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Article

Chemical Characterization of *Guiera senegalensis* Leaves Organic Extracts and Their Antibacterial Activity: A Plant of the Senegalese Pharmacopeia

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ABSTRACT

Guiera senegalensis is a plant from the Senegalese pharmacopeia, current study dealt with the evaluation of crude extracts of *G. senegalensis* leaves antibacterial activity. The phytochemical study was carried out using coloring and precipitation tests. For the antibacterial evaluation, the minimum inhibitory concentration was determined using the double dilution method of concentrations expressed as (g/mL). Two strains of bacteria were used: *Escherichia coli* and *Staphylococcus aureus*. In terms of results, phytochemical analysis revealed the presence of polyphenols, flavonoids, alkaloids, and hydrolyzable tannins in all extracts. However, catchy tannins are absent in the methanolic extract.

On the other hand, sensitivity studies of crude extracts of *G. senegalensis leaves* showed that *E. coli* was very sensitive to the methanolic extract and moderately susceptible to *S. aureus*. Chloroform extracts also showed significant sensitivity to *S. aureus* and less sensitivity to *E. coli*. However, the cyclohexane extract showed no sensitivity to the bacteria tested. Interestingly, the antibacterial activity of crude extracts of *G. senegalensis leaves* showed that the chloroform extract had a bactericidal effect on *S. aureus* with an antibacterial power <4 and has a bacteriostatic effect on *E. coli*. However, the methanol and cyclohexane extracts had bacteriostatic effects on the strains studied.

Key words: Antibacterial activities, *Guiera senegalensis*, Phytochemical tests.

1. INTRODUCTION

Guiera senegalensis is a medicinal plant highly coveted in Africa for the local treatment of certain infections. These leaves, widely recognized in traditional African medicine, as universal remedy and have a bitter taste. These leaves, widely recognized in traditional African medicine, are like a universal remedy and have a bitter taste. These leaves are opposite, oval, orbicular, or elliptical, 3.5 cm long by 2.5 cm wide with a greyish-green color. They are rounded or slightly curved with a grey base and can give a grayish appearance to the shrubs. The white hairs often give a green tint to shrubs when fresh. The petiole, short and frozen, 2–4 mm long, is generally pubescent as are the young branches. They are widely used by traditional healers for the treatment of various disorders such as diarrheal, dysentery, malaria, cough, and microbial infections in the form of decoctions or food preparations [1-12]). Phytochemical screening showed that extracts of leaves, roots, stems and galls possess carbohydrates, steroids, saponins, flavonoids, alkaloids, tannins, saponins, cardiac glycosides, coumarins, anthraquinones, ascorbic acid, cardiac glycosides, cyanogens, and terpenoids. Numerous studies have revealed that *G. senegalensis* possesses antibacterial properties. Bassène [6], using the method of Mitscher and Coll, demonstrated that the aqueous macerates of the leaves of *G. senegalensis* contain antibacterial properties against *Bacillus subtilis*, *Escherichia coli*, *Corynebacterium*, *Pseudomonas aeruginosa.*

This activity is generally linked to the presence of secondary metabolites such as alkaloids, tannins, flavonoids, and essential oils [11].

The objective of this study is to evaluating the antimicrobial effectiveness against *E. coli* and *Staphylococcus aureus* organic extracts from the leaves of *G. senegalensis*

2. MATERIALS AND METHODS

2.1. Plant Material

Leaves of *G. senegalensis* plant species were collected in the commune of Nguénième (Thiès/Senegal) and dried in the shade, away from the sun, humidity, and at room temperature for 2 weeks. After drying, the sample was crushed, and the powder obtained was used for the extractions.

2.2. Bacteria

Bacterial strains *S. aureus* (gram +) *and E. coli* (gram−) were used in this study. The strains were provided by the laboratory of the Ecole Supérieure Polytechnique of Dakar to evaluate the antibacterial activity of crude extracts of *G. senegalensis* leaves.

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2.3. Extraction

Successive cold maceration [2] extraction method was adopted using solvents with a gradient of increasing polarity (cyclohexane, chloroform, and methanol). Briefly, approximately 100 g of ground sample was soaked in 750 mL of cyclohexane in a screw cap bottle for 72 h at room temperature. The produced filtrate was obtained using vacuum filtration. Then, the filtrate was concentrated using a rotary evaporator between 45°C and 50°C, and the extract was transferred to a sampling bottle to be dried with a shredder and in the refrigerator at 4°C for possible analyses.

