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(RESEARCH ARTICLE)

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Evaluation of the antibacterial activity of total alkaloid extracts from the leaves and bark of *Anogeissus leiocarpus* (Combretaceae), a plant widely used in traditional Senegalese medicine

Mamadou Latyr Ndour [1], El Hadji Gorgui DIOUF [1,*], Alioune Diouf [2], Nouhou Diaby [3], Abdou Sarr [4], Adama Faye [1], Mamadou Kébé [1] and Talibouya Ndior [1]

[1] *Department of Chemistry, Laboratory of Natural Products, Faculty of Science and Technology, Cheikh Anta Diop University of Dakar, Senegal.*
[2] *Department of Chemistry, Organic chemistry and bioorganics laboratory, Faculty of Science and Technology, Cheikh Anta Diop University of Dakar, Senegal.*
[3] *Waste water Treatment Laboratory, IFAN Cheikh Anta Diop, Dakar, Senegal.*
[4] *Department of Pharmacy, Pharmacognosy and Botanic Laboratory, UCAD, Senegal*

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Abstract

Anogeissus leiocarpus is a plant in the Combretaceae family. Its various parts are used in a wide range of fields, including ecology, economics and health.

Bacteria are a real public health problem because of their involvement in many diseases. Their resistance to antibiotics has become one of the most important problems in the fight against infectious diseases worldwide.

The aim of our study was to evaluate the antibacterial activity of dichloromethane total alkaloid extracts from *Anogeissus leiocarpus* leaves and bark.

Firstly, total alkaloids were extracted from the leaves and roots using the alkaline extraction method. The results showed a low extraction rate. They also showed that the leaves were richer in alkaloids than the barks.

The total alkaloids were then revealed by thin layer chromatography (TLC) after spraying with Dragendorff's reagent, which gives orange-red spots characteristic of alkaloids.

Finally, antibacterial tests of the total alkaloid extracts were carried out on two strains, *Staphylococcus aureus* "Gram +" and *Escherichia coli* "Gram -". The results of the antibacterial parameters such as MIC, CMB and PA showed that the extracts from the leaves and roots of *Anogeissus leiocarpus* had a bacteriostatic effect on the strains tested.

Keywords: Antibacterial activity; Alkaloids; *Anogeissus leiocarpus*

1. Introduction

Throughout the centuries, human traditions have developed the knowledge and use of medicinal plants with the aim of overcoming human suffering and improving human health. (Iserin, 2001). Plants have long been used by people all over the world to treat themselves. Today, they are still the leading source of therapeutic substances in developing countries

* Corresponding author: El Hadji Gorgui DIOUF

(WHO, 2014). The WHO estimates that 40% of all medicines contain a natural product as an active ingredient, and that around 80% of the world's population rely on plants for treatment.

Worldwide, pharmacological studies on plant extracts and secondary metabolites have demonstrated their effectiveness against parasites, lava bacteria, etc. In this context, the large family of alkaloids occupies a prime position, with more than 27,000 compounds identified and/or isolated since the discovery of morphine in 1806 (Jin, 2005).

The advent of antibiotics has been a real consolation for mankind, as they have helped to considerably reduce the spread of these pathogens. However, several conventional antibiotics have developed resistance to bacteria (Ben, et al., 2005). These resistances contribute to making pathogens linked to microbes the leading cause of death in the world, killing more than 50,000 people every day (Iqbal & Anna, 2001). This has led to a search for new active molecules. A number of studies have investigated the antibacterial activity of different plants in order to identify and isolate their principles.

To build on previous work on the antibacterial activity of *Anogeissus leiocarpus*, we decided to assess the antibacterial activity of dichloromethane total alkaloid extracts from *Anogeissus leiocarpus* leaves and bark on pathogenic bacterial strains (*E. coli* and *Staphylococcus aureus*) responsible for various human diseases.

2. Material and methods

2.1. Plant material

The material used for this work is a plant often used in traditional Senegalese medicine, namely *Anogeissus leiocarpus* (Combretaceae). It was collected in the commune of Nguénième (Mbour's department). The samples were air-dried in a ventilated area away from direct sunlight and humidity for eight (08) weeks. After drying, they were ground to a relatively fine powder using an electric grinder at the Faculty of Medicine, Pharmacy and Odontology at Cheikh Anta Diop University in Dakar.

