

# Antibacterial and Antifungal Activity Essay of Alkaloids Extracted from Four *Combretaceae* Used in Senegal

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Received: November 12, 2021 Accepted: June 15, 2022 Online Published: October 30, 2022

doi:10.5539/ijc.v14n2p59

URL: <https://doi.org/10.5539/ijc.v14n2p59>

## Abstract

The use of medicinal plants remains important in West Africa where *Combretaceae* are among the most widely used for the treatment and herbal teas. The objective of this study is to extract total alkaloids from four plants of the *Combretaceae* family and test their antibacterial and antifungal activities. The results of the extraction showed that *Combretum glutinosum* is the richest with 0.61% in alkaloids. It is followed by *Anogeissus leiocarpus*, 0.13%. The study of antimicrobial activity has globally shown that alkaloids are more active against fungi than against bacteria. In this study, the lowest MIC, 93µg/ml, was obtained with alkaloids from *Guiera senegalensis* against *Staphylococcus aureus*. On the other hand, the total alkaloids extracted from *Combretum glutinosum* are the only ones that are active against all strains, both bacterial and fungal.

**Keywords:** leaves, alkaloids, extraction, *Combretaceae*, antifungal activity, antibacterial activity

## 1. Introduction

The year 2020 will undoubtedly be remembered not for its aesthetics but because of the appearance of the coronavirus (COVID-19) infecting thousands of people. This virus, which originated in China, spread rapidly throughout the world. Because of its many mutations (Zhang, et al, 2020), COVID-19 is resistant to almost all antiviral drugs used so far (Qing, et al). It has even had to be treated with anti-malarial drugs (Touret, et al, 2020). This is mainly due to the lack of new drugs on the market. The fact that chloroquine, a derivative of quinine, is used to treat a virus shows the importance of medicinal plants (Kluska, et al, 2016) as a source of bioactive molecules to prevent such a situation from recurring.

Medicinal plants are widely used in West Africa and *Combretaceae* are among the most prized. These plants, *Combretum glutinosum*, *Combretum micranthum*, *Guiera senegalensis*, and *Anogeissus leiocarpus* are used as well for their medicinal virtue for the treatment of different diseases, as in the consumption as herbal tea (Diatta, et al, 2019). Different studies have been done on plant extracts for their antibacterial activity (Bawa, et al, 2019), antioxidant activity (Mabozou, et al, 2019), nephroprotective activity (Mabozou et al, 2019) and antifungal activities (Gurama et al, 2020).

## 2. Experimental

### 2.1 Plant Material

Plants were collected from the municipality of Niassene (13°55'44.2"N 16°12'16.7"W). The plant material used consists of green leaves of *Combretum glutinosum*, *Combretum micranthum*, *Guiera senegalensis*, and *Anogeissus leiocarpus*. The leaves of *Combretum glutinosum*, *Combretum micranthum*, *Guiera senegalensis*, and *Anogeissus leiocarpus*, used in this study were harvested. These different samples were dried and then ground into fine powder.

### 2.2 Alkaloids Extraction

The weighed plant material 15 g was extracted with 200 ml chloroform–ether–alcohol (90%; 23 + 8 + 2.5) for 10 min. Six millilitres of dilute ammonia was then added to the reaction mixture, after which the flask was shaken for 1 hour and allowed to incubate for 8 hours with intermittent mixing. To this, 10 ml water was added, and the mixture was

shaken vigorously. Upon settlement of our target compound 100 ml of the solution was drawn out and filtered into a separator and washed with a few millilitres of a mixture of solvent ether and chloroform, and lastly shaken with 0.5 N sulphuric acid to complete the extraction. The combined acid extract was filtered and made alkaline with dilute ammonia solution. The alkaloids are liberated and subsequently washed with chloroform. The chloroform washes and the extract were then poured into a tarred conical flask after which the chloroform is distilled off and the solvent is removed completely in a vacuum desiccator. Five millilitres of alcohol were added to the residue, and the solvent was again removed. The evaporation with alcohol was repeated, and the residue was dried to constant weight in a vacuum desiccator and massed as total alkaloids (*Physicochemical Standards of Unani Formulations* (1986) Part 1, Central Council for Research in Unani Medicine, New Delhi, India, Pp 229–230 Numéro OCLC: 622665107). The extraction was repeated twice. The total alkaloids obtained are dissolved in dimethyl sulfoxide (DMSO) at a concentration of 6 mg/mL.

### 2.3 Antimicrobial Activity

The microbial strains used in this study were obtained from the Bacteriology and Virology Laboratory of the Aristide Le Dantec hospital at Dakar. Those studied consist of reference strains: *Staphylococcus aureus* (ATCC 29213), *Pseudomonas aeruginosa* (ATCC 29853), *Trichophyton rubrum* and *Candida albican*. The bacterial species were planted in a nutrient medium and incubated at 37 °C for 24 h. The culture media used in this study are Mueller-Hinton broth (MH) for bacterial tests and Sabouraud broth for fungal tests.

The reference antibiotics used are amoxicillin (United States Pharmacopoeia standard lot N° L0K369), cloxacillin sodium (European Pharmacopoeia lot N°3a) and neomycin B sulfate (International Chemical Reference Substance Control N° 193178). Each of these substances is dissolved in dimethyl sulfoxide (DMSO) at a concentration of 6 mg/mL.

