



(RESEARCH ARTICLE)



## Antidermatophyte activity of ethanolic extracts of *Daniellia oliveri* (Fabaceae) and *Parinari curatellifolia* (Chrysobalanaceae) in Chad

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GSC Advanced Research and Reviews, 2022, 13(02), 170–179

Publication history: Received on 30 September 2022; revised on 06 November 2022; accepted on 09 November 2022

Article DOI: <https://doi.org/10.30574/gscarr.2022.13.2.0299>

### Abstract

**Introduction:** Skin health disease is still a public health problem. Concerning this situation, the study was conducted with the objective of enhancing the Chadian pharmacopoeia medicinal plants of *Daniellia oliveri*, *Parinari curatellifolia* who traditionally used in the treatment of fungal diseases particular dermatophytosis.

**Methods:** The agar incorporation method was used to determine the antidermatophyte activity of the ethanolic extracts of the barks of *Daniellia oliveri* and *Parinari curatellifolia*. In addition, a phytochemical study was carried out to link the structure to the activity.

**Results:** The phytochemical results revealed that the ethanolic extracts of the barks of *Daniellia oliveri* and *Parinari curatellifolia* are richer in secondary metabolites (flavonoids, anthocyanins, tannins, alkaloids, saponosides, glycosides, anthraquinones and free quinones). These ethanolic extracts of the barks of *Daniellia oliveri* and *Parinari curatellifolia* rich in secondary metabolites inhibited the growth of dermatophytes isolated from patients, for *Trichophyton Schoenleinii*, at the minimum inhibitory concentration (MIC) of 0.75 mg/ml and *Trichophyton rubrum* and *Microsporum canis* at the same MIC of 1.5 mg/ml.

**Conclusion:** From this study, the bioactivity compounds highlighted that these plants could be a possible source for phytomedicinal developing against fungal infections (Dermatophytoses).

**Keywords:** *Daniellia oliveri*; *Parinari curatellifolia*; Phytochemistry; Antidermatophyte

### 1. Introduction

Skin health is still a relevant public health issue in a context where the skin is exposed to external threats (Favier, 2003). Skin diseases such as mycoses, stretch marks, eczema, acne and ageing of multifactorial origin are the most common pathologies encountered in humans (Rock, 2003). Superficial mycoses are diseases of the skin, mucous membranes and phanera caused by microscopic fungi (Khadka et al., 2016), they are very common and have been reported worldwide (Hay, 2017; Grover et al., 2016), where they are a significant public health concern and lead to tremendous morbidity especially in developing countries (Nweze, 2010; Chikoi et al., 2018).

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The worldwide estimated prevalence of superficial mycoses is 20 to 25% of skin diseases (Coulibaly, 2018; Chikoi et al., 2018). Epidemiological studies have shown that almost all people living with HIV experience skin diseases at some point during their illness (Fulgence et al., 2013). They are seen in almost 80% of AIDS patients and 60% of early-stage patients (Patel et al., 2009). The prevalence in Europe has decreased significantly due to improved living conditions (Patel et al., 2009). The prevalence of scalp ringworm is estimated to be 20% in West Africa, and about 10-70% in other parts of Africa among school-age children (Léon et al., 1960). In tropical countries such as Senegal, dermatosis affects 30% of the population in rural areas (Bitew, 2018). The most common dermatophytosis is ringworm and is almost a disease of childhood (3-15 years), mostly found in African and Caribbean children (Intra et al., 2018; Adesiji et al., 2019).

La prévalence de la teigne était de 34%, 13,9% et 39,3% respectivement au Nigeria, en Côte d'Ivoire et au Mali (Adesiji et al., 2019 ; Zika, 2011 ; Coulibaly et al., 2018 ). Ces chiffres sont inquiétants. Bien qu'on dispose aujourd'hui de médicaments antifongiques, le traitement des mycoses reste difficile d'une part du fait du nombre limité de principes actifs réellement efficaces et de leur coût très élevé et d'autre part lié à l'émergence de souches résistantes à certains antimycosiques usuels (Adesiji et al., 2019).

