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Research Article

In vitro anti-inflammatory and antimicrobial activity of *Securidaca longepedunculata* and *Annona senegalensis* hydro-alcoholic extract

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Abstract

Annona senegalensis and *Securidaca longepedunculata* are two plants traditionally used in inflammation and wounds infection treatment after snakebites.

This study aims to investigate the in vitro anti-inflammatory and antimicrobial activities of the hydroalcoholic extracts of *Annona senegalensis* and *Securidaca longepedunculata*.

Antimicrobial activity of the two plant extracts was examined against five bacterial strains with the well diffusion method and the inhibition zones diameters (IZD), minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined using the 96-well microplate dilution method. While antiinflammatory activity was assessed by the albumin denaturation method.

The results obtained showed that the hydroalcoholic extract of *Annona senegalensis* has antimicrobial property against *Staphylococcus aureus* (IZD=12.22 ± 0.24 mm, MIC=62.5 mg/mL, MBC=125 mg/mL) and against *Pseudomonas aeruginosa* (IZD=12.06 ± 0.06 mm, MIC=125 mg/mL, MBC=250 mg/mL). *Securidaca longepedunculata* also showed its antimicrobial activity against *Staphylococcus aureus* (IZD=12.03 ± 0.03 mm, MIC=125 mg/mL, MBC=250 mg/mL) and *Candida albicans* (IZD=12.12 ± 0.07 mm, MIC=62.5 mg/mL, MFC=125 mg/mL).

In the other hand, *Annona senegalensis* and *Securidaca longepedunculata* exhibited concentration-dependent anti-inflammatory activity by reducing significantly ($p<0.001$) the denaturation of BSA. In addition *S. longepedunculata* inhibited haemolysis significantly ($p<0.001$) more than Diclofenac sodium at 200 and 400 μ g/mL.

Hence, it was concluded that *Annona senegalensis* and *Securidaca longepedunculata* possessed anti-inflammatory and antimicrobial properties and can be used in the treatment of inflammation and wounds infection after snakebites.

Keywords: *Annona senegalensis*, *Securidaca longepedunculata*, anti-inflammatory, antimicrobial, BSA.

INTRODUCTION

Inflammation and wound infection occurring after snakebite complicate treatment of victims. To treat snake bites in Africa, few victims use modern health facilities¹. The microbes resistance to available antibiotics, the blow of antibiotics and anti-inflammatory drugs available on the market leads to the search for new therapeutic molecules with antimicrobial and anti-inflammatory properties. Medicinal plants are an adequate solution to these problems. Victims often use medicinal plants. *Annona senegalensis* (*A. senegalensis*) and *S. longepedunculata* are medicinal plants used in envenomation management in Togo².

A. senegalensis is a small tree that is widely distributed in Africa³. It is usually 2 to 6 meters tall and has an aromatic

flower that is used to flavour foods. Its ripe fruit has a yellow colour and a pleasant smell. Its fruit is edible⁴. Previous studies have shown that this plant has anti venom^{4,5}, anti diarrhea⁶, anti cancer⁷, Spermatogenic⁸, anti convulsion⁹ properties. A methanol root extract has analgesic and anti-inflammatory effects¹⁰. Ethanol and methanol extracts from roots, leaves and bark have antimicrobial effects¹¹.

Securidaca longepedunculata is also called «snake tree». It is a 7 m or 10 m tall shrub with a smooth bark and grey branches. Its pink to purple flowers make it easy to recognize during its flowering season¹². Previous studies have shown that this plant has: antioxidant¹³, antiplasmodiale¹⁴, antiparasitic¹⁵ properties. The methanol extract, petroleum ether fraction and methanol fraction have

anti-inflammatory effects ¹⁶. The methanol extract and chloroform fraction have antibacterial properties ¹⁷.

The control of inflammation and wound infection caused by snakebites is important in envenomation management. Anti-inflammatory Medicinal plants are used to treat several adverse effects of synthetic anti-inflammatory drugs. Antimicrobial plants are the source of new molecules that can counteract microbial resistance. The aim of this study is to evaluate in vitro anti-inflammatory and antimicrobial properties of *S. longepedunculata* and *A. senegalensis* using their hydroalcoholic extracts.

MATERIAL AND METHODS

Plants materials

Roots of *A. senegalensis* were collected from Gblainvié in Zio prefecture (Togo), located at 30 km north of Lomé. The roots of *S. longepedunculata* were collected from Anié locality in the prefecture of Anié (Togo). This locality is located 187,7 Km north of Lomé.