2.2. Biological materials

The antibacterial activity was studied using *Escherichia coli* and *Staphylococcus aureus* as bacterial strains.

2.3. Methods

2.3.1. Extraction

The extraction method used in this study is based on the techniques described in the work of Bassene in 2012 and Vercauteren (2013-2014).

2.3.2. 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 in a rotary evaporator under vacuum. The concentrate obtained is a crude dichloromethane extract. This concentrate is acidified three times with 250 mL of a 10% dilute hydrochloric acid solution.

2.3.3. Extraction of total alkaloids from Anogeissus liocarpus leaves and roots

After this preliminary stage, the acidic solution is alkalinized by adding a 50% dilute ammonia solution to obtain a basic solution with a pH of between 9 and 10. The alkaloids are then converted to the basic state.

Extraction with dichloromethane

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 ($MgSO_4$) and finally evaporated under reduced pressure using a rotavapor to give the total dichloromethane alkaloid extract.

2.4. Confirmation test for alkaloids in extracts of *Anogeissus leiocarpus* leaves and roots by TLC: Operating mode

We identified the total alkaloids in our extracts by Thin Layer Chromatography (TLC).

The eluent is a mixture of cyclohexane and methanol. The spots made on 1 cm of the lower edge of the plate are air-dried and then introduced into the migration tank containing the eluent. The eluent migrates throughout the stationary phase.

2.5. Evaluation of the antimicrobial activity of total alkaloid extracts

2.5.1. Determination of extract sterility

To better assess the sterility of these extracts, the following procedure was adopted.

A mass of 2.3 g of nutrient agar and 6.5 g of sabour and agar were dissolved in two graduated glass broths each containing 100 mL of distilled water. After they had cooled, they were first placed in eight petri dishes each containing agar and then dried in a host.

0.1 g of each dichloromethane alkaloid extract was dissolved in four test tubes containing 10 mL of the thioglycolate solution and then placed in an oven at 37°C for 24 hours.

After this incubation period, the broth was inoculated onto petri dishes containing 10 mL of the thioglycolate solution and then placed in an oven at 37°C for 24 hours.

After this incubation period, the broth was inoculated onto petri dishes containing nutrient agar and another containing sabouraud agar. The petri dishes were then incubated in an oven at 37°C. After 24 hours of incubation, a sterility test was carried out on the dishes and those that were contaminated were discarded.

2.5.2. Method for assessing 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.

Operating mode

The medium used for the culture is Muller-Hinton agar, which is prepared by weighing

3.8 g of agar dissolved in 100 mL of distilled water and then autoclaving the mixture at 121°C for 15 minutes. Once the agar has been prepared, it is poured into petri dishes and the preparation is solidified at room temperature in a host. After drying at room temperature, the Muller-Hinton agar was inoculated into the plates, which were then dried at room temperature. After drying, cups were made by inserting the large end of a Pasteur pipette into the agar and then these cups were filled with 50 µL of alkaloid extracts.

Determining the diameters of the inhibition zones

After this incubation period, the zones of inhibition were read to the point where no obvious growth was observed with the naked eye when the plate was held at a distance of approximately 30 cm from the eye. The diameters of the zones of inhibition around each cup were measured to the nearest millimetre using a calliper. The efficacy of these alkaloid extracts was assessed according to the criteria of Tsurnirindravo *et al.,* (2009).

Table 1 Standards used to read extract sensitivity test results

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)

2.5.3. Method for determining antibacterial power (AP)

Determination of antibacterial parameters such as MIC and BMC were carried out by dilution in liquid medium using the technique described in the work of Kouadio et al. (2015).

Determination of Minimum Inhibitory Concentrations (MICs) of extracts

The concentration range was determined using the double dilution method. A 100 mg/mL solution of alkaloid extracts had already been prepared.

1 mL of bacterial inoculum is introduced into 28 experimental tubes. After this incubation period, observation is made with the naked eye. The lowest concentration for which no bacterial growth is observed corresponds to the inhibitory concentration.

Determination of Minimum Bactericidal Concentrations (MBC)

The starting inoculum was diluted from 10 to 10. These different dilutions were first inoculated into a petri dish. containing Muller-Hinton agar using a 2 microliters calibration in a 5 cm long series, then incubated at 37o C in an oven for 24 hours.