### 2.4 Determination of the Minimum Inhibitory Concentration (MIC)

With microplates containing 100 µL of broth per well, dilution series ranging from 3 to 6 µg/mL are performed with the extract and antibiotic standard solutions used to perform the activity tests. Then each well 10 µL bacterial culture suspension at 10<sup>5</sup> UFC was filed. Clear staining of the well was interpreted as a lack of growth and wells showing a cloudy appearance were considered positive because of the growth of bacteria.

## 3. Results and Discussion

Alkaloids are among the most important molecules in plants and have led to the discovery of several molecules against different pathologies (Hamid, et al, 2017) *Combretaceae* are plants rich in alkaloids (Niass, et al, 2017). This richness can vary from one species to another. Table 1 gives the yield of total alkaloid extracts as a percentage of 30 g of vegetable powder. Among the four species of *Combretaceae*, *Combretum glutinosum* is the richest, 0.61%, up to 5 times richer than *Anogeissus leiocarpus* which shows an alkaloid content of 0.13%. *Combretum micranthum* and *Guiera senegalensis* showed the lowest levels of 0.08 and 0.07%, respectively (Table 1).

Table 1. Total alkaloid extracts yield as a percentage of 30 g of vegetable powder

Plants	Alkaloids weight (mg) ±0.02	Percentage, %
<i>Combretum glutinosum</i>	184.4	0.61
<i>Guiera senegalensis</i>	21.8	0.07
<i>Combretum micranthum</i>	24.0	0.08
<i>Anogeissus leiocarpus</i>	41.0	0.13

Several scientific studies have shown that alkaloids may have antibacterial (Rinaldi, et al, 2017), anti-hyperglycemic (Mohamed, et al, 2016), or antifungal (Sytykiewicz, et al, 2015) activities. The study of antimicrobial activity has generally shown that alkaloids are more active against fungi than against bacteria (Table 2). The total alkaloids extracted from *Combretum glutinosum* are the only ones active against all strains of both bacteria and fungi. Among the minimal inhibitory concentrates (MICs) those of the total alkaloids of *Guiera senegalensis* against *Staphylococcus aureus* are the most important for a value of 93 µg/mL. This MIC value is the same as that of neomycin B sulphate, one of the reference antibiotics used, against the same bacterial strain (Table 2). Total alkaloids from *Combretum glutinosum* follow with a MIC of 375 µg/mL. Despite this, some bacterial strains show resistance to alkaloids extracted from *Guiera senegalensis* and *Anogeissus leiocarpus*, such as *Pseudomonas aeruginosa* and *Staphylococcus aureus*. As well as *Combretum micranthum* against *Staphylococcus aureus*. Reference antibiotics such as amoxicillin and cloxacillin used have MICs higher than those of most total alkaloids against *Staphylococcus aureus* (Table 2). All total alkaloids are active against all fungal strains, *Candida albican* and *Trichophyton rubrum*. The latter are sensitive to the total

alkaloids of the three plants with the lowest MIC values, equal to 375 µg/mL (Table 2). However, the two reference antibiotics used, amoxicillin and cloxacillin, are much less active than the total alkaloids against this strain (Table 2). *Candida albican* is more sensitive to the total alkaloids of *Guiera senegalensis* and *Combretum micranthum* with MIC values equal to 375 µg/mL (Table 2) at the time neomycin B sulfate gave the lowest MIC equal to 187 µg/mL (Table 2). With the same fungal strains, these alkaloids remain more active than the extracts studied by Ifedolapo *et al.*, 2019. These results show that alkaloids from *Guiera senegalensis* are more active than aqueous extracts against *Candida albican*, but much lower in the case of *Anogeissus leiocarpus* (Diatta, et al, 2019). If the total alkaloids of *Combretum glutinosum* are the most active in this study, they are less active than the methanolic extracts of the same plant with the same bacterial strains (Niass, et al., 2016). On the other hand, the antifungal activity remains more important than that obtained in other studies (Sytykiewicz, et al., 2015 & Jindal, et al., 2012). These results are important compared with some apolar fractions used (Cimanga, K., et al., 1998).

Table 2. Value of minimum inhibitory concentration (MIC) in µg/mL of different total alkaloids and the antibiotic standards

Plants	Value of minimum inhibitory concentration (MIC) in µg/mL			
	<i>Pseudomonas aeruginosa</i>	<i>Candida albican</i>	<i>Staphylococcus aureus</i>	<i>Trichophyton rubrum</i>
<i>Combretum glutinosum</i>	3000	750	375	375
<i>Guiera senegalensis</i>	-	375	93	375
<i>Combretum micranthum</i>	3000	375	-	375
<i>Anogeissus leiocarpus</i>	-	1500	1500	750
Amoxicillin	375	750	<1.5	>3000
Cloxacillin	187	750	<1.5	>3000
Neomycin B sulfate	141	187	93	1500

#### 4. Conclusion

The total alkaloids extracted from the four *Combretaceae* were more active against fungi than against bacteria. These results constitute an important avenue for the treatment of certain pathologies, including aesthetic ones. Further research in the sense of isolation and characterization of the active ingredients is necessary so that drugs can be developed.

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