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Research Article

# Antifungal Activity of the Ethanol Extract of *Terminalia laxiflora* Engl. (Combretaceae) from the Eastern Logone Province of Chad

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## Abstract

**Introduction:** Infections caused by pathogenic filamentous fungi are a real public health problem. These germs develop resistance to standard antifungals. Medicinal plants are an important source of secondary metabolites. To address these issues, this study was carried out to evaluate the antifungal activity of the ethanolic extract of Terminalia laxiflora Engl (Combretaceae) from the Eastern Logone province in Chad. **Materials and methods:** For this study, we used the bark and roots of Terminalia laxiflora Engl. The extract was obtained by maceration from these different parts of the plant. The antifungal activity was evaluated using the agar incorporation method to determine the minimum inhibitory concentration (MIC) and the minimum fungicidal concentration (MFC). Additionally, a phytochemical analysis was carried out to establish a correlation between the chemical structure of the compounds and their biological activities. **Results:** the extraction yield ranged from 9.89 to 18.37%. Phytochemical screening of the bark and roots of Terminalia laxiflora Engl. revealed the presence of flavonoids, gallic tannins, saponins, alkaloids, heterosides, free quinones, anthraquinones, sterols, terpenoids, and anthocyanins. These extracts inhibited the growth of Aspergillus flavus, Aspergillus niger, Trichophyton rubrum, Trichophyton soudanense, and Microsporum canis, with minimum inhibitory concentrations (MIC) ranging from 1 mg/mL to 3 mg/mL. **Conclusion:** The extract from Terminalia laxiflora Engl. will be a potential source of secondary metabolites with antifungal activity.

**Keywords:** activity, antifungal, Terminalia laxiflora Engl., Eastern Logone, Chad

## INTRODUCTION

Fungal infections are a real public health problem in many developing countries. Over the past 20 years, the incidence of fungal infections has increased significantly. These conditions most often occur in vulnerable patients. [1]. These infections are caused by dermatophytes, aspergillus. These dermatophytes are responsible for damage to the skin, nails, and hair [2]. Some species of the *Aspergillus* genus, like *Aspergillus flavus*, produce aflatoxins that cause severe liver toxicity and liver cancer [3].

Around the world, these pathogenic filamentous fungi are responsible for a large number of deaths and disease cases, with over 2.5 million deaths per year [4]. In tropical countries like Senegal, skin diseases affect 30% of the rural population [5]. The most common dermatophytosis is ringworm and it is almost a childhood disease (3-15 years old), especially found in children of African and Caribbean origin [6]. Its prevalence was 34%, 13.9%, and 39.3% in Nigeria, Côte d'Ivoire, and Mali, respectively [7,8]

The treatment of fungal infections mainly relies on taking synthetic antifungals. Despite the many antifungals available, fungal infections are still common

because fungi have developed resistance to these antifungals [9], as well as by their toxic effects and high cost [10]. From then on, finding new treatment options that are effective, safe, and accessible seems like a must [11].

To tackle this problem, natural products, especially medicinal plants, are getting more attention as possible sources of bioactive molecules. In fact, the World Health Organization (WHO) points out that about 80% of the world's population, especially in Africa, rely on traditional medicine for primary healthcare because it's accessible and relatively affordable [10]. In this context, medicinal plants play a very important role in the search for new antifungal molecules. They are an important source of bioactive compounds such as polyphenols, saponins, and alkaloids known for their antimicrobial properties [12].

In Chad, several plant species are empirically used to treat various infections, including fungal infections. Among these, Terminalia laxiflora Engl. (Combretaceae) is widely spread and used for its therapeutic properties. Previous studies have shown that the genus Terminalia is known for its high pharmacological potential, linked to the presence of secondary metabolites with antimicrobial properties [13].

So, with the aim of promoting medicinal plants from the Chadian pharmacopeia, especially Terminalia laxiflora Engl. which is traditionally used to treat microbial infections, this study was carried out to evaluate their antifungal activity with a view to creating traditionally enhanced herbal medicines.

## MATERIALS AND METHODS

### Study Framework, Type, and Period

Different parts of Terminalia laxiflora Engl (trunk bark, roots) were collected in January 2026 in the Eastern Logone province, more precisely in Ndaba Sadoro in the Kouh-West Department (Southern Chad). The antifungal activity was carried out at the Bacteriology Unit of the Laboratory for Diagnostic Research and Scientific Expertise (LaboReDES) at the Faculty of Human Health Sciences (FSSH), University of N'Djamena. The Pharmacology Unit of the Laboratory for Diagnostic Research and Scientific Expertise (LaboReDES) at the Faculty of Human Health Sciences (FSSH), University of N'Djamena, was used for the extraction of the ethanolic extract of the studied plant. This was a prospective and experimental study that lasted 4 months, from January 2026 to April 2026.

### Biological material

The biological material consisted of bark from the trunk and roots of Terminalia laxiflora Engl, as well as fungi isolated from samples of films, urine, and pus. Antifungal tests were carried out with 5 fungal strains: *Aspergillus flavus, Aspergillus niger, Trichophyton rubrum, Trichophyton soudanense,* and *Microsporum canis*, isolated from samples taken from patients hospitalized at the National Reference University Hospital Complex of Chad (CHU-RN).