Both plants have been identified in Botany and Plant Ecology Laboratory of Faculty of Science (University of Lome), where voucher specimen was deposited in the herbarium under the number TOGO 15673 (*A. senegalensis*) and TOGO 15676 (*S. longepedunculata*).

Roots of the plants were cleaned out with water, cut into small pieces, dried at the Animal Physiology laboratory at 22°C and then reduced into powder using THOMAS-Wiley, LABORATORY MILL, Model 4 mill.

Preparation of the hydro-alcoholic extracts

400g of powder from each plant were extracted in 4L of an ethanol/water mixture (50:50, V/V) for 72 hours under intermittent manual agitation. The crude extract was filtered on Whatman paper and evaporated in vacuum at 45°C using a Rotavapor (Heidolph2, Germany).

The extracts were in the form of crystals and have been stored in the refrigerator at 4°C.

Phytochemical Study of *A. senegalensis* and *S. longepedunculata*

Phytochemical screening

The phytochemical analysis was performed for detection of phyto-constituents present in the extracts using standard procedure by ¹⁸.

Determination of total phenols and tannins

Total phenols were measured in the extracts by the Folin-Ciocalteu method and for the determination of tannins; a second dosage of the phenols was performed after fixing tannins by PVP (Polyvinyl pyrrolidone). Total tannins content was determined by absorbance difference between the first and second assay according to the method of Maksimovic et al., ¹⁹.

Determination of total flavonoids

Flavonoids content was determined according to the method used by Mimica-dukic ²⁰. Briefly, to 2 ml of extract / rutin at different concentrations (5-100 µg/ml), 2 ml of aluminum chloride (20 mg/ml) and 6 ml of sodium acetate (50 mg/ml) were added. Absorbance was read at 440 nm after 2.5 hours of incubation.

In vitro antimicrobial property of *A. senegalensis* and *S. longepedunculata* extracts

Microbial strains

The pathogenic bacterial strains assayed in this study were *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 700603, including the yeast *Candida albicans* ATCC 35659. These strains come from bacteriology laboratory strain bank of the National Institute of Hygiene (Togo).

Microbial suspensions preparation

Grown 24 hours old Microorganisms were used. To obtain these young colonies of microorganisms, the selected germs were isolated on Muller-Hinton agar (MHA) for bacteria and on Sabouraud Chloramphenicol agar (SCA) for *Candida albicans*. Incubation was done at 37°C for bacteria and 25°C for *Candida albicans* for 18 to 24 hours.

Microbial suspensions of densities of 0.5 MC Farland diluted 10⁻¹ were prepared in normal physiological saline with young colonies of microorganisms.

The *S. longepedunculata* and *A. senegalensis* extracts solutions were prepared at a concentration of 250 mg/mL in distilled water and then sterilized on millipore membrane of 0.45 µm porosity and 47 mm in diameter ²¹.

Presumptive Test

It is a presumptive test that has made it possible to identify the active extracts starting from a high concentration. The antibiotic susceptibility testing was performed by the agar well diffusion method with some changes ²¹. The high concentration of extract in this study is 250 mg/mL.

The microbial suspensions used were equal to 0.5 Mac Farland (~108 CFU/mL). The inoculum was introduced on culture medium prepared under standard conditions. These were MHA for bacteria and SCA for *Candida*. The quality of these medium was evaluated by sterility and fertility tests before use. After inoculation of the medium, wells of 6 mm in diameter were made using a sterile hollow punch concentrically in the agar. Gentamicin solution's 30 µg/mL (for *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*) and Nystatin solution's 250 mg/mL for *Candida albicans* were used as reference drugs. For negative controls, sterile distilled water was used in place of the extract. After 30 minutes of pre-diffusion at laboratory temperature, the Petri dishes were incubated for 24 h at 35°C for the bacteria and 25°C for the yeasts. The microbial growth inhibition zone diameter's was measured using an electronic reading chart. Extracts having an inhibition diameter ≥ 12 mm (including disc) were used for the determination of MIC and MBC/MFC. The tests were repeated in triplicate ²².

Minimum Inhibitory Concentrations (MIC) and Bactericidal/Fungicidal (MBC/MFC) determination

Extracts that showed a growth inhibition diameter of 12 mm according to our presumptive test were used for the MIC and MBC/MFC determination.

