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Phytochemical screening and evaluation of the antibacterial activity of extracts dichloromethane of total alkaloids from the leaves and roots of *Guiera senegalensis* (Combretaceae), a plant widely used in traditional Senegalese medicine

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Abstract

The present study involved extracting total alkaloids from the leaves and roots of *Guiera senegalensis*, a plant widely used in traditional Senegalese medicine. The results showed a low extraction rate.

A phytochemical screening of these alkaloid extracts was carried out and the results showed the presence of alkaloids and a total absence of other chemical groups, in particular polyphenols, flavonoids, catechic tannins and gall tannins.

Finally, antibacterial activity tests of alkaloid extracts from *Guiera senegalensis* leaves and roots were carried out on two pathogenic bacterial strains, namely "Gram +"*Staphylococcus aureus* and "Gram -" *Escherichia coli*. The results showed an antibacterial effect of the leaf extract on *Staphylococcus aureus* and *Escherichia coli* with a lower antibacterial power 4. The root alkaloid extract showed an antibacterial effect on Staphylococcus aureus with an even lower antibacterial potency 4. On the other hand, the root extract showed a bacteriostatic effect on *Escherichia coli* with an antibacterial potency greater than 4.

Keywords: *Guiera senegalensis*; Alkaloids; Antibacterial activity; Phytochemical tests

1. Introduction

Plants have long been used by people all over the world to treat themselves. Today, they still represent the leading source of therapeutic substances in developing countries (WHO, 2014). According to Legani *et al.,* (2010), the WHO estimates that 40% of medicines have a natural product as their active ingredient and that around 80% of the world's population depends on plants.

In recent decades, there has been a great deal of interest in the study of medicinal plants and their use in traditional medicine in different parts of the world. Of the 1,335 molecules that obtained marketing authorization between 1980 and 2010, 364 were derived directly from natural products, 202 were biomolecules that were generally peptides, and 324 were inspired by or mimicked natural products (Cragg, 2012)

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Infectious diseases are caused by different types of pathogenic microorganisms such as bacteria, viruses or fungi (WHO, 2015). These infectious diseases are responsible for more than 70% of deaths per year worldwide and account for 43% of deaths in developing countries (WHO, 2015). Bacterial infections are responsible for 70% of these deaths (Gangoue, 2003). The discovery of antibiotics helped to significantly reduce the spread of these pathogens. The remarkable effectiveness of these antibiotics has been accompanied by their massive and abusive use, leading to the emergence of resistance.

A number of studies have been carried out on different plants to assess their antibacterial activity. This study focused on *Guiera senegalensis*, a plant widely used by local people to treat various infections such as coughs and malaria. With a view to isolating the active principles responsible for the antibacterial activity of *Guiera senegalensis*, this work focuses on characterising the plant's chemical groups and evaluating the antibacterial activity of extracts of total dichloromethane alkaloids from the leaves and roots of *Guiera senegalensis*.

2. Material and methods

2.1. Material

2.1.1. Plant material

The plant material used in this work consisted of samples of leaves and roots of *Guiera senegalensis*. The species was collected at Thiolaye, a locality in the commune of Nguénième (Mbour department/Thiès region/Senegal). The harvested samples were air-dried in a ventilated area away from direct sunlight and humidity for two months. After drying, they were ground to a relatively fine powder using an electric mill fitted with a sieve at the Faculty of Medicine, Pharmacy and Odontology at Cheikh Anta Diop University in Dakar. The powder obtained was stored in plastic buckets for extraction of the alkaloids.

2.2. Biological material

The biological material consisted of two strains of bacteria, *E. coli* ATCC 35218 and Staphylococcus aureus ATCC 29213, which were supplied by the laboratory of the Ecole Supérieure Polytechnique (ESP) in Dakar. These bacteria were chosen because they represent the Gram-positive and Gram-negative species most commonly responsible for nosocomial infections and resistant to antibiotics.