This plant was harvested in January 2026 in the Logone Oriental province, specifically in Ndaba Sadoro in the Kouh-Ouest Department (southern Chad), and identified at the Herbarium of the Institute for Livestock Research for Development (HIRED) in Chad under number 41/HIRED/TCHAD

### Preparation of the extract

50 g of plant material were placed in a closed vial and mixed with 500 ml of 10% ethanol, then left to macerate under electric stirring for 24 hours. The macerate was filtered through cotton wool and then through Whatman 1 filter paper using a Buchner funnel, a vacuum flask, and a vacuum pump. The resulting filtrate was evaporated under vacuum at 45-50°C using a rotary evaporator. The concentrated filtrate was placed in a beaker and dried under ventilation at room temperature. The residue was weighed and stored in a dry vial in the refrigerator at 4°C [14].

### Extract yield

The extraction yield was obtained using the following formula:

$$Yield\ (\%) = \frac{dry\ weight\ of\ the\ extract\ (g)}{\text{weight of dry plant material (g)}} X\ 100$$

### Phytochemical screening

The analysis of the chemical composition in secondary metabolites of the powder from different species, to justify the antibacterial activity, was done according to the protocols described by Bauer and Tittel in 1996 [15] ; Jha and Sit in 2022[16]; Trease and Evans, in 1989 [17] and Sofowora, in 2008[18].

### Antifungal activity

After preparing the solutions of the extract and the reference antifungal, the antifungal activity was determined using the agar incorporation method as described by De Campos et al., in 2000 [19].

### Incorporation into the agar

The Sabouraud medium with chloramphenicol-2 was diluted with the previously prepared ethanolic and aqueous extract solutions. The media were poured into 90 mm Petri dishes at 20 ml per dish and left under the hood until the agar solidified.

### Fungus inoculation

A 6 mm mycelial disk was taken from the growing edge of a 3- to 4-day-old culture and placed in the center of the previously prepared Petri dishes. Incubated at 37°C, the mycelial growth was monitored by measuring two perpendicular diameters at the same time each day for 7 days, and averaging these 2 diameters minus the diameter of the explant [20].

The antifungal activity of the extracts was evaluated by calculating the inhibition percentage using the following formula:

$$\%\ I = \frac{Dt - Dx}{Dt}\ X\ 100$$

% I: inhibition percentage, Dt: average growth diameter of the mushroom in the control dish and Dx: average growth diameter of the mushroom in the test box.

**Determination of Minimum Inhibitory Concentrations (MIC) and Minimum Fungicidal Concentrations (MFC)**

After 7 days of incubation in the presence of the extracts, the MIC and MFC were measured for the explants that did not grow. Fungicidal activity was determined by the MFC/MIC ratio.

**Statistical Analysis**

The results of the fungal analyses obtained were processed using Microsoft Word and Excel for graph plotting.

## RESULTS

**Mapping of the Study Area**

The plant sample was collected in Ndaba, in the Kouh-Ouest department, in the Eastern Logone province in southern Chad (Figure 1). It is located approximately between 8°24' and 8°39' North latitude and between 16°58' and 17°09' East longitude. This location is in the Sudanian zone with a humid tropical climate, characterized by a rainy season from May to October and a dry season from November to April**.** The town of Ndaba has a great wealth of plant life, including *Terminalia laxiflora Engl.,* a medicinal plant commonly used by local people to treat fungal infections. The abundance of this plant resource, along with the favorable ecological conditions of the area, explains why this town was chosen as a study area for analyzing the biological activities of these medicinal species.