This test was performed using 96-well microplate dilution method ^{23, 24, 25}. From a stock extract solution of 250 mg/mL. A successive dilutions series of the two plants extracts (250, 125, 62.5, 31.25, 15.625, 7.8125 and 3.90625 mg/mL) were placed in Mueller Hinton Broth (MHB). The wells were inoculated with a microbial suspension at 6x10⁵ CFU/mL. Quality control was performed with MHB (not inoculated).

Another control was performed with MHB seeds to facilitated reading. The tests were performed in a sterile environment. The preparations were covered with parafilm and incubated at the appropriate temperature for 24 h. After incubation, the wells were observed with the naked eye. Turbidity presence corresponded to microbial culture presence. The MIC of the extract on tested strain corresponds to extract lowest concentration for which no culture was observed. Then, 100 μ L was taken from wells that did not show visible microbial growth to the naked eye and plated on MHA for bacteria and SCA for *Candida albicans*. Incubation was carried out at the appropriate temperature for 24 h. The tests were performed in triplicate. The lowest concentration for which no colonies were found was considered the MBC or MFC of the extract on the strain tested. The MBC/MIC ratio was used to determine the antibiotic activity of the extract on the microbial strain.

Assessment of the *in vitro* anti inflammatory activity of *A. senegalensis* and *S. longepedunculata* extracts

Membrane stabilisation assay

The method described by Javed et al.,²⁶ and Joshi et al.,²⁷ was used to perform this test. Wistar rats were anesthetized using light diethyl ether and blood was collected from the retro-orbital sinus in heparinised tubes. The collected blood was centrifuged at 1500 rpm for 10 min and washed three times with the same volume of normal saline. The reconstitution of red blood cells (RBC) with saline solution was 10% v/v suspension. To 1 mL of the RBC suspension were added 1 mL of plant extract or the reference drug Diclofenac sodium at different concentrations (50-1600 μ g/mL) and 2 mL of hyposaline solution (0.36%). After 30 min of incubation at 37 °C, centrifugation (3000 rpm) was performed for 20 min. The assay was performed in three replicates for each concentration and the membrane stabilisation percentage reflecting anti-inflammatory activity was determined after reading the absorbance at 560 nm.

The inhibition percentage was calculated by following formula.

$$\text{Anti-inflammatory activity (\%)} = (\text{A}0 - \text{A}t / \text{A}0) \times 100$$

A0 was the control absorbance (without extract) and At was the absorbance of extract or drug presence.

Inhibition of bovine protein serum albumin (BSA) denaturation

The anti-inflammatory effect of extracts was investigated using Saleem et al., method²⁸. To 0.45 mL of bovine serum albumin (BSA) solution (5% w/v), 0.05 mL of extract at different concentration or reference drugs (Diclofenac sodium) were added (1600; 800; 400; 200; 10 and 50 μ g/ml). Incubation took place twice : at 37°C during 20 min

incubation, and at 70°C during 5 min. After cooling, 2.5 mL PBS (pH 6.3) was added to each sample. The test was carried out in three replicates for each concentration and the protein denaturing inhibition percentage that reflects anti-inflammatory activity was determined after reading the absorbance at 660 nm.

The inhibition percentage was calculated by following formula.

$$\text{Anti-inflammatory activity (\%)} = (\text{A}0 - \text{A}t / \text{A}0) \times 100$$

A0 was the control absorbance (without extract) and At was the absorbance of extract or drug presence.

Data Analysis

Data were expressed as Mean \pm SD (standard deviation) for presumptive test and Mean \pm SEM (standard error of the Mean) for anti-inflammatory tests using the GraphPad Prism 7 software. Statistical differences between groups were determined by ANOVA followed by Dunnett test and considered significant for $p < 0.05$.

RESULTS

Phytochemical Study of *A. senegalensis* and *S. longepedunculata*

Phytochemical screening

Phytochemical analysis of hydroalcoholic extracts *A. senegalensis* and *S. longepedunculata* revealed the presence of flavonoids, tannins, carbohydrates, alkaloids, phenols, and saponosides. The results of phytochemical screening are resumed in table 1.

Table 1 : Phytochemical screening

Metabolites	<i>A. senegalensis</i>	<i>S. longepedunculata</i>
Alkaloids	+	+
Tannins	+	+
Flavonoids	+	+
carbohydrates	+	+
Phenols	+	+
Saponosides	+	+

+ : presence

Determination of total flavonoids, phenols and tannins

Table 2 shows that *A. senegalensis* and *S. longepedunculata* contain phenolic compounds.