2.3. Methods

2.3.1. Extraction of total alkaloids from Guiera senegalensis leaves and roots

Preparation of the crude extract

Before the actual extraction of the alkaloids, 100 g of plant powder was macerated in 1000 mL of dichloromethane for 72 hours at room temperature. After filtration, the solvent was evaporated under vacuum using a rotary evaporator. The concentrate obtained is a crude dichloromethane extract. This concentrate is acidified three times with 250 mL of a 10% hydrochloric acid solution diluted with distilled water to equal volume at a pH of between 1 and 2. As a result, the alkaloids are found in the form of alkaloid salts in the acidic aqueous phase.

Extraction of total alkaloids from *Guiera senegalensis* leaves and roots

- Preparation of the crude extract

After this preliminary step (III.1.1 Preparation of the crude extract), the acidic solution is alkalinised by adding a 50% dilute ammonia solution with distilled water to obtain a basic solution with a pH between 9 and 10. The alkaloids are then converted to the basic state and can be extracted using non-polar organic solvents.

- Extraction of total alkaloids

The above basic solution was extracted with dichloromethane (3×150 mL). The dichloromethane phases are first combined and then washed several times with water until neutral, then dried over anhydrous magnesium sulphate (MgSO4) and finally evaporated under reduced pressure using a rotavapor to give the total alkaloid extract.

2.4. Tests to confirm the presence of alkaloids in the various extracts using thin layer chromatography (TLC) and color reactions

In this part, the aim was to highlight the presence of alkaloids from the leaves and roots in the extracts, while validating the protocol used. This also enabled us to check for the absence of other chemical groups that might be present.

2.4.1. Characterisation reaction

Chemical groups were characterized using techniques described in the work of Ronchetti and Rosso (1971), Hedgnauer (1973), Wagner (1983) and Békro et al (2007). However, the alkaloids were identified using staining reactions and Thin Layer Chromatography (TLC).

Characterization of polyphenols

To identify the polyphenols in the total alkaloid extracts, an infusion was made by dissolving 1 mg of total extract in 20 ml of distilled water. The mixture was left to stand for 25 min before filtering. A 2 ml sample of the filtrate was taken and placed in test tubes. A few drops of an alcoholic solution of Fe Cl3 were added. The same filtrate was also used to characterize flavonoids and tannins.

Characterization of flavonoids

To reveal the flavonoids, 1 ml of the previous infusion is added to 1 ml of hydrochloric alcohol with a few shavings of magnesium. The appearance of a pink-orange color indicates the presence of flavones, while a purplish-pink color indicates the presence of flavanones and a red color indicates the presence of flavonols.

Tannins

- Catechic tannins or condensed tannins or non-hydrolysable tannins

To characterise catechic tannins, 1 mL of the infusion was added to 0.5 mL of Stiasny's reagent (a mixture of methanal and hydrochloric acid). The resulting mixture was heated to 90°C for 15 min. The appearance of a precipitate indicates the presence of catechic tannins.

- Gallic or condensed tannins

For the revelation of gallic tannins, the contents of the tubes after the catechin tannin test were filtered. Next, 0.5 mL of the filtrate was saturated with sodium acetate and 0.2 mL of a 2% solution of Fe C_{l3} was added to this mixture. The reaction gave a dark brown coloration in the presence of the gallic tannins.

- Alkaloids

1 mg of each dry extract was dissolved in 20 mL of 10% concentrated sulphuric acid. A few drops of Dragendoff's reagent were added to this mixture. The appearance of an orange-red precipitate indicates the presence of alkaloids.

Characterization by thin layer chromatography (TLC)

A few microliters of the alkaloid extract and a control were deposited at different points on the silica-coated plate. The eluent was a mixture of cyclohexane (9V) and methanol (1V). The spots deposited on 1 cm of the lower edge of the plate were air-dried and then introduced into the migration tank containing the eluent. After drying in an oven at 100 °C, the plates were sprayed.

With a typical alkaloid developer, Dragendorff's reagent (mercuric-potassium iodide solution), which gives an orange-red precipitate in the presence of alkaloids (Figure 1).

2.5. Evaluation of the bactericidal activity of total alkaloid extracts

2.5.1. Determination of extract sterility

Preparation of the culture medium

A mass of 0.1425 g of thioglycolate was first added to 50 mL of distilled water. The resulting solution was autoclaved at 121 °C for 15 min. A mass of 2.3 g of nutrient agar and 6.5 g of sabouraud agar were dissolved in two graduated glass bottles each containing 100 mL of distilled water. The two solutions obtained were autoclaved at 121°C for 15 minutes.