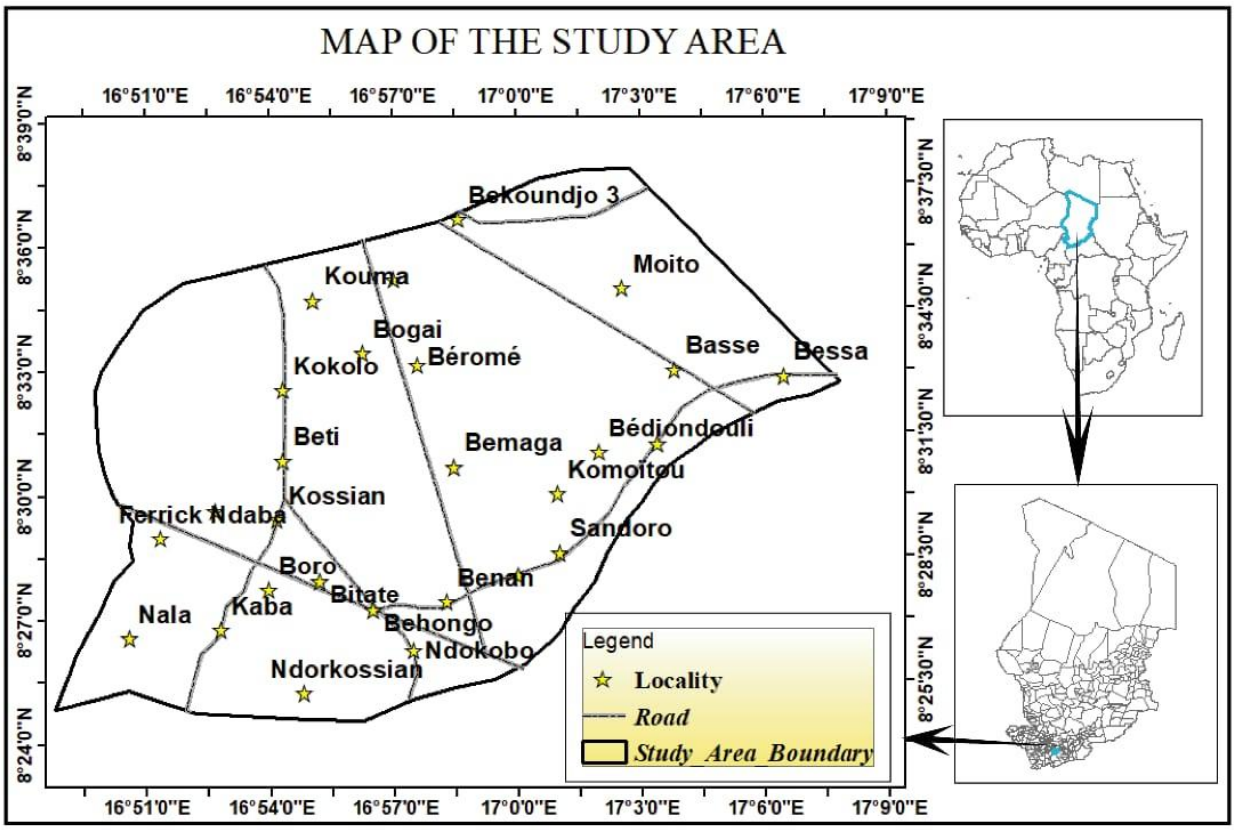


**Figure 1**: Map of the Plant Organ Collection Area for the Study

**Extraction Yields**

The yields of the ethanolic extract are summarized in Table I.

Table I: Yields of the ethanolic extract from the roots and bark of *Terminalia laxiflora Engl.,*

| Species | Plant organ | Solvent | Yield (%) |
|---|---|---|---|
| *Terminalia laxiflora Engl* | Bark | Ethanol | 9,89 |
| *Terminalia laxiflora Engl* | Roots | Ethanol | 18,37 |

Table I shows that yields range from 9.89% to 18.37%, depending on the different parts of the plant. The highest yield was from the roots of *Terminalia laxiflora Engl.*, at 18.37%.

**Phytochemical Screening**

Phytochemical analysis of the powder from the bark and roots of Terminalia laxiflora Engl. revealed the presence of the secondary metabolites listed in Table II.

Table II: Phytochemical analysis of the powder from the bark and roots of *Terminalia laxiflora Engl.*

| Secondary metabolites | Bark | Roots |
|---|---|---|
| Saponins | + + + | + + + + |
| Alkaloids | + + + | + + |
| Flavonoids | + + + | + + + |
| Sterols and terpenoids | + + + | + + |
| Anthraquinones | + + | + + + + |
| Free quinones | + | + + + |
| Catechic tannins | – | – |
| Gallotannins | + + + + | + + + |
| Anthocyanins | + + + | + + + |
| Cardiotonic glycosides | – | – |

Legend: (-) absent; (+) present in low concentrations; (++) present in moderate concentrations; (+++) present in high concentrations; (++++) present in very high concentrations.

Following the phytochemical analysis, the powder from the bark and roots of Terminalia laxiflora Engl. was found to be rich in saponins; alkaloids; flavonoids; sterols and terpenoids; anthraquinones; free quinones; gallic tannins; and anthocyanins. Catechin tannins and cardiotonic glycosides were absent (Table II).

## Antifungal Activity

### Antifungal Activity of the Ethanol Extract from the Bark and Roots of Terminalia laxiflora Engl.

The antifungal effect of the ethanolic extract of the bark and roots of *Terminalia laxiflora Engl.* on fungal strains (*Aspergillus flavus, Aspergillus niger, Trichophyton rubrum, Trichophyton soudanense,* and *Microsporum canis*) is shown in Figures 2 and 3.