Table 2 : Total phenols, flavonoids and tannins content

Extracts	Total flavonoids (mgRE/g)	Total phenols (mgGAE/g)	Total tannins (mgGAE/g)
<i>A. senegalensis</i>	15.62 \pm 0.71	31.77 \pm 2.24	5.15 \pm 2.24
<i>S. longepedunculata</i>	29.62 \pm 0.95	11.09 \pm 0.62	1.07 \pm 0.62

Total phenols and tannins are expressed in mg Gallic Acid Equivalent/g extract. Flavonoids are expressed in mg Rutin Equivalent/g extract.

In vitro antimicrobial property of *A. senegalensis* and *S. longepedunculata* extracts

Antimicrobial presumptive test

The results showed that *A. senegalensis* extract was active on *Staphylococcus aureus* (*S. aureus*) with IZD = 12.22 ± 0.24 mm and on *Pseudomonas aeruginosa* (*P. aeruginosa*) with IZD

= 12.06 ± 0.06 mm. *A. senegalensis* root extract has not active on : *Klebsiella pneumonia* (*K. pneumonia*), *Escherichia coli* (*E. coli*) and *Candida albicans* (*C. albicans*). In addition, *S. longepedunculata* extract was active on *S. aureus* with IZD = 12.03 ± 0.03 mm and *C. albicans* with IZD = 12.12 ± 0.07 mm. *S. longepedunculata* root extract has not active on *K. pneumoniae*, *E. coli* and *P. aeruginosa*. (Table 3)

Table 3 : inhibition zone diameter obtain in presumptive test

	IZD (mm)			
	<i>A. Senegalensis</i>	<i>S. longepedunculata</i>	GN	NY
	250 mg/mL	250 mg/mL	30 µg/mL	250 mg/mL
<i>P. aeruginosa</i> ATCC 27853	12.06 ± 0.06	NA	14.80 ± 0.79	
<i>K. pneumonia</i> ATCC 700603	NA	NA	11.84 ± 0.34	
<i>S. aureus</i> ATCC 29213	12.22 ± 0.24	12.03 ± 0.03	27.30 ± 0.81	
<i>E. coli</i> ATCC 25922	NA	NA	20.65 ± 0.06	
<i>C. albicans</i> ATCC 35659	NA	12.12 ± 0.07		29.09 ± 0.04

IZD = inhibition zone diameter, GN = Gentamicin, NY = Nystatin, NA = not active

Determination of Minimum Inhibitory Concentrations (MIC) and Bactericidal/Fungicidal (MBC/MFC)

MICs and MBCs/MFCs were determined for germs that were susceptible to extra ts with inhibition diameters ≥ 12 mm. The bacteriostatic and bactericidal effects of the extracts on the germs were determined by the ratio of MBC/MIC or MFC/MIC ≤ 1 (Bactericidal); MBC/MIC or MFC/MIC ≥ 2 (Bacteriostatic).

A. senegalensis extract was active on *S. aureus* with MIC = 62.5 mg/mL and MBC = 125 mg/mL. He is also active on *P. aeruginosa* with MIC = 125 mg/mL and MBC = 250 mg/mL. The MBC/MIC ratio is 2 for the two bacteria on which *A. senegalensis* extract was active. *S. longepedunculata* extract was active on *C. albicans* with MIC = 62.5 mg/mL and MBC = 125 mg/mL. He is also active on *S. aureus* with MIC = 125 mg/mL and MBC = 250 mg/mL. The MFC/MIC or the MBC/MIC ratio is 2 for the two bacteria on which *S. longepedunculata* extract was active. (Table 4)

Table 4 : MIC and MBC of microorganisms

Germs	<i>A. senegalensis</i>			<i>S. longepedunculata</i>		
	CMI (mg/ml)	CMB (mg/ml)	MBC/CMI	CMI (mg/ml)	CMB (mg/ml)	MBC/CMI
			MFC/CMI			MFC/CMI
<i>P. aeruginosa</i>	125	250	2	0	0	0
<i>K. pneumonia</i>	0	0	0	0	0	0
<i>S. aureus</i>	62.5	125	2	125	250	2
<i>E. coli</i>	0	0	0	0	0	0
<i>C. albicans</i>	0	0	0	62.5	125	2

Assessment of the *in vitro* anti inflammatory activity of *A. senegalensis* and *S. longepedunculata* extracts

Membrane stabilisation assay

The analysis showed concentration-dependent protection of the cell membrane by the hydroalcoholic extracts of *A. senegalensis* and *S. longepedunculata*. For example, at the dose of 50 µg/mL, *A. senegalensis* and *S. longepedunculata*

significantly ($p < 0.001$) inhibited haemolysis compared to diclofenac.