Therefore, the search for natural bioactive molecules seems to be one of the priorities in the last years. One of the strategies for this research is to explore traditional medicine plants. The WHO estimates that up to 80% of the world's population in general, and particularly the African population, rely on traditional herbal medicine to meet their health needs due to its accessibility and low cost (WHO, 2000).

With the aim of valuing the Chadian pharmacopoeia's medicinal plants, most notably *Daniellia oliveri* and *Parinari curatellifolia*, which are traditionally used to treat microbial diseases, this study was carried out to evaluate the antidermatophyte activity for the purpose of formulating traditionally improved phytomedicines.

## 2. Material and methods

### 2.1. Vegetal material

The plant material used in this study was made up of *Daniellia oliveri* and *Parinari curatellifolia* trunk barks, collected in Pala on 10 June 2021 in the Department of Mayo-Dallah located between 921'48"N and 14°54'36"E in Chad, which receives an average of 606.9 mm of rainfall per year. These plants were selected based on an ethnobotanical study and identified at the Herbarium de l'Institut de Recherche en Elevage pour le Développement (HIRED) where the specimens were stored under the identification numbers: *Daniellia oliveri* (4578/HIRED/Chad) and *Parinari curatellifolia* (2523/HIRED/Chad).

### 2.2. Fungal material

The fungal material consisted of three strains of dermatophytes (*Trichophyton rubrum*, *Trichophyton schoenleinii*, and *Microsporum canis*) collected from patients seen in the Dermatology Department of the Centre Hospitalier Universitaire de Référence Nationale (CHURN) after verbal consent.

### 2.3. Preparing plant extracts

#### 2.3.1. Principle

The extraction is the preparation of the active parts of plants by selectively using the solvents by standard procedures to obtain an extract. The obtained products from plants are complex mixtures of secondary metabolites (crude extract) in liquid, semi-solid or powder format and are used either orally or externally. This includes preparation types known as decoction, infusion, maceration, and infleurage (Gerbino, 2006). The powdered maceration of organs was chosen for this study.

### 2.4. Procedure for the ethanolic extract

50 g of plant material from each plant was put in a closed bottle and mixed with 500 ml of ethanol (10%) and left to macerate under electric shaking for 24 hours. The macerate was filtered on cotton wool and then on Wattmans1 filter paper using a Buchner, vacuum flask and aspirator. The obtained filtrate was evaporated under vacuum of 45-50 °C using a Rotative Evaporator. The concentrated filtrate was placed in a beaker and dried under ventilation at ambient temperature. The residual was weighed and stored in a dry bottle in the refrigerator at 4 °C (Gerbino, 2006).

### 2.4.1. Extraction performance

The extraction yield was obtained by the following formula:

$$\text{Rendement (\%)} = \frac{M_2 - M_1}{M_0} \times 100$$

M2 = mass of the beaker containing the residue of the extract,

M1 = mass of the empty beaker

M0 = mass of the used powder (Gerbino, 2006).

### 2.5. Phytochemical study

The analysis of the chemical composition in secondary metabolites of ethanolic extracts of *Daniellia oliveri* and *Parinari curatellifolia* trunk barks, for the purpose of justifying the antidermatophyte activity, was carried out according to the protocols described by (Harbone, 1976) at the Laboratoire de Pharmacologie et Toxicologie of the Faculté des Sciences de la Santé Humaine (FSSH) of the University of N'Djamena (Chad).

### 2.6. Determination of antidermatophyte activity

The evaluation of the antidermatophytic activity was determined by the agar incorporation method following the protocol described by (De Billerbeck, 2000) which consisted in evaluating the percentage of inhibition of the growth diameter of the dermatophyte strains (*Trichophyton rubrum*, *Trichophyton schoenleinii*, and *Microsporum canis*) towards ethanolic extracts of *Daniellia oliveri* and *Parinari curatellifolia* trunk bark, in order to determine the inhibition parameters, the Minimum Inhibitory Concentration (MIC) and the Minimum Fungicidal Concentration (MFC) of the extracts on these dermatophyte strains.