Once they had cooled, they were placed in eight petri dishes containing agar. They were then dried in a host. After drying, a few microliters of thioglycolate broth were added to the plates, which were then incubated in an oven at 37°C for 24 hours.

Preparation of extracts

0.1 g of each dichloromethane alkaloid extract was dissolved in four test tubes containing 10 ml of thioglycolate solution (broth). The whole mixture was placed in a 37% oven for 24 hours before use.

Inoculation

After the incubation period, the broth was inoculated into petri dishes containing nutrient agar and another containing Sabouraud agar. These petri dishes were incubated in an oven at 370 °C. After 24 hours of incubation, a sterility test was carried out on the petri dishes to eliminate any contaminated plates.

2.5.2. Evaluation of the sensitivity of bacterial strains to extracts

The agar diffusion method described by Tsurnirindravo *et al.,* (2009) was used to assess the antimicrobial activity of our extracts.

By dilution in a solid medium

- Preparation of the alkaloid extract

Distilled water was used as a solvent to dilute all the extracts obtained. 100 mg of dry extract was introduced into a test tube by adding 1 ml of distilled water before vortexing.

- Preparation of the culture medium

Muller Hinton (MH) agar is used as a culture medium. It is prepared by weighing 3.8 g of the agar dissolved in 100 mL of distilled water, then the mixture obtained is put in an autoclave at 121 °C for 15 min. Once the agar had been prepared, it was poured into petri dishes and solidified at room temperature. After drying, cups were hollowed out and incubated at 37 °C.

- Preparation of the bacterial inoculum

From a culture of microorganisms (Escherichia coli, Staphylococcus aureus, etc.) taken the day before the analysis. A fresh colony or colonies of bacteria were first taken and then suspended in a tube containing a sterile normal saline solution (physiological water) and vortexed. The microbial suspension was then visually compared with the Mac Farland 0.5 turbidity standard and adjusted, if necessary, by adding more saline solution or cells. The final solution obtained constitutes the bacterial inoculum, estimated at 108 bacteria/mL

- Seeding

After drying the plates, Muller Hinton agar was inoculated into the petri dishes, which were then dried at room temperature. After drying, the plates were swabbed with bacterial inoculum and left to dry for around ten minutes under a host.

- Distribution of alkaloid extracts and cups (wells)

After drying, cups were first hollowed out by inserting the large end of a Pasteur pipette into the agar and then these cups were filled with 50 µl of alkaloid extracts. In addition, an alcoholic solution was used as a positive control and distilled water was used as a negative control. Finally, the cups were incubated in an oven at 37 °C for 24 hours.

- Determination of the diameters of the inhibition zones

After this incubation period, the zones of inhibition were read at the point where no obvious growth was observed with the naked eye. The diameters of the zones of inhibition around each cup were measured to the nearest millimeter using a caliper (Table II). The efficacy of the alkaloid extracts was assessed according to the criteria described by Tsurnirindravo *et al.,* (2009).

Table 1 Standards used to read the results of sensitivity tests on dichloromethane alkaloid extracts

Determination of inhibition halo (A)	Degree of sensitivity of the extract
A < 7 mm	Insensitive
7 mm ≤ A < 8 mm	Sensitive
8 mm ≤ A < 9 mm	Fairly sensitive
A ≥ 9 mm	Very sensitive

Source: WHO 2002; Tsirinirindravo et al., (2009)

- Preparation of the bacterial inoculum

Two 24-hour-old bacterial colonies were first sampled using a pasteur pipette, then emulsified in a test tube containing 10 ml of sterile Muller-Hinton broth, and finally the mixture was incubated at 37°C in an oven for 3 hours. After this incubation period, a 0.3 ml suspension was taken from this pre-culture and diluted in 10 ml of sterile Muller-Hinton broth, then the mixture was homogenized.

- Preparation of the extract concentration range

The concentration range was determined using the double dilution method.