These results provide evidence for the membrane stabilizing effect of both extracts as an additional mechanism for their anti-inflammatory activity (Figure 1).

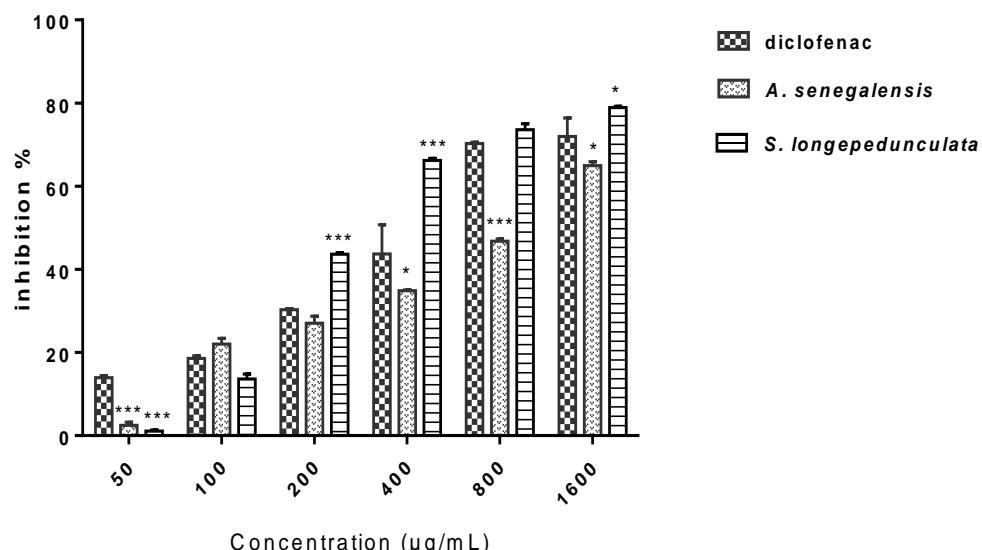


Figure 1: Effet of *A. senegalensis* and *S. longepedunculata* hydroalcoholic extracts on red blood cell membrane stabilisation

*** $p < 0.001$; * $p < 0.05$ (compared to diclofenac). % inhibition = $(A_0 - A_t) / A_0 \times 100$. A_0 was the control absorbance (without extract) and A_t was the absorbance of extract or drug presence.

Inhibition of bovine protein serum albumin (BSA) denaturation

The results in figure 4 showed that At 50 µg/mL, hydroalcoholic extracts of *A. senegalensis* and *S.*

longepedunculata significantly ($p < 0.05$) inhibited protein denaturation compared to Diclofenac. In addition, at the dose of 400 µg/mL, the inhibition of BSA denaturation by the two plants were more significant ($p < 0.001$) than Diclofenac (Figure 2).