### 2.7. Principle

This method is based on the ability of a micro-organism to grow on an agar medium, supplemented with extracts whose activity is to be tested at specific concentrations (De Billerbeck, 2000). The Sabouraud substrate was prepared according to standard laboratory methods and autoclaved at 121.1 °C for 30 min.

### 2.8. Preparing the stock and control solutions for the test

One hundred milligrams of each crude extract was diluted to give a stock solution of 10 mg/ml concentration. Fluconazole and griseofulvin were prepared under the same conditions and used as positive controls. Stock solutions (10 mg/ml) of the ethanolic extracts were prepared under sterile conditions in a laminar flow hood. Then a series of dilutions were made in Sabouraud's chloramphenicol medium to obtain the concentrations: 0.25 mg/ml, 0.5 mg/ml, 0.75 mg/ml, 1 mg/ml, 1.5 mg/ml, 2 mg/ml and 2.5mg/ml from the stock solution. The same procedure was used for the preparation of a series of solutions for the reference molecules (fluconazole and griseofulvin).

### 2.9. Method of operation

This process consists of diluting the Sabouraud chloramphenicol solution with the extract/ethanol solution prepared beforehand in order to obtain the desired concentration. Each tube was homogenised and poured into an identified Petri dish. The mixture was left to stand in the fume hood until the agar solidified. Inoculation was performed by depositing a 7 mm diameter explant from the growth front of a 3-4 day old culture. Petri dishes were then sealed and incubated for 7 days at room temperature. Each trial was performed in triplicate. Mycelial growth on each dish was observed and the diameter of the dermatophyte mycelial growth zone was measured in two perpendicular directions through the centre of the explant to determine the percentage inhibition (PI). The antifungal activity of the extracts was assessed by calculating the percentage inhibition according to the formula (Zacchino *et al.*, 1999)

$$PI = \frac{dt - dx}{dt} \times 100$$

PI= inhibition percentage expressed as a percentage, dt = average diameter of the mycelium in the "negative control" box (without antifungal agent) and dx = average diameter of the mycelium in the test box containing the antifungal agent.

### 2.10. MIC determination (Minimum Inhibitory Concentration)

After 7 days of incubation, Petri dishes in which no visible growth of explants was observed gave us an idea of the minimum inhibitory concentration (MIC).

### 2.11. Determining the MFC (minimum fungicide concentration)

Explants that did not grow during the entire incubation period because they were inhibited by the extract, were re-inoculated into petri dishes containing the substrate not supplemented with extract to determine the nature of the inhibition. When growth restarts, the extract is fungistatic and when it does not, the extract is fungicidal, and the parameter evaluated is the MFC (minimum fungicide concentration). The fungicide was determined by the MFC/MIC ratio.

### 2.12. Statistical analysis

Excel: *In vitro* test results are expressed as average  $\pm$  SD: Standard deviation.

## 3. Results

### 3.1. Extraction performance

The extraction yields of ethanolic extracts from the bark of the trunks of *Daniellia oliveri* and *Parinari curatellifolia* two plants are presented in Table 1.

**Table 1** Extraction yields

Plant species	Yield (%) m/m
Bark of <i>Daniellia oliveri</i>	35.7
Bark of <i>Parinari curatellifolia</i>	11.2

Table 1 shows that the yield varies from 11.2 to 35.7% depending on the plant type and the best performance is for the ethanolic extract of the bark of the *Daniellia oliveri* trunk with 35.7%.