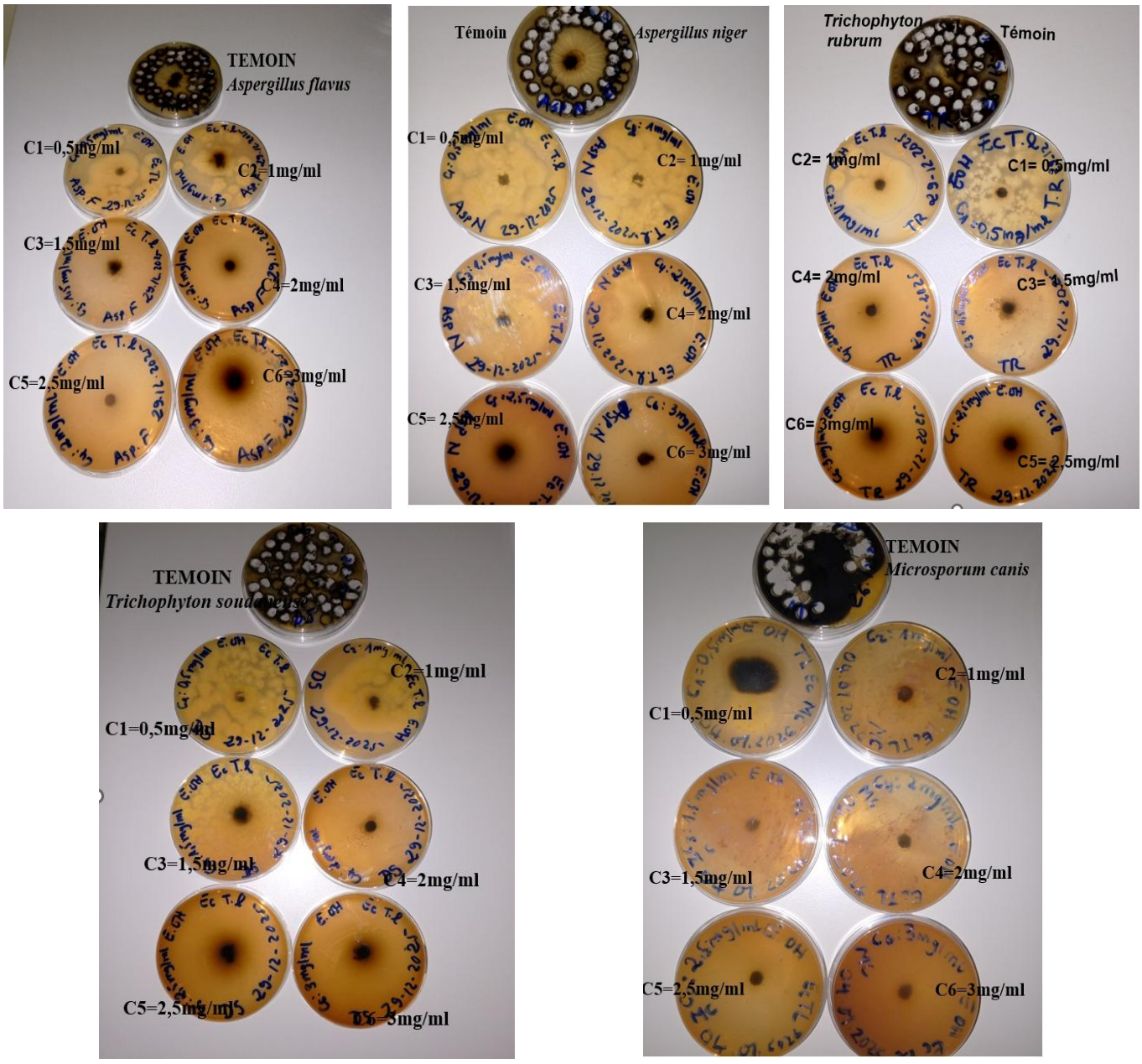


**Figure 2:** Inhibition of the growth of Aspergillus flavus, Aspergillus niger, Trichophyton rubrum, Trichophyton soudanense, and Microsporum canis at various concentrations of an ethanolic extract from the bark of Terminalia laxiflora Engl.

AF: *Aspergillus flavus*, AN: *Aspergillus niger*, TR: *Trichophyton rubum*, TS: *Trichophyton soudanense,* MC: *Microsporum canis ;* Tl *: Terminalia laxiflora Engl*

Figure 2 illustrates the inhibition of growth of *Aspergillus flavus, Aspergillus niger, Trichophyton rubrum, Trichophyton soudanense*, and *Microsporum canis* in the presence of different concentrations of an ethanolic extract from the bark of *Terminalia laxiflora Engl*. A 100% inhibition rate was observed at a concentration of 1 mg/mL for *Microsporum canis*; 2 mg/mL for *Aspergillus niger, Aspergillus flavus*, and *Trichophyton rubrum*; and 2.5 mg/mL for *Trichophyton soudanense*, demonstrating the efficacy of the ethanolic extract of *Terminalia laxiflora Engl.* bark.