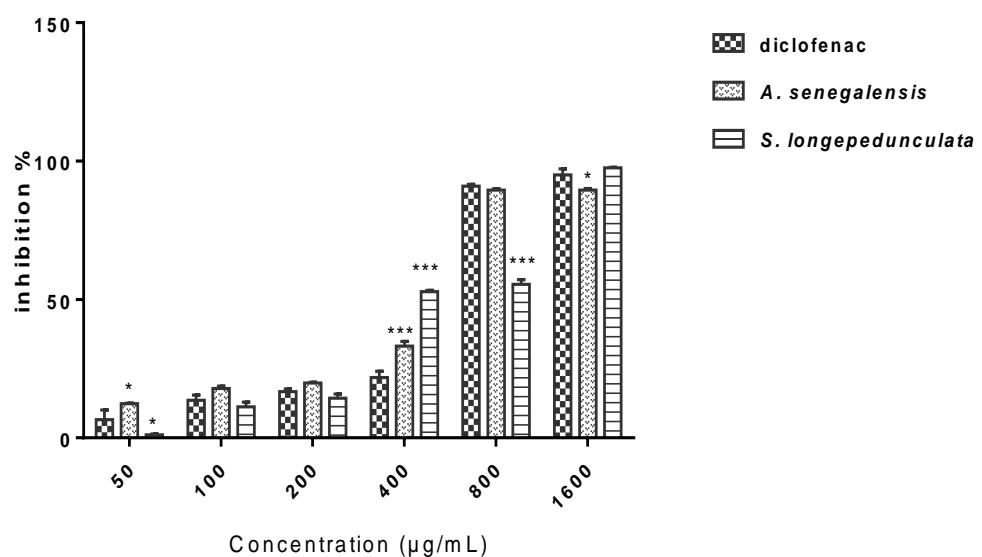


Figure 2 : Effet of *A. senegalensis* and *S. longepedunculata* hydro-alcoholic extract on BSA denaturation

*** $p < 0.001$; * $p < 0.05$ (compared to diclofenac). % inhibition = $(A_0 - A_t) / A_0 \times 100$. A_0 was the control absorbance (without extract) and A_t was the absorbance of extract or drug presence.

DISCUSSION

The search for natural anti-inflammatory and antimicrobial agents with fewer side effects has crucially increased nowadays. This study was then investigated in order to evaluate the antiinflammatory and antimicrobial activities of hydroalcoholic extracts of *A. senegalensis* and *S. longepedunculata*.

The antimicrobial assay was performed in order to detect the sensitivity of five germs (*Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 700603; *Candida albicans* ATCC 35659) in the presence of *A. senegalensis* and *S. longepedunculata* hydro-alcoholic extracts. Results revealed that 40% of the germs tested were

susceptible to hydro-alcoholic extracts of *A. senegalensis* and *S. longepedunculata*.

A. senegalensis extract was found to be active on *Staphylococcus aureus* (*S. aureus*) and *Pseudomonas aeruginosa* (*P. aeruginosa*) with inhibition zone diameters of 12.22 mm and 12.06 mm respectively. For the two bacteria on which *A. senegalensis* extract was active, the MIC = 62.5 mg/mL for *S. aureus* and MIC = 125 mg/mL for *P. aeruginosa*. The MBC extract for these two bacteria is 125 mg/mL for *S. aureus* and 250 mg/mL for *P. aeruginosa*. However, this extract was not active on *E. coli*, *K. pneumoniae* and *C. albicans*. Our results corroborate with the work which showed that Kaurenoic Acid isolated from *A. senegalensis* root bark inhibited *S. aureus* and *P. aeruginosa* but has no effect on *Klebsiella pneumoniae* and *Escherichia coli* ²⁹. This also corroborates with those of More et al., which showed that *A. senegalensis* methanol extract is not active on *Candida albicans* ³⁰. Our results are different from the work of Awa et al ³¹ in which methanol extract from *A. senegalensis* stem bark acted on *E. coli* with inhibition zone diameter equals 12 mm. Lino and Deogracious ³² showed that the aqueous extract inhibited *S. aureus* with a diameter of 18 mm. Similarly, the raw flavonoids isolated from stem bark of *A. senegalensis* inhibited *E. coli* an inhibition zone diameter equal to 18 mm ³³. The MIC and MBC obtained with methanol and ethanol extracts of this plant are different from the values obtained with hydro-alcoholic extract in this study. Thus, *A. senegalensis* methanol extract was active on *S. aureus* with MIC=250 µg/ml and MBC>1000 µg/ml ¹¹. The ethanol extract from *A. senegalensis* root bark acted on *S. aureus* with a MIC=500 µg/ml ³⁴.

In addition, *S. longepedunculata* extract was active on *S. aureus* and *C. albicans* respectively with 12.03 mm and 12.12 mm inhibition diameters. For the two microbes on which *A. senegalensis* extract was active, the MIC are 125 mg/mL for *S. aureus* and 62.5 mg/mL for *C. albicans*. *S. longepedunculata* extract has an MBC=250 mg/mL for *S. aureus* and an MFC=125 mg/mL for *C. albicans*. However *S. longepedunculata* is not found to be active on *E. coli*, *P. aeruginosa* and *K. pneumoniae*. Our results are same with those obtained by ³⁵ which showed that *S. longepedunculata* roots ethyl acetate fraction and n-butanol fraction have inhibitory effects on *S. aureus* and *C. albicans*. The two fractions had an inhibition zone diameter of 12 mm each on both germs. Acqueous extract yielded 13 mm inhibition zone diameter with *S. aureus* and MIC=1000 mg/mL ³². The lack of sensitivity of the microorganisms used in this work to the extracts of *A. senegalensis* and *S. longepedunculata* could be explained by: an inaccessibility of the molecules contained in these two extracts to the microbial cell due to the impermeability of the membrane of the microbes to the molecules contained in these extracts; or an affinity of the molecules contained in these two extracts for the bacterial target or an expulsion of the antibiotic molecules contained in these extracts by chromosomal efflux pumps.