### 3.2. Phytochemistry

**Table 2** Phytochemical analysis of plant ethyl extracts

Secondary Metabolites	Ethanolic extracts from bark	
	<i>Daniellia oliveri</i>	<i>Parinari curatellifolia</i>
Flavonoids	++++	++++
Sterols and Terpenoids	++++	++++
Gallic tannins	-	-
Catechic tannins	++++	++++
Free Quinones	++++	++++
Antraquinones	+	+
Alkaloids	++++	++++
Heterosides	++	++
Anthocyanins	++++	++++
Saponosides	++++	++++

Caption: (-) absent; (+) found in low concentrations; (++) found in medium concentrations; (+++) found in high concentrations; (++++) found in very high concentrations.

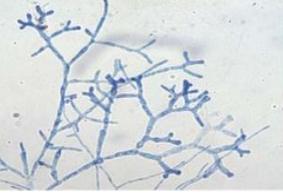
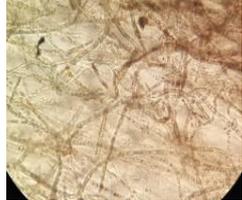
The phytochemical analysis of the ethanolic extracts of *Daniellia oliveri* and *Parinari curatellifolia* trunk barks has revealed the presence of secondary metabolites listed in table 2.

Except for the Gallic tannins which are not present, the ethanolic extracts of *Daniellia oliveri* (Fabaceae) and *Parinari curatellifolia* (Chrysobalanaceae) trunk barks are rich in alkaloids, sterols, terpenoids, Gallic tannins, catechic tannins, free quinones, anthraquinones, heterosides, anthocyanins and saponosides and have almost the same chemical composition which indicates a common chemotaxis approach although these two plants belong to two different families.

### 3.3. Antidermatophyte activity

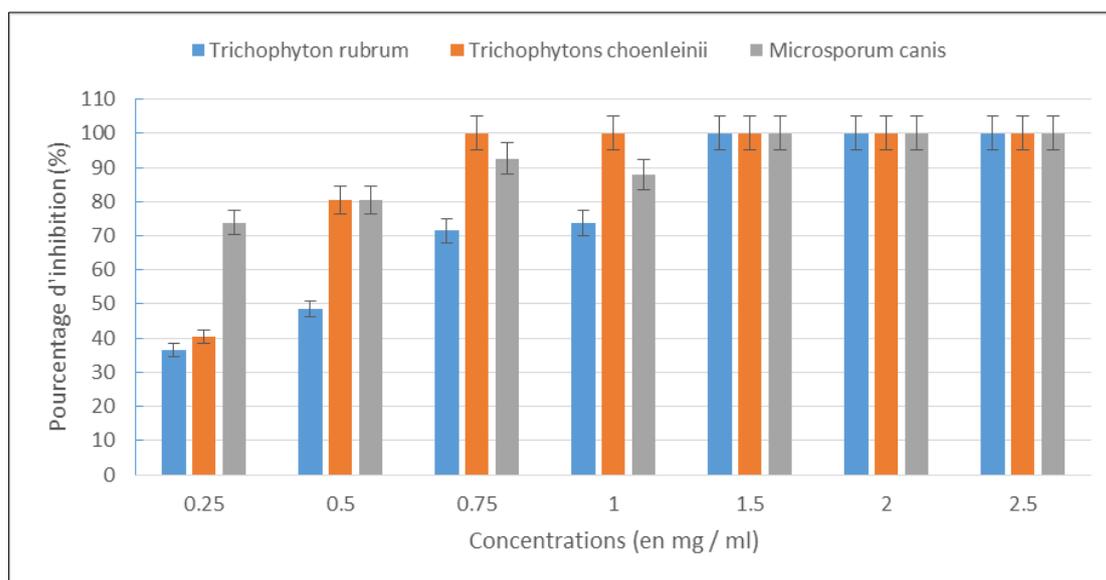
#### 3.3.1. Isolation and identification

After cultivation of fungal material taken from the patients (four patients), isolated and re-cultured each different colony macroscopically observed in new petri dishes containing Sabouraud substrate with chloramphenicol. This process (replating) is repeated several times to obtain pure strains. After three to seven days of culture, microscopic observation is repeated to ensure that the microscopic observations of the images are like those obtained at the beginning of the samples. The cultures of the samples on Sabouraud medium with chloramphenicol allowed the isolation of three different strains of dermatophytes after observation under the electronic microscope at X40 magnification. **Figure 1** shows the presence of these strains in our samples. This table considers the macroscopic and microscopic aspect of the fungal strains isolated from the patients and the reference pictures for comparison.