A 100 mg/ml solution of alkaloid extracts has already been prepared (III.4.2.1.2). A series of twofold dilutions was carried out on this same solution to obtain concentration ranges from 100 to 1.5625 mg/ml.

2.5.3. Determination of antibacterial power (BP)

Determination of antibacterial parameters of extracts

Determination of antibacterial parameters such as MIC and MBC were used to assess the antibacterial potency (BP) of our extracts.

Determination of MICs for the various extracts

In 28 experimental tubes, 1 ml of each alkaloid extract concentration range is introduced into contact with 1 ml of bacterial inoculum. In a first growth control tube, 1 ml of sterile distilled water and 1 ml of bacterial inoculum were first introduced, followed by 2 ml of sterile Muller-Hinton broth (MBH) in a second sterile control tube. All the tubes were incubated at 37o C in an oven for 24 hours (Figure III). After this incubation period, the tubes were observed with the naked eye. The lowest concentration for which no bacterial growth was observed with the naked eye corresponded to the MIC.

Determination of Minimum Bactericidal Concentrations (MBC) II.1.3.1.2.1 Numbering of the bacterial inoculum

The starting inoculum was diluted 10^{-four} (four dilutions were obtained: 10^{-1}, 10^{-2}, 10^{-3} and $10)^{-4}$.

- Seeding

These different dilutions were first inoculated into a petri dish (A and A') containing Muller- Hinton agar using a 2-microliter calibrator in a 5 cm long series, then incubated at 37 °C in an oven for 24 hours.

- Numbering of dichloromethane alkaloid extracts

After reading the tubes for MIC determination, the contents of these tubes were used to inoculate the Muller-Hinton agar contained in the petri dishes (B and B') in series 5 cm long. The BMC/MIC ratio is used to assess the mode of action of the Fauchere's extract in 2002.

3. Results

3.1. Extraction rate of total alkaloids

Table 1 below gives the dry extract mass and extraction rate.

Table 2 Extraction rate of total alkaloid extracts from *Guiera senegalensis*

Extracts	Sample weights (g)	Dry extract weight (g)	Extraction rate (%)
Leaves	300	4	1.3
Roots	300	3	1

3.2. Phytochemical screening of total alkaloid extracts

3.2.1. Characterization reactions

The chemical groups identified after phytochemical tests carried out on dichloromethane alkaloid extracts from *Guiera senegalensis* leaves and roots were alkaloids. However, no other groups were identified (Table 2).

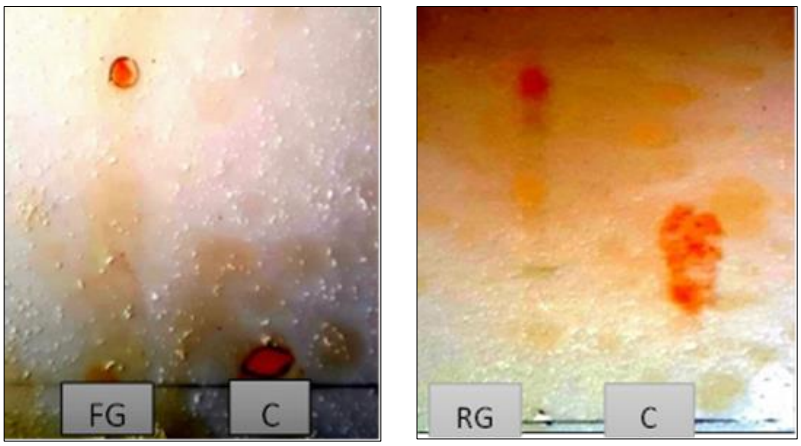
Table 3 Identification of chemical groups in the leaves and roots of *Guiera senegalensis*

Extracts	Polyphenols	Flavonoids	Condensedtannins	Hydrolysabletannins	Alkaloids
Leaves	-	-	-	-	+++
Roots	-	-	-	-	++

- = Negative ++ = average presence ++ + = strong presence

3.2.2. Characterization of total alkaloids by TLC

Figures and show photographs of thin layer chromatograms performed with the total alkaloid extracts to confirm the results obtained with the coloration and precipitation tests in test tubes. The TLCs show the presence of alkaloids in the extracts with an orange-red coloration.