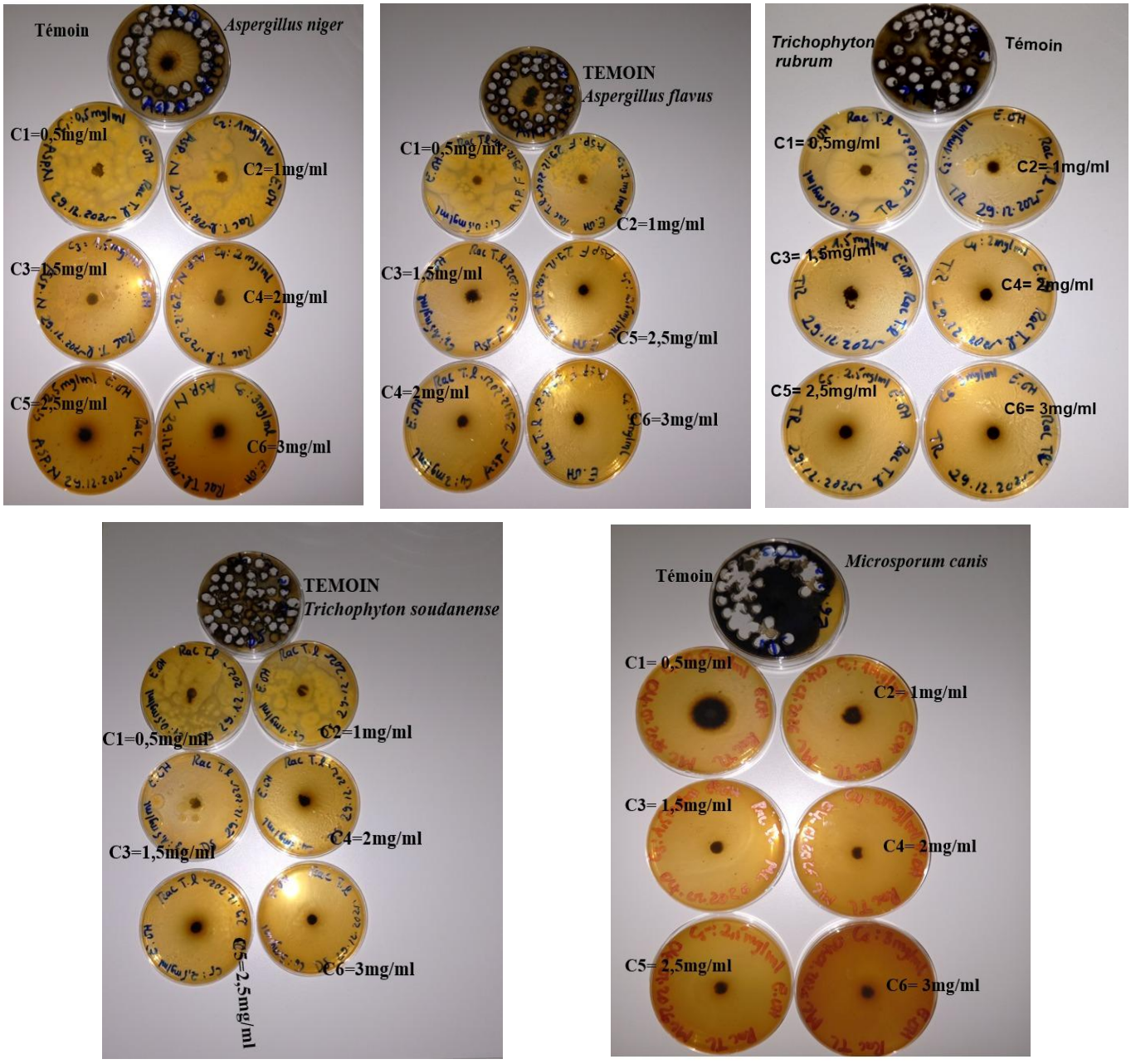


**Figure 3 :** Inhibition of the growth of Aspergillus flavus, Aspergillus niger, *Trichophyton rubrum, Trichophyton soudanense,* and *Microsporum canis* at various concentrations of an ethanolic extract from the roots of *Terminalia laxiflora Engl.* AF: *Aspergillus flavus*, AN: *Aspergillus niger*, TR: *Trichophyton rubrum*, TS: *Trichophyton soudanense,* MC: *Microsporum canis,* Tl*: Terminalia laxiflora Engl*

Figure 3 shows that the ethanolic extract of *Terminalia laxiflora Engl.* roots exhibited 100% inhibition at a concentration of 1.5 mg/mL against *Microsporum canis* and *Trichophyton rubrum*, and at 2 mg/mL against *Aspergillus niger, Aspergillus flavus*, and *Trichophyton soudanense.*

The antifungal activity of the ethanolic extract of the bark and roots of *Terminalia laxiflora Engl.* against the fungal strains (*Aspergillus flavus, Aspergillus niger, Trichophyton rubrum, Trichophyton soudanense*, and *Microsporum canis*, as shown in Figures 4 and 5) reveals inhibition of these strains at various concentrations.