Genre	Macroscopic aspect		Microscopic view at magnification 40 X	Reference picture
	Recto	Verso		
<i>Microsporum</i>			 <i>Microsporm canis</i>	 <i>Microsporm canis</i> (Chabasse et al., 2004)
<i>Trichophyton</i>			 <i>Trichophyton schoenleinii</i>	 <i>Trichophyton schoenleinii</i> (Dufresne,2021)
<i>Trichophyton</i>			 <i>Trichophyton rubrum</i>	 <i>Trichophyton Rubrum</i> (Rippon, 1987)

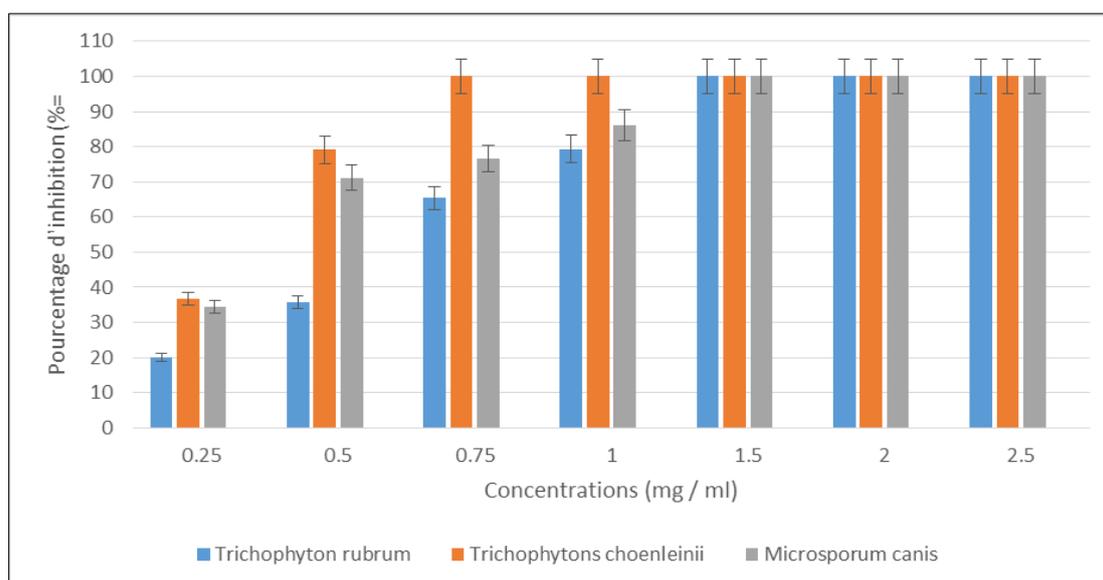
**Figure 1** Macroscopic and microscopic characteristics of fungal strains isolated from patients

Antidermatophyte activity of ethanolic extracts of *Daniellia oliveri* and *Parinari curatellifolia*.



**Figure 2** Antifungal activity of ethanolic extract of *Daniellia oliveri* on *Trichophyton Schoenleinii*, *Trichophyton rubrum* and *Microsporium canis*

The antidermatophyte activity of ethanolic extracts of *Daniellia oliveri* and *Parinari curatellifolia* trunk barks on the isolated strains (names of the strains) shown in Figures 2 and 3 reveals their inhibition at different concentrations.