FG : *Guiera senegalensis* leaves, RG: *Guiera senegalensis* root, C : colchicine

Figure 1 TLC photo of total alkaloid extracts from *Guiera senegalensis* leaves and roots

3.3. Sterility tests on extracts of total dichloromethane alkaloids

The various results of sterility tests on dichloromethane alkaloid extracts from *Guiera senegalensis* leaves and roots showed no germs (Figure 2).

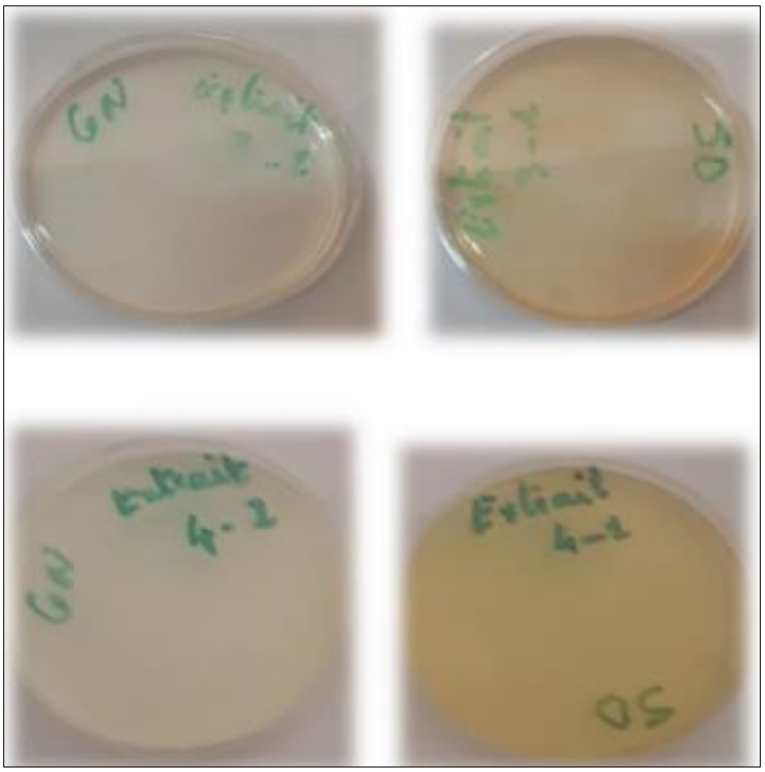


Figure 2 Germ-free extracts of dichloromethane alkaloids

3.4. Sensitivity testing of alkaloid extracts

The results obtained after the inhibition test on the two bacterial strains of the different extracts are presented in Figure 3. These results showed that the alkaloid extract of *Guiera senegalensis* leaves is more active on Staphylococcus aureus than on *E. coli*.

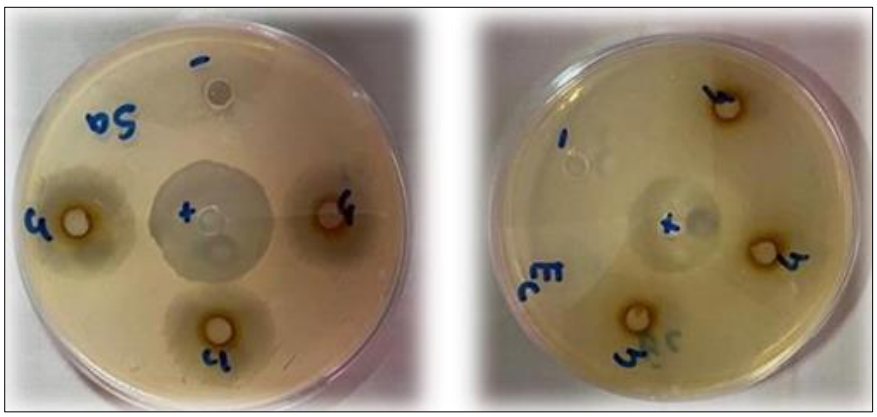


Figure 3 Presence of leaf sensitivity to *Staphylococcus aureus* and *E. coli*

These results showed a slight difference in the sensitivity of the alkaloid extract from *Guiera senegalensis* roots to *Staphylococcus aureus* and *E. coli*.