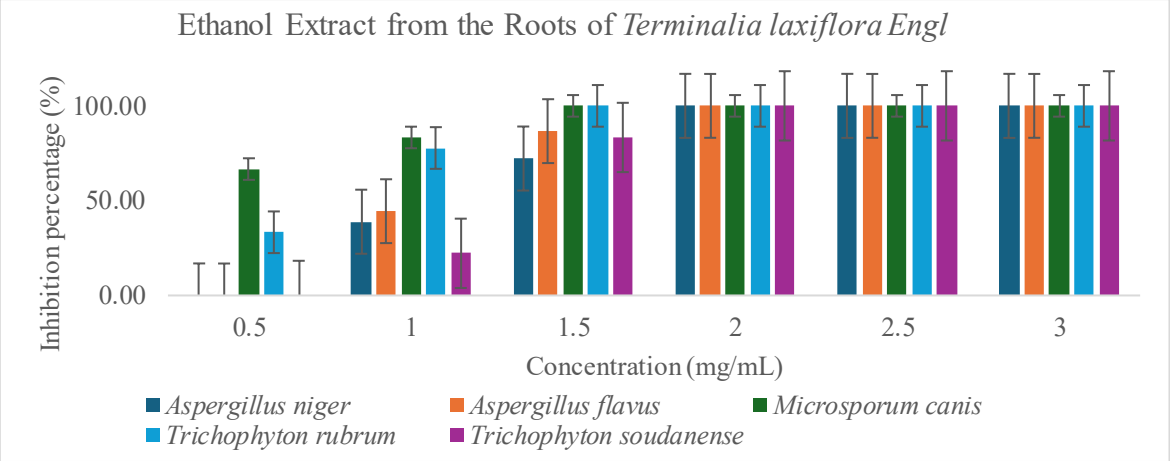


**Figure 4:** Antifungal Activity of the Ethanol Extract from the Roots of *Terminalia laxiflora Engl.*

Figure 4 shows that the percentage of inhibition against *Microsporum canis, Trichophyton rubrum, Aspergillus niger, Aspergillus flavus*, and *Trichophyton soudanense* increased with the concentration of extracts in the culture media. Sensitivity varied among strains and reached 100% inhibition at a concentration of 1.5 mg/mL of the ethanolic extract of *Terminalia laxiflora Engl.* roots for *Microsporum canis* and *Trichophyton rubrum* and at 2 mg/mL for *Aspergillus niger, Aspergillus flavus,* and *Trichophyton soudanense*, corresponding, respectively, to the minimum inhibitory concentrations (MICs).

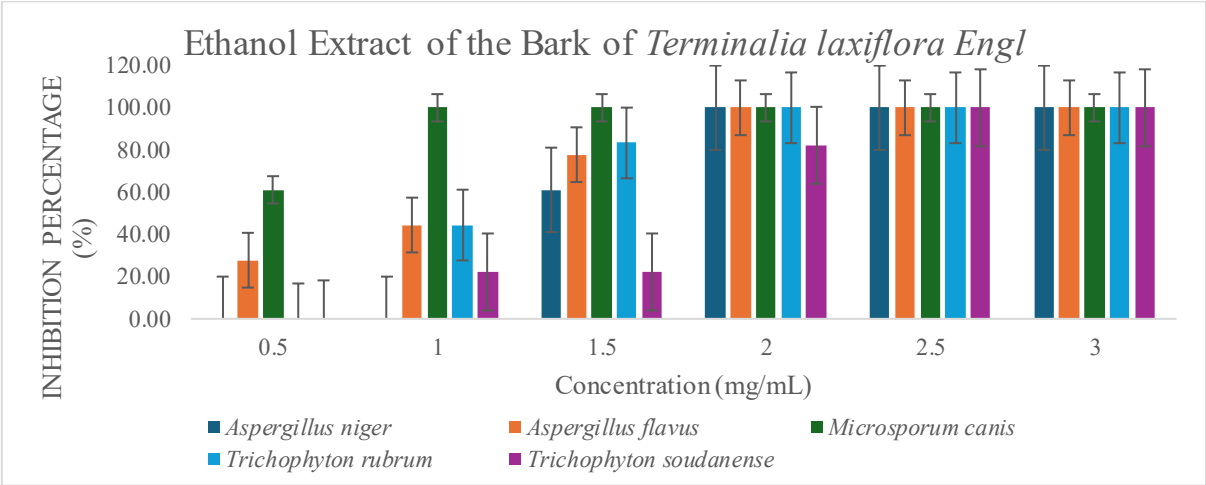


**Figure 5:** Antifungal Activity of the Ethanol Extract from the Bark of *Terminalia laxiflora Engl.*

Figure 5 shows that the ethanolic extract of *Terminalia laxiflora Engl*. bark inhibits the growth of *Microsporum canis* at 1 mg/mL, *Aspergillus niger, Aspergillus flavus*, and *Trichophyton rubrum* at 2 mg/mL, and *Trichophyton soudanense* at 2.5 mg/mL. These concentrations correspond to the minimum inhibitory concentrations (MIC).

Since the subcultures of *Microsporum canis, Trichophyton rubrum, Aspergillus niger, Aspergillus flavus*, and *Trichophyton soudanense* showed no growth, it was possible to determine the minimum fungicidal concentration and the MFC/MIC ratio, which are recorded in Tables III and IV.

**Table III:** Summary Table of CMI, CMF, and CMF/CMI

| **Ethanolic extract from the roots of Terminalia laxiflora Engl.** | | | |
|---|---|---|---|
| **Settings** | **MIC (mg/mL)** | **MCF (mg/mL)** | **MCF/MIC** |
| *Microsporum canis* | 1,5 | 3 | 2 |
| *Trichophyton rubrum* | 1,5 | 3 | 2 |
| *Aspergillus niger* | 2 | 3 | 1,5 |
| *Aspergillus flavus* | 2 | 3 | 1,5 |
| *Trichophyton soudanense* | 2 | 3 | 1,5 |
| **MCF: Minimum Fungicidal Concentration; MIC: Minimum Inhibitory Concentration.** | | | |

**Table IV**: Summary Table of MIC, MCF, and MCF/MIC

| Ethanolic extract of the bark of Terminalia laxiflora Engl. | | | |
|---|---|---|---|
| **Settings** | **MIC (mg/mL)** | **MCF (mg/mL)** | **MCF/MIC** |
| *Microsporum canis* | 1 | 3 | 3 |
| *Trichophyton rubrum* | 2 | 3 | 1,5 |
| *Aspergillus niger* | 2 | 3 | 1,5 |
| *Aspergillus flavus* | 2 | 3 | 1,5 |
| *Trichophyton soudanense* | 2,5 | 3 | 1,2 |
| **MCF: Minimum Fungicidal Concentration; MIC: Minimum Inhibitory Concentration.** | | | |

The MCF/MIC ratios in Tables III and IV are strictly less than 4 (MCF/MIC < 4), indicating that the ethanolic extract of the bark and roots of *Terminalia laxiflora Engl.* exhibits fungicidal activity against *Microsporum canis*, *Trichophyton rubrum*, *Aspergillus niger*, *Aspergillus flavus*, and *Trichophyton soudanense* [21].