After drying the residue, the extraction was repeated with chloroform and finally with methanol for 72 h each. The extracts are concentrated in a rotary evaporator then stored in the refrigerator pending analysis and other subsequent uses.

2.4. Phytochemical Screening

The noise extracts were subjected to the following analyzes to verify the presence of bioactive substances in the leaves of *G. senegalensis*:

2.4.1. Polyphenols

Approximately 1 mg of extract is diluted in 2–4 mL of hot water in a test tube then a few drops of $FeCl_3$ (2%) are added. The blue-blackish coloring indicates the presence of polyphenols [6,15].

2.4.2. Flavonoids

One mg of extract previously was taken and a volume of 1 mL hydrochloric alcohol was added with a few magnesium shavings. The reddish-pink coloring indicates the presence of flavonoids [3].

2.4.3. Alkaloids

Alkaloids are revealed by the Dragendorff precipitation reaction. Approximately 1 mg of extract is dissolved in 4 mL of 10% sulfuric acid then a few drops of Dragendorff reagent are added. The appearance of precipitate indicates the presence of alkaloids.

2.4.4. Tannins

Tannins are detected by the Stiasny reagent. One mg of extract was diluted with hot water; a few drops of Stiasny reagent are added. After heating in a water bath, a brown precipitate forms, hence the presence of condensed tannins. The precipitate obtained is then filtered using cotton and the filtrate is saturated with sodium acetate. The appearance of brown-blackish color after the addition of 2% $FeCl_3$ indicates the presence of hydrolysable tannins, not precipitable by Stiasny's reagent [4].

2.5. Antibacterial Tests

2.5.1. Sensitivity test of G. senegalensis extracts

The evaluation of the sensitivity of *G. senegalensis* extracts was made by referring to the work described by Tsirnirindravo and Andrianarisoa [19].

2.5.2. Evaluation of antibacterial parameters

The minimum inhibitory concentration (MIC) is defined as the lowest concentration of the sample that inhibits the visible growth of a microbe. The liquid medium consists of Mueller–Hinton broth for the two strains tested. The determination of the MICs was carried out using the double dilution method of concentrations expressed in mg/ml. After 24 h of incubation of the tubes at 37°C, the test tubes with broth without extract with inoculum and test tubes without inoculum were used as positive and negative controls. Clear tube staining was considered no growth and tubes showing a cloudy appearance were interpreted as positive due to bacterial growth.

After reading the MICs, the contents of the tubes in which there was no visible growth allowed Muller–Hinton agar to be inoculated in 5 cm streaks. The concentration minimal bactericide (CMB) was evaluated by comparing the bacterial growth of the petri dishes. The CBM/CMI report made it possible to specify the mode of action of a substance. If the ratio is <2 the substance is called bactericidal, on the other hand, if it is >2, the substance is called bacteriostatic.

3. RESULTS

3.1. Phytochemical Composition of Extracts

The result of phytochemical screening of organic extracts of *G. senegalensis leaves* revealed the presence of the chemical substances as shown in Table 1.

The results showed that the methanolic extract contains a high presence of polyphenols and alkaloids and a low presence of hydrolyzable flavonoids and tannins. However, it is devoid of condensed tannins. The chloroform extract only has Polyphenols as well as the cyclohexene extract.

3.2. Antibacterial Activity of G. senegalensis Extracts (Inhibition Zone; mm)

The sensitivity of confirmed clinical bacterial isolates to the methanolic extract of *G. senegalensis leaves* was determined by measuring the zones of inhibition formed around the cupules (wells) impregnated with different concentrations of leaf extracts by the agar diffusion method. The results are shown in [Table 1 and Figures 1-3].

The methanolic extract has a high sensitivity to *E. coli* with an inhibition zone of 15.05 mm and an average sensitivity to *S. aureus* with an inhibition zone of 9.23 mm. The chloroform extract has a significant sensitivity to *S. aureus* with an inhibition zone of 13.10 mm but there is a low sensitivity to *E. coli* with 6.33 mm. However, the cyclohexane extract gave low sensitivity on the two bacterial strains tested.