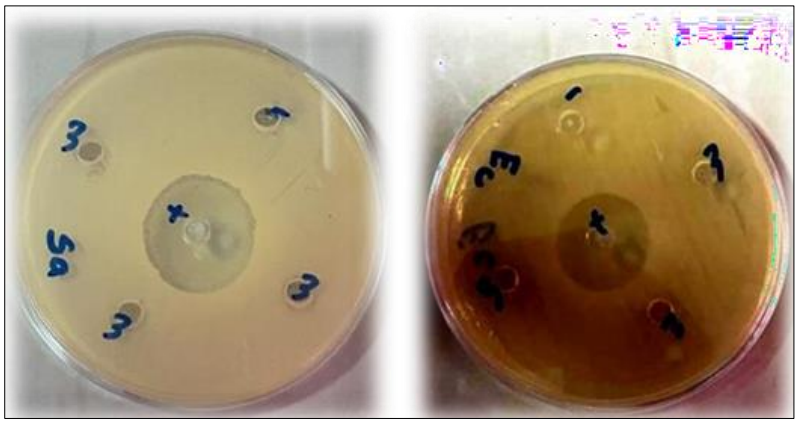


Figure 4 Presence and absence of root sensitivity to *Staphylococcus aureus* and *E. coli* respectively

Table 4 Zones of inhibition for the different extracts

Bacterial strains	***Guiera senegalensis***	
	Leaves	**Roots**
Escherichia coli	10.05 mm	6.75 mm
Staphylococcus aureus	22.23 mm	7.65 mm

3.5. Determination of antibacterial parameters of extracts

3.5.1. Determination of MICs for the various extracts

The different results obtained after measuring the MICs of the alkaloid extracts on the two strains tested in liquid medium are presented in Table 4.

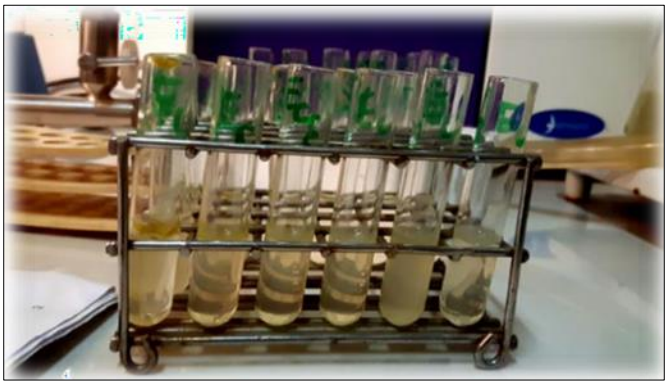


Figure 5 MIC readings from different test tubes after a 24-hour incubation period at 37 °C

The results of MIC tests on alkaloid extracts from *Guiera senegalensis* leaves and roots are shown in Table 4. The leaf extract gave a MIC of 1.5625 mg/mL for Staphylococcus aureus and 3.125 mg/mL for Escherichia coli.

Table 5 Determination of the different MICs for the various extracts

Extracts	***Escherichia coli***	***Staphylococcus aureus***
03	1,5625	3,125
04	3,125	1,5625

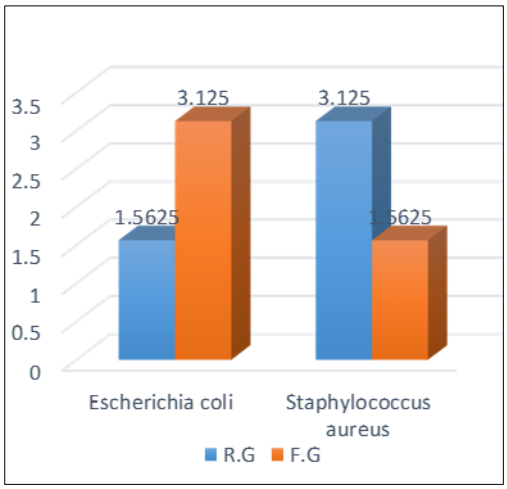


Figure 6 MIC diagram for different extracts

The diagram above shows the MICs of the various alkaloid extracts from *Guiera senegalensis* leaves on Staphylococcus aureus and *E. coli*. The leaf extract gave the lowest MIC on Staphylococcus aureus with 1.5625 mg/mL than on *E. coli* with 3.125mg/mL. The root extract, on the other hand, showed the opposite effect on the two strains tested.