After reading the test tubes in which no bacterial growth was observed with the naked eye for MIC determination, the contents of these tubes were used to inoculate the Muller-Hinton agar contained in the petri dishes on 5 cm long Fauchere series in 2002.

3. Results

3.1. Extraction of total alkaloids from *Anogeissus leiocarpus*

The results obtained after extraction of total alkaloids from the leaves and barks of *Anogeisssus leiocarpus* are shown in Table 1. These results showed that the leaves are richer in alkaloids with an extraction rate of 0.93% compared to the barks with an extraction rate of 0.83%.

Table 2 Extraction rate of total alkaloids in *Anogeissus leiocarpus*

Extracts	Sample weights (g)	Dry extract weight (g)	Extraction rate (%)
Leaves	300	2.8	0.93
Barks	300	2.5	0.83

3.2. Identification of total alkaloids in extracts by TLC

The results of the alkaloid characterization are shown in the photo below. These results showed orange spots characteristic of alkaloids.

FAl = *Anogeissus leiocarpus* leaves; EAl = *Anogeissus leiocarpus* barks; C = colchicine

Figure 1 Identification of alkaloids by TLC

3.3. Sterility tests on extracts

The various results of sterility tests on extracts of dichloromethane alkaloids from leaves and bark of *Anogeissus leiocarpus* showed no germs after an incubation period of 72 hours at 37°C. These results are illustrated in Figure IV.

Figure 2 Total alkaloid extracts after 24 hours incubation at 37 °C

3.4. Sensitivity tests on the various extracts

The results obtained after the inhibition test on the two bacterial strains of the different dichloromethane alkaloid extracts and the controls are presented in tables 3 and 4. These results showed that the positive control was much more sensitive to *Staphylococcus aureus* with 25.95 mm than to *Escherichia coli* with 13.75 mm.

Leaf and root extracts showed virtually the same sensitivity to the two germs tested.

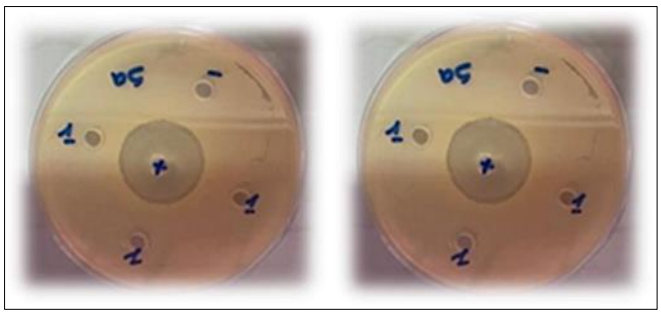

Figure 3 Lack of sensitivity to *E. coli* and *Staphylococcus aureus*

Table 3 Determination of the diameters of the zones of inhibition of the various controls

Witnesses	***Escherichia coli***	***Staphylococcus aureus***
Positive control: alcoholic solution	13.75 mm	25.95 mm
Negative control: Distilled water	0	0

Table 4 Determination of the diameters of the inhibition zones of the various extracts

Bacterial strains	*Anogeissus leiocarpus*	
	Leaves	Barks
Escherichia coli	6.42 mm	5.56 mm
Staphylococcus aureus	6.05 mm	6.45 mm

3.5. MICs of different extracts of total alkaloids from dichloromethane

The results obtained after determination of the MICs are presented in Table 5. The extracts gave the same MICs with 3.125 mg/mL on the strains tested.

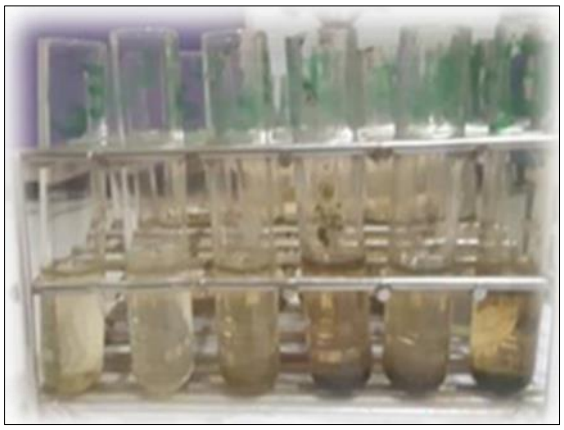

Figure 4 Test tubes after a 24-hour incubation period

Table 5 MICs for different extracts of total alkaloids from *Anogeissus leiocarpus*