3.3. MIC of Different Extracts of G. senegalensis

The results of the determination of MICs of the organic extracts were grouped in Table 2 and Figure 4. The methanolic extract showed the lowest MIC of 1.5625 mg/mL on *S. aureus* and 100 mg/mL on *E. coli.* The chloroform extract presented MICs of 12.25 mg/mL and 3.125 mg/mL, respectively on *E. coli* and *S. aureus.* Finally, the cyclohexane extract gave MICs of 100 mg/mL on *E. coli* and 25 mg/mL on *S. aureus.*

3.4. CMB of Different Extracts of G. senegalensis

The results of the CMB of the organic extracts of the leaves of *G. senegalensis* were recorded in [Table 3 and Figures 5-7].

The results show that the cyclohexanic extract presents the only minimum bactericidal concentration (MBC) of 12.25 mg/mL on *S. aureus* and an absence on *E. coli.* However, the chloroform and methanolic extract did not present any CMB on the strains tested.

The diagram above shows that the chloroform extract presents the only MBC of 12.25 mg/mL on *S. aureus* and a total absence of the methanolic and cyclohexanic extract on the strains tested [Figure 8].

Table 1: Standards used for reading the results of sensitivity tests on extracts of *Guiera senegalensis.*

Inhibition zone	Degree of sensitivity of the extract
A <7 mm	Insensitive
7 mm ≤A <8 mm	Sensitive
8 mm ≤A <9 mm	Quite sensitive
A ≥9 mm	Very sensitive

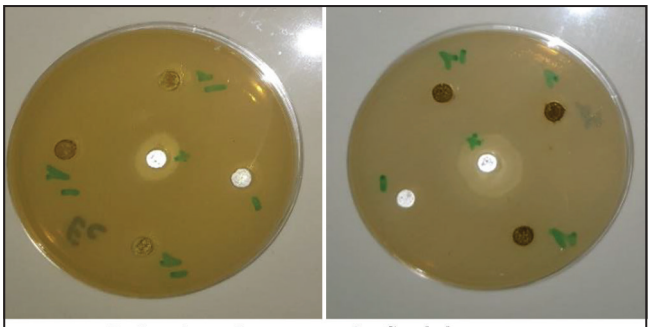

Figure 1: Sensitivity *Escherichia coli* and *Staphylococcus aureus* against cyclohexane extract (1').

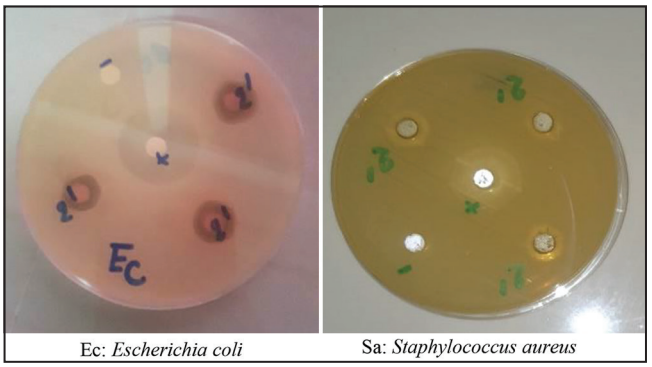

Figure 2: Sensitivity of *Escherichia coli* and *Staphylococcus aureus* against chloroform extract (2').

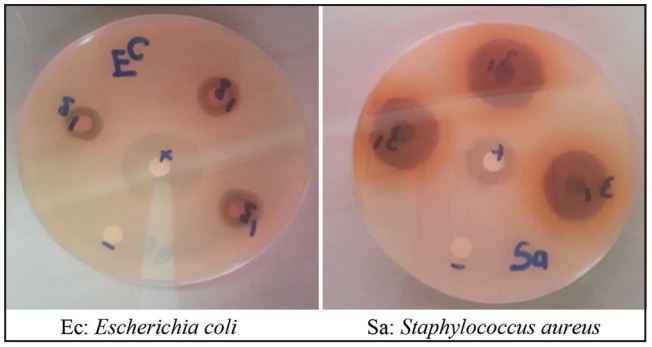

Figure 3: Sensitivity *Escherichia coli* and *Staphylococcus aureus* against of the methanolic extract (3').

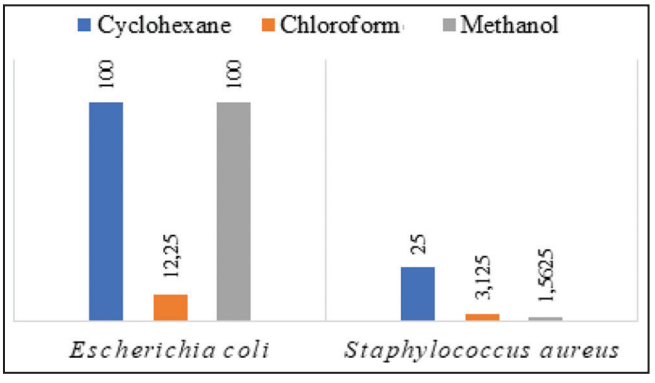

Figure 4: Minimum inhibitory concentration of the different crude extracts of *Guiera senegalensis*.