**Antifungal Activity of the Two Reference Compounds (Fluconazole and Griseofulvin)**

The reference compounds were tested on five (5) fungal strains (*Aspergillus flavus, Aspergillus niger, Trichophyton rubrum, Trichophyton soudanense*, and *Microsporum canis*). Figures 6 and 7 illustrate the growth inhibition of these different strains by fluconazole and griseofulvin.

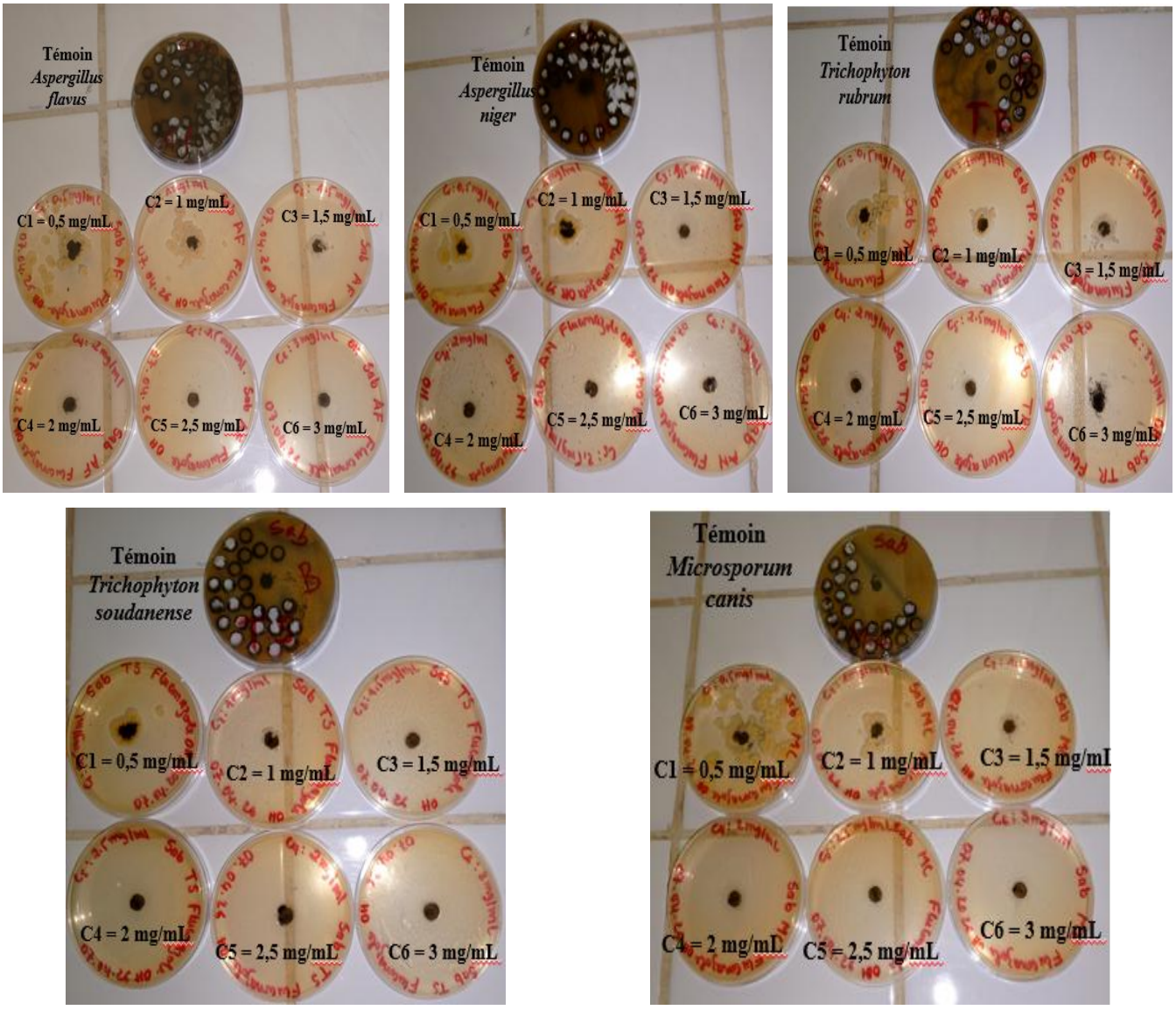


**Figure 6:** Effect of fluconazole on the growth of *Aspergillus flavus, Aspergillus niger, Trichophyton rubrum, Trichophyton soudanense* and *Microsporum canis.*

Figure 6 shows the inhibition of *Aspergillus flavus, Aspergillus niger, Trichophyton rubrum, Trichophyton soudanense*, and *Microsporum canis* by fluconazole. The results indicate that inhibition is complete (100%) at fluconazole concentrations of 2 mg/mL and 2.5 mg/mL. We can therefore conclude that the minimum inhibitory concentration (MIC) of fluconazole against *Aspergillus niger, Aspergillus flavus, Trichophyton rubrum*, and *Trichophyton soudanense* is 2 mg/mL; the MIC for *Microsporum canis* is 2.5 mg/mL.