Extracts	*Escherichia coli*	*Staphylococcus aureus*
Leaves	3.125	3.125
Barks	3.125	3.125

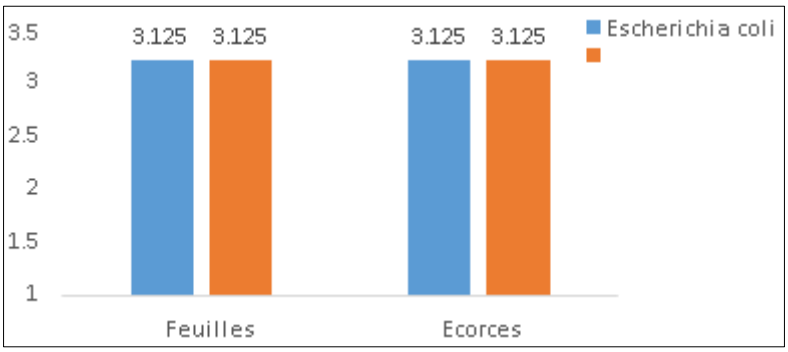

Figure 5 MIC diagram for different *Anogeissus leiocarpus* extracts

The diagram below shows the different MICs of the alkaloid extracts from the leaves and bark of *Anogeissis leiocarpus* on *Escherichia coli* and *Staphylococcus aureus*. The alkaloid extracts from the leaves and roots showed the same MICs against the two strains tested.

3.6. BMC results for the various extracts of total alkaloids from *Anogeissus leiocarpus*

The CMB results for the different extracts were first illustrated in the photos in Figures 8 and 9, then grouped together in Table 6.

The alkaloid extracts from the leaves and barks showed the same CMB with 100 mg/mL on *Escherichia coli*, but none of the alkaloid extracts gave any CMB on the two germs tested.

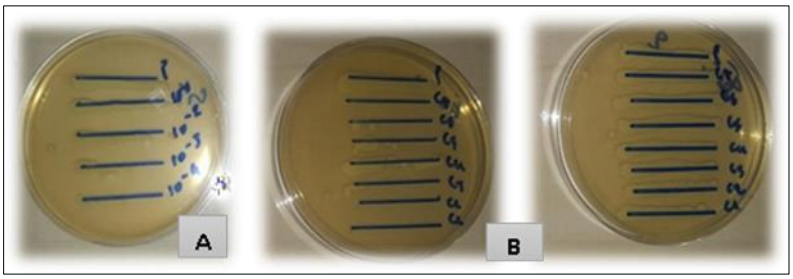

Figure 6 Bacterial inoculum and different alkaloid extracts

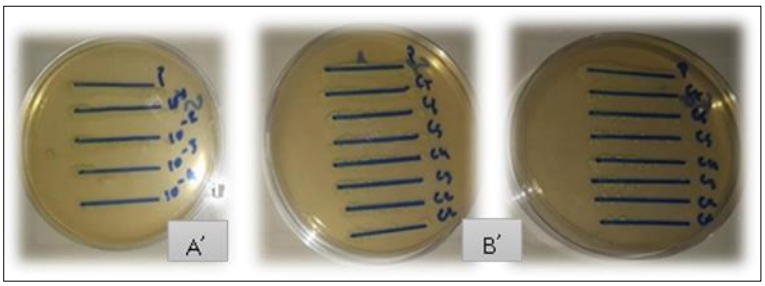

Figure 7 Numbering of bacterial inoculum and alkaloid extracts

Table 6 BMC of different total alkaloid extracts from *Anogeissus leiocarpus*

Extracts	***Escherichia coli***	***Staphylococcus aureus***
Leaves	100	0
Barks	100	0