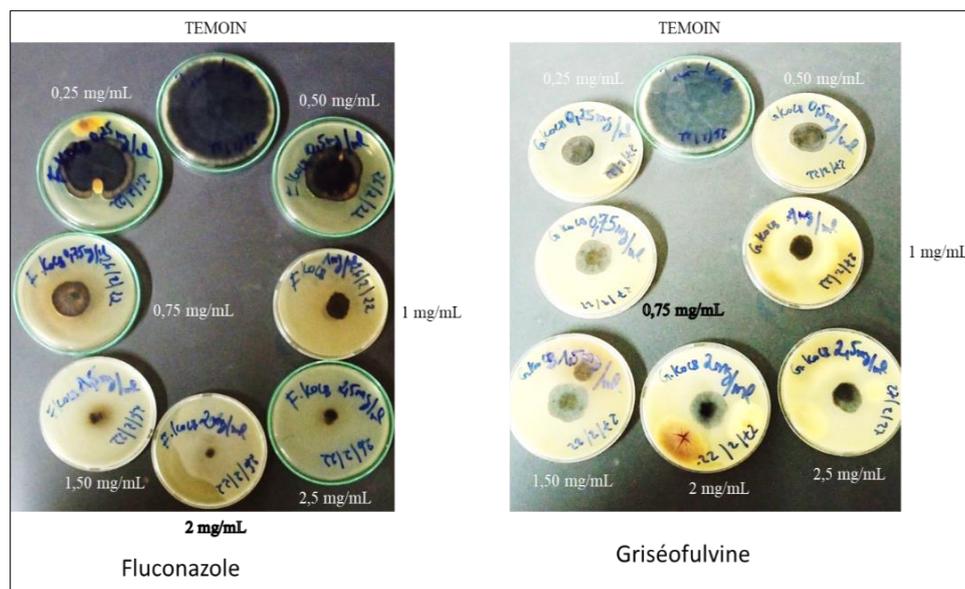
**Figure 3** Antifungal activity of ethanolic extract of *Parinari curatellifolia* on *Trichophyton Schoenleinii*, *Trichophyton rubrum* and *Microsporium canis*

Figures 2 and 3 show that the percentage of inhibition on *Trichophyton rubrum*, *Microsporium canis*, and *Trichophyton schoenleinii* species increases with the concentration of extracts in the growing medium. The sensitivity is variable according to the strains and reaches 100% inhibition at a concentration of 0.75 mg/ml ethanolic extract of *Daniellia oliveri* bark on *Trichophyton schoenleinii*, and at 1.5mg/ml for *Trichophyton rubrum*, and *Microsporium canis* corresponding respectively to the minimum inhibitory concentrations (MIC). Ethanolic extract of *Parinari* bark at 1.5 mg/ml inhibits the growth of *Trichophyton rubrum* and *Microsporium canis*. Ethanolic extract of *Daniellia oliveri* bark, *Parinari curatellifolia* and inhibits the growth of *Trichophyton schoenleinii* at a MIC of 0.75 mg/ml

### 3.3.2. Antidermatophyte activity of two reference molecules

Fluconazole and Griseofulvin.

The reference molecules were tested on the three strains *Trichophyton rubrum*, *Trichophyton schoenleinii*, *Microsporum canis*. Figure 4 shows the growth inhibition of *Microsporum canis* by Fluconazole and Griseofulvin for illustration.



**Figure 4** Action of Fluconazole and Griseofulvin on the growth of *Microsporum canis*

These results show that the inhibition is complete (100%) from the concentration of 2mg/ml for fluconazole. Therefore, we can deduce that the minimum inhibitory concentration of fluconazole on *Microsporum canis* is 2mg/ml. Griseofulvin did not inhibit the growth of *Microsporum canis*.

In comparison with the MICs obtained, ethanolic extracts of *Daniellia oliveri* and *Parinari curatellifolia* trunk bark were more active than the reference molecules as they inhibited the growth of those three strains of dermatophytes at MIC ranging from 0.75 to 1.5 mg/ml in comparison to fluconazole which inhibited the growth of