**Figure 7:** Effect of griseofulvin on the growth of *Aspergillus flavus, Aspergillus niger, Trichophyton rubrum, Trichophyton soudanense* and *Microsporum canis.*

In the case of griseofulvin, there is no inhibition. The fungal strain grows on all the plates. It therefore appears that griseofulvin has no effect on Aspergillus flavus, Aspergillus niger, Trichophyton rubum, Trichophyton soudanense, and Microsporum canis.

Compared with the MICs obtained, the ethanolic extract of the bark and roots of *Terminalia laxiflora Engl.* appears to be more active than the reference compounds, as it inhibited the growth of these five strains of dermatophytes and *Aspergillus* at MICs ranging from 1 to 3 mg/mL, compared to fluconazole, which inhibited the growth of *Microsporum canis* at an MIC of 2 mg/mL. Griseofulvin did not inhibit any of these strains, demonstrating the resistance of *Aspergillus flavus, Aspergillus niger, Trichophyton rubrum, Trichophyton soudanense*, and *Microsporum canis* to this compound.

## DISCUSSION

The evaluation of the antifungal activity of the ethanolic extract of the roots and bark of Terminalia laxiflora Engl. has attracted considerable interest due to the increase in strains resistant to synthetic antifungals. The purpose of this discussion is therefore to analyse the results obtained by comparing them with data reported in the scientific literature.

During the extraction process, yields ranged from 9.89% to 18.37% depending on the plant parts, with the highest yield 18.37% obtained from the ethanolic extract of the roots of *Terminalia laxiflora Engl*. These

results corroborate those of N'do et al. (2024)[22] who also showed that polar solvents, particularly ethanol, yield higher extraction yields (10.65 ± 0.01%) in species of the genus *Terminalia.* The observed variability in extraction yield is likely related to the chemical composition of the plant parts used, the harvest period, the dry matter content of the plant material, and the chemical composition of the different plant parts used; soil factors could also explain this difference, according to Phillipson (2007) [23].

The bark and roots of Terminalia laxiflora Engl. contain saponosides, alkaloids, flavonoids, anthraquinones, free quinones, tannins, and anthocyanins. These results are consistent with those of Wissam in 2022[24], who isolated these chemical compounds from species in the Combretaceae family, as well as with the work of N'do et al. in 2024 [25] on several species of the genus *Terminalia*.

The variations observed in the secondary metabolite composition of these plants can be explained by the combined influence of ecological, genetic, and environmental factors. Indeed, climatic conditions and abiotic stresses, such as drought and high temperatures, modulate the biosynthesis of bioactive compounds, particularly polyphenols and flavonoids[26].

*Terminalia laxiflora* Engl., which is rich in secondary metabolites, is likely to exhibit antifungal properties, as demonstrated against pathogenic filamentous fungi.[27].

The antifungal activity of the ethanolic extract of Terminalia laxiflora Engl. showed that this extract inhibits the growth of Aspergillus flavus, Aspergillus niger, Trichophyton rubrum, Trichophyton soudanense, and Microsporum canis at minimum inhibitory concentrations (MICs) ranging from 1 mg/mL to 3 mg/mL. These values indicate promising efficacy, suggesting that this plant species is a potential source of antifungal compounds.

This observed activity can be attributed to the high content of flavonoids, tannins, saponins, and alkaloids in these plants, which were identified during phytochemical screening. These metabolites are known for their antifungal effects through several mechanisms, including disruption of the fungal cell membrane, inhibition of essential enzymes, and induction of oxidative stress. Saponins interact with membrane sterols, which can cause membrane disruption. Polyphenols inhibit fungal growth by disrupting metabolic processes [28].

Although scientific data on the evaluation of the antifungal activity of Terminalia laxiflora Engl. remain limited, several studies conducted on species of the genus Terminalia have demonstrated significant antifungal activity. For example, the study by Ouattara et al. in 2023[29] used the agar incorporation method to demonstrate inhibition of mycelial growth in dermatophytes.

The susceptibility of the pathogenic filamentous fungi tested highlights a broad spectrum of antifungal activity. This property is particularly clinically relevant, given that these microorganisms are involved in common infections, ranging from superficial fungal infections to opportunistic infections. The fact that the extracts are active against these different types of fungi reinforces their pharmacological interest.

These results confirm the value of these medicinal plants in traditional Chadian pharmacopoeia and support their empirical use in the treatment of fungal infections. They also open up promising prospects for the development of new naturally derived antifungal agents.

## CONCLUSION

The ethanolic extract of the bark and roots of *Terminalia laxiflora Engl*., rich in secondary metabolites, is believed to be responsible for the antifungal activity demonstrated by this plant in the Chadian pharmacopoeia. This plant could be utilised as a potential source for the development of herbal medicines to combat infectious diseases, particularly pathogenic filamentous fungi.

**Conflicts of Interest:** The authors declare that they have no conflicts of interest.

**Authors' contributions:** Bégoto Mbailassem collected the samples, performed the laboratory procedures, and drafted the manuscript. Roberto Alladoum contributed to the laboratory procedures and proofreading. Mahamat Hadja Ibrahim contributed to the laboratory procedures. Nadlaou Bessimbaye provided guidance on the laboratory procedures. Issakou Bakarnga-Via coordinated the entire project.

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