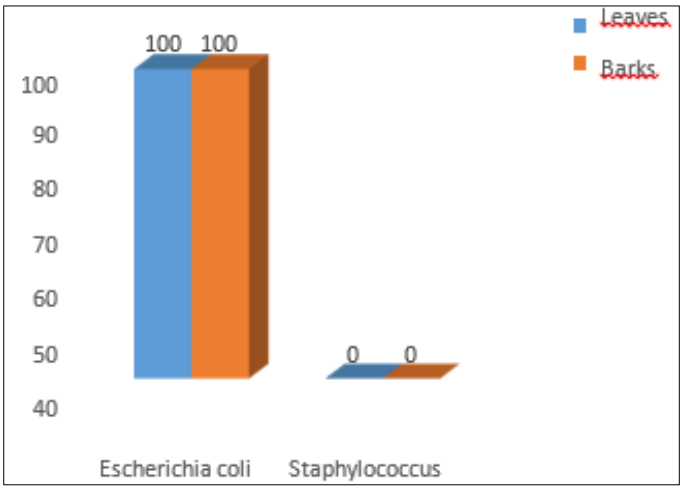

Figure 8 BMC diagram for different alkaloid extracts from *Anogeissus leiocarpus*

The diagram above shows the BMCs of the various extracts of total alkaloids from the leaves and roots of *Anogeissus leiocarpus* on *Escherichia coli* and *Staphylococcus aureus*. The leaf and root extracts gave the same BMC on *Escherichia coli*. However, none of the extracts gave a BMC on *Staphylococcus aureus*.

Table 8 Evaluation of the antibacterial power of total extracts of *Anogeissus leiocarpus*

Extracts	***Escherichia coli***			***Staphylococcus aureus***		
	CMB	**CMI**	**PA**	**CMB**	**CMI**	**PA**
Leaves	100	3.125	32	0	3.125	0
Barks	100	3.125	32	0	3.125	0

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

These results showed that the antibacterial power of the alkaloid extracts from the leaves and roots is equal on *Escherichia coli* with an AP of 32. On the other hand, leaf and root extracts have zero AP on *Staphylococcus aureus*.

4. Discussion

The extraction of total alkaloids from *Anogeissus leiocarpus* leaves and barks using dichloromethane showed a low extraction rate of 0.93% for leaves and 0.83% for roots for 300 g of dry powder. It should also be noted that the leaves are richer in alkaloids than the barks.

This difference in alkaloid concentration in the plant could be linked to the harvesting period or location.

The phytochemical study of dichloromethane alkaloid extracts revealed only the presence of alkaloids by coloring reactions and thin layer chromatography. These results differ from those of Salau et al. (2013) carried out on crude extracts of *Anogeissus leiocarpus* trunk bark. These results could be justified by the fact that the extracts are obtained by selective extraction of the alkaloids.

Susceptibility testing of dichloromethane alkaloid extracts from the leaves and bark of *Anogeissus leiocarpus* at a concentration of 100 mg/ml was carried out on two bacterial strains, namely *E. coli* and *Staphylococcus aureus*. The results of the sensitivity tests were not active on any of the germs tested, with zones of inhibition of less than 7 mm. These results are similar to those of Mann et al (2003). However, they differ from those obtained from Ikram et al. (2015). The lack of antibacterial activity observed in the extracts would then be linked to the absence of a synergistic effect between the different chemical groups absent in the extracts, such as polyphenols, flavonoids and tannins.

With regard to the results of the antibacterial parameters, in particular the MICs and MBCs of the various dichloromethane alkaloid extracts. The leaf and bark extracts showed the same MICs (3.125 mg/ml) for the germs tested. The BMC results for the leaf and root extracts were equal for *E. coli*. However, the leaf and root extracts had no BMC on *Staphylococcus aureus*. Consequently, the extracts are bacteriostatic on the two strains tested with PA > 4 ((Kouadio, et al., 2015)).

5. Conclusion

The work carried out on phytochemical screening of *Anogeissus leiocarpus* leaf and root extracts confirmed the presence of alkaloids and also the effectiveness of the method used, despite a fairly low extraction rate.

The study carried out in this document confirmed the antibacterial activity of *Anogeissus leiocarpus* leaf and root extracts on the bacterial strains E. coli and *Staphylococcus aureus*. Determination of MICs and BMCs revealed a bacteriostatic effect of these extracts on the various strains tested. In view of these results, this species could be a remedy for a number of microbial diseases that have become a real threat to public health.

Further investigation is therefore required to identify and/or isolate the active ingredients responsible for the bacteriostatic effect observed in the extracts.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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