3.5.2. Determination of the BMCs of the various alkaloid extracts

These results are shown in Table 5 after an incubation period of 24 hours.

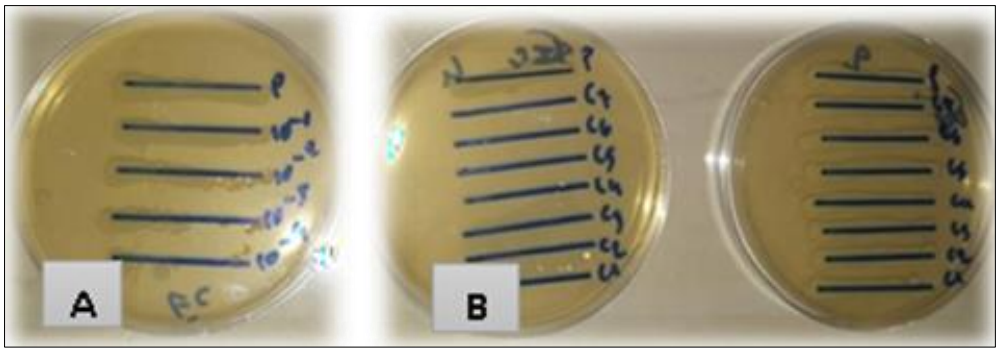


Figure 7 Bacterial inoculum and root extracts

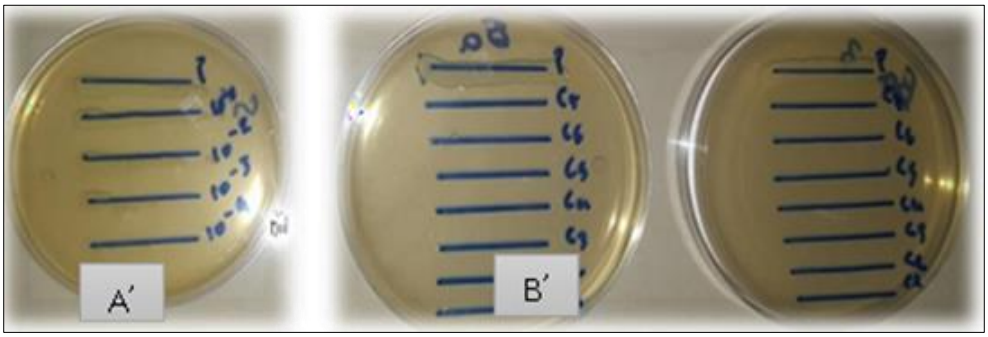


Figure 8 Numbering of bacterial inoculum and leaf extracts

Table 6 Determination of BMCs for extracts

Extracts	*E. coli*	*Staphylococcus aureus*
03	0	1.5625
04	1.5625	1.5625

03: Root *Guiera senegalensis* (R. G) 04: Leaves *Guiera senegalensis* (F. G)

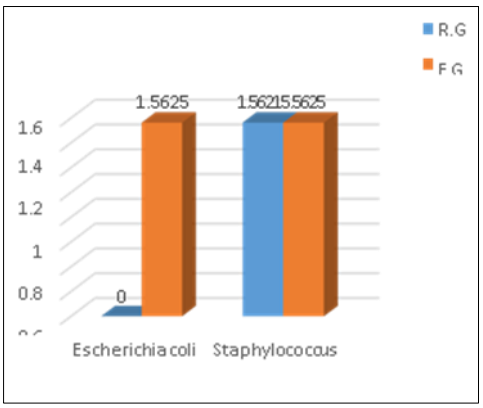


Figure 9 CMB diagram for the various extracts

The results of the BMCs, MICs and determination of the antibacterial potency (BP) of the alkaloid extracts are shown in Table 6.