The diversity observed in the sensitivity of *K. pneumoniae* to the extracts could be explained first by the capsule surrounding the bacteria presence as a natural protection ^[36]. Difference between our results and those obtained with methanol and ethanol extracts is explained by the fact that hydro-alcoholic extract (50:50) would not have been able to extract sufficient the active ingredients extracted acting microbe with methanol and ethanol. These active ingredients would increase the bactericidal potency of the two extracts when methanol and ethanol are used as maceration solvents.

Inflammation is a physiological process that defends body against aggression that results in tissue alteration. Inflammation primary function is to eliminate the aggressor and allow tissue repair. Short-term inflammation known as acute inflammation is a beneficial phenomenon of the body that allows it to regain its physiological integrity. Whereas the negative inflammation aspect occurs when it lasts and becomes a chronic inflammation. In this case, inflammatory reaction should be controlled by the drugs ³⁷.

In this study, the effect of *S. longepedunculata* and *A. senegalensis* extracts on membrane stabilization was indexed via the ability to protect RBCs from heat-induced haemolysis.

The red blood cell membrane stabilization can be extrapolated to the lysosomal membrane because the two membranes are analogous. The haemolytic effect of a hypotonic solution is expressing by excessive accumulation of fluid inside the cell that caused membrane rupture ³⁸.

Results showed that *S. longepedunculata* and *A. senegalensis* protected red blood cell membrane against haemolysis. This effect occurred in dose dependent manner. However, *S. longepedunculata* showed a higher protective capacity than the reference drug used for the 200 µg/mL dose. This could be explained by the control of surface/volume ratio of cells or by strengthening of the red blood cells membrane by the plants.

Protein denaturation is a process by which proteins lose their structure due to the presence of other compounds, external stress, or heat, thus leading them to lose their biological functionality. Therefore, denaturation of tissue proteins is recognized as a marker of inflammation. In our study, Both extracts inhibited BSA protein denaturation in concentration dependent manner. It is possible that bioactive compounds in the extracts protect lysosomal membranes against injury by interfering with activation of phospholipases. The extracts may block an exaggerated release of pro-inflammatory molecules, including histamine, serotonin, tachykinine, bradykinine and complement proteins ³⁹. Our results corroborate with other studies that had demonstrated the anti-inflammatory effect of *A. senegalensis* ^{40,41} and *S. longepedunculata* ^{42,43}.

To better understand the effect of the two plants, their preliminary phytochemical study was carried out. With phytochemical screening, the presence of alkaloids, tannins, flavonoids, carbohydrates, saponosides and phenols were revealed in the two extracts. This is in accordance to the works of ⁴⁴ and ⁴⁵. Quantitative tests confirmed the presence of these phenolic compounds. The phenolic compounds present in the plants could explain their antimicrobial and anti-inflammatory effects because many studies demonstrated the antibacterial properties of tannins ⁴⁶ and flavonoids ⁴⁷; anti-inflammatory properties of phenols such as coumarin ⁴⁸, flavonoids ⁴⁹ and tannins ⁵⁰. In fact, these compounds present in the extract having functional groups serve as electron donors by breaking the free radical chain. Flavonoids also have the property of stabilizing the structure of biological membranes. Phenolic compounds also are able to inhibit either the production or the action of pro-inflammatory mediators, resulting in anti-inflammatory capacity.

CONCLUSION

In summary, *A. senegalensis* and *S. longepedunculata* have remarkable antimicrobial and anti-inflammatory properties. These properties could be related to the phytochemical compounds present in the hydroalcoholic

extracts of *A. senegalensis* and *S. longepedunculata*. Taken together, these two medicinal plants can be used in the patients suffering of inflammation and microbial infection of wounds by snake bites.

However, further studies are required to understand the exact mechanism of action of various constituents present in the two plants.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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