mL for ABTS and DPPH respectively, whereas ascorbic acid used as a reference had IC_{50} of 0.04 and 0.06 mg/mL. Over all the extracts studied, the IC_{50} were lower with the ABTS test than with the DPPH test, thus showing the more sensitive nature of the ABTS cation radical in relation to our extracts. This difference in the sensitivity of the two tests can be explained by the mechanisms involved. In the DDPH test we find the radical hydrogen transfer

mechanism, whereas in the ABTS test we find the electron and hydrogen transfer mechanisms. It has been described that during microbial infections, reactive oxygen species such as ROS and RNS derived from NO are largely produced [35, 36]. These are responsible for the pain experienced, oxidative stress and inflammatory factors. Antioxidants are one of the most important biological molecules protecting the body against the dangers of endogenous and exogenous oxidants [37]. The use of secondary plant metabolites in the management of microbial infections could help prevent the dangerous consequences of oxidants.

5. Conclusion

The search for active ingredients from plant organs is still going strong. Plants continue to provide mankind with remedies for the various diseases with which people are confronted. With this in mind, extracts from the leaves of *Morelia senegalensis*, a Rubiaceae found in Senegalese flora, were studied against four reference strains of bacteria. The results of this preliminary study show that the polar extracts are the most active on all the parameters studied, demonstrating the importance of a probable synergistic action of all the chemical constituents of these extracts. The bactericidal activity of all the extracts studied shows an interesting spectrum of action of the secondary metabolites of this plant. In the future, it will be necessary to isolate and characterize the compounds responsible for the antibacterial and antioxidant activities observed.

Competing Interests

Authors have declared that no competing interests exist concerning this manuscript.

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