Table 7 Evaluation of the antibacterial power of extracts

Extracts	*E. coli*			*Staphylococcus aureus*		
	CMB	**CMI**	**PA**	**CMB**	**CMI**	**PA**
03	/	1.5625	/	1.5625	3.125	0.5
04	1.5625	3.125	1	1.5625	1.5625	1

/ = absence; PA = CMB/CMI; PA with* = bacteriostatic power; without* = bactericidal; CMB and CMI in mg/mL

4. Discussion

The extraction of total alkaloids from the dichloromethane of the leaves and roots of *Guiera senegalensis* showed a low level. Moreover, the leaves were richer in alkaloids than the roots, with a slight superiority of 1.13% for the leaves and 1% for the roots for a sample mass of 300

g. These results could be explained by a difference in alkaloid concentration linked to the date of collection or the biotope of the harvesting environment.

The search for chemical groups, with phytochemical tests of coloration and precipitation in test tubes in dichloromethane alkaloid extracts, revealed that alkaloids were present. Consequently, this phytochemical constituent is responsible for the antimicrobial effects observed in *Guiera senegalensis* leaf and root extracts. Many alkaloids are known for their antimicrobial effects, notably Berberine and Sanguinarine.

Their presence in the leaves and roots of this species could explain why this plant is used to treat coughs (Diatta, et al. 2007).

Sterility tests on dichloromethane alkaloid extracts from *Guiera senegalensis* leaves and roots did not reveal any germs. Consequently, the extracts were not contaminated by any external agent that might be responsible for the antibacterial activity of the strains tested.

Evaluation of the antibacterial activity of dichloromethane alkaloid extracts from the leaves and roots of *Guiera senegalensis* was carried out on two bacterial strains, namely *E. coli* and *Staphylococcus aureus*. Sensitivity tests showed that the root extract was inactive against

E. coli, with a zone of inhibition of 6.75 mm, while it was slightly sensitive against Staphylococcus aureus, with a zone of inhibition of 7.65 mm. Leaf extract was not very sensitive to *E. coli*, with a zone of inhibition of 10.05 mm, but was highly sensitive to Staphylococcus aureus, with a zone of inhibition of 22.23 mm. These results differ from those of Salihu and Ikram in 2015, who were able to show that the crude dichloromethane extract of *Guiera senegalensis* leaves was not sensitive to either of the two strains tested. These differences can be explained by a difference in the composition of the active substances. If the results obtained are anything to go by, the inhibitory effect of the leaf extract on the germs tested would be linked to the presence of alkaloids. Furthermore, the antibiotics used showed more significant activity than all the extracts tested, with inhibition zones of 13.75 mm and

25.95 mm. This could be explained by the fact that the antibiotics are isolated, pure molecules with a well-known concentration Sourabie *et al.,* (2010) whereas the dichloromethane alkaloid extracts are mixtures that have not been purified of their active substances.

Antibacterial parameters, i.e. MICs and BMCs, were determined on extracts of dichloromethane alkaloids from the leaves and roots of *Guiera senegalensis*. The leaf extract was found to have bactericidal activity on the germs tested, as the AP was less than or equal to

4 (Kamanzi 2002). On the other hand, the root extract has a bactericidal effect only on *Staphylococcus aureus* with an AP less than or equal to 4 and is bacteriostatic on *Escherichia coli*.

5. Conclusion

The alkaloid extraction method used in this study shows a fairly low extraction rate.

The present work involved phytochemical screening of the extracts obtained. The results show that these extracts contain only alkaloids, which could be responsible for the antibacterial effect observed during the tests.

The study also highlighted the antibacterial properties of the various extracts on the strains tested. These strains are implicated in various pathologies responsible for a number of diseases in humans. The results of the antibacterial tests on the leaf extract all showed an antibacterial effect on the germs tested. However, the root extract only showed an antibacterial effect on Staphylococcus aureus and a bacteriostatic effect on *Escherichia coli.*

It would therefore be interesting to undertake more in-depth research to isolate and identify the molecules responsible for the antibacterial activity of these extracts.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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