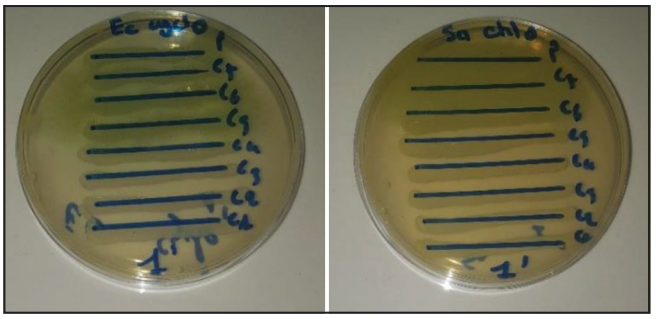

Figure 5: Absence of concentration minimal bactericide from the cyclohexanic extract on *Escherichia coli* and *Staphylococcus aureus*

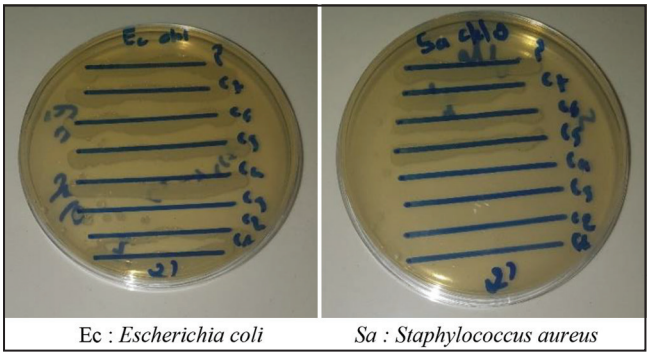

Figure 6: Presence and absence of concentration minimal bactericide from the chloroform extract on *Escherichia coli* and *Staphylococcus aureus*

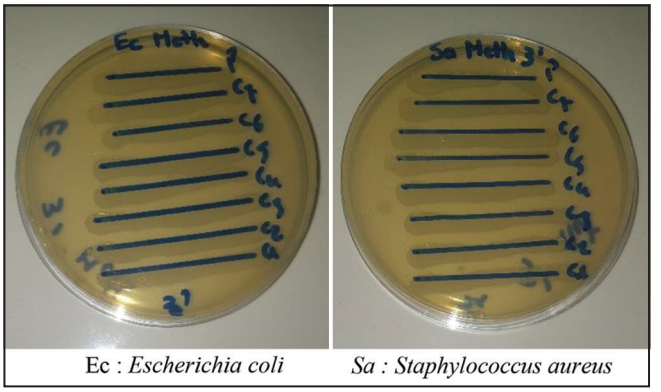

Figure 7: Absence of concentration minimal bactericide from the methanolic extract on *Escherichia coli* and *Staphylococcus aureus.*

4. DISCUSSION

Phytochemical screening of organic extracts of *G. senegalensis* showed the presence of secondary metabolites such as polyphenols, flavonoids, cathetic tannins, and hydrolyzable tannins. These results obtained coincide with those obtained in the work of Faso [9,14], *G. senegalensis* contains anthroquinolones, ascorbic acid, alkaloids, and tannins which explain its potential.

These phytocompounds could be responsible for the antibacterial, antioxidant, and antitussive activity of *G. senegalensis* leaves.

The results of sensitivity tests (Table 4) of *G. senegalensis leaf extracts* that were carried out on bacterial strains (*E. coli* and *S. aureus*) showed

Table 2: Concentration of different extracts.

Dilution	Concentration of extracts (mg/mL)
100	100
100/2	50
100/4	25
100/8	12.5
100/16	6.25
100/32	3.125
100/64	1.5425

Table 3: Phytochemical constituents of *Guiera senegalensis* leaves.

Phytochemicals	Cyclohexane	Chloroform	Methanol
Polyphenols	+	+	+++
Flavonoids	-	-	+
Alkaloids	-	-	+++
Condensed tannins	-	-	-
Hydrolyzable tannins	-	-	+

- Absent. + Little present. +++ very present

Table 4: Results of sensitivity tests.

Excerpts	*Escherichia coli* (mm)	*Staphylococcus aureus* (mm)
Cyclohexane	8.02	7.01
Chloroform	6.33	13.10
Methanol	15.05	9.23

Table 5: Recovery table of the different MICs of raw extracts of *Guiera senegalensis*.

Excerpts	Bacteria	MIC (mg/ml)
Cyclohexanic	*Escherichia coli*	100
	Staphylococcus aureus	25
Chloroformic	*Escherichia coli*	12.25
	Staphylococcus aureus	3,125
Methanolic	*Escherichia coli*	100
	Staphylococcus aureus	1.5625

MICs: Minimum inhibitory concentration

Table 6: CMB of the different organic extracts of *Guiera senegalensis*.

Excerpts	Bacteria	CMB
Cyclohexanic	*Escherichia coli*	-
	Staphylococcus aureus	-
Chloroformic	*Escherichia coli*	-
	Staphylococcus aureus	12.25
Methanolic	*Escherichia coli*	-
	Staphylococcus aureus	-

CMB: Concentration minimal bactericide

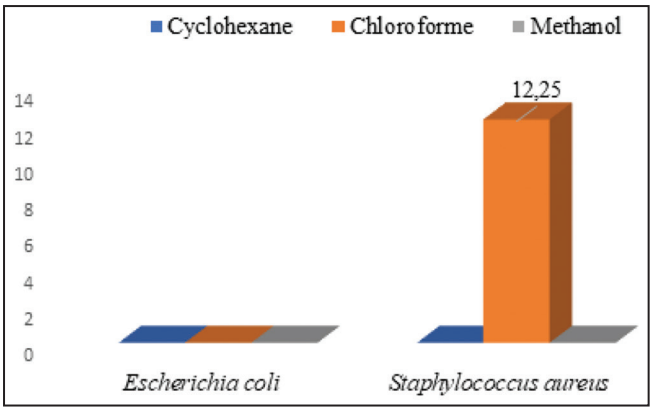

Figure 8: Histogram of concentration minimal bactericides of the different extracts of *Guiera senegalensis* leaves.

that the methanolic extract was more inhibitory to *E. coli* than on *S. aureus* with zones of inhibition, respectively, of 15, 65 mm and 9.51 mm. These results are consistent with those obtained in the work of Williams *et al.* [5] and differ with those of Salihu and Usmann [16]. The chloroform extract was only active against *S. aureus* with an inhibition zone of 13.17 mm. These results obtained are different from those obtained by Salihu and Usmann [16]. Finally, the cyclohexane extract showed no sensitivity toward the two strains tested. All of these results show that secondary metabolites such as polyphenols, flavonoids, alkaloids, and tannins highlighted by phytochemical screening could be responsible for the antibacterial activity observed in the extracts.

The results of the antibacterial parameters in particular the MICs (Table 5 and Table 6) and the CMBs of the extracts of the leaves of *G. senegalensis* showed that the chloroform extract had a bactericidal effect on *S. aureus* with an antibacterial power >2 [13,17-19] and an effect bacteriostatic on *E. coli.* This during the *methanolic and cyclohexane* extracts presented a bacteriostatic effect on the germs tested.

In-depth studies could be carried out later in these extracts for the structural determination of the compounds responsible for the bacteriostatic and bactericidal effect on the strains tested.

5. CONCLUSION

In the present work, we first opted for the chemical composition of the crude extracts of *G. senegalensis leaves.* Phytochemical screening revealed the presence of polyphenols, flavonoids, alkaloids, and hydrolyzable tannins in the methanolic extract and an absence of cathetic tannins. The cyclohexane and chloroform extract also showed a low presence of polyphenols and an absence of other secondary metabolites such as polyphenols, flavonoids, alkaloids, and hydrolyzable tannins.

The study of the sensitivity of the crude extracts of the leaves of *G. senegalensis* showed that the methanolic extract revealed a high sensitivity of methanolic extract on *E. coli* and average sensitivity to *S. aureus.* Evaluation of the antibacterial activity of crude extracts of *G. senegalensis* leaves revealed that the chloroform extract has a bactericidal effect on *S. aureus* and a bacteriostatic effect on *E. coli*. However, the methanolic extracts and cyclohexane have a bacteriostatic effect on the strains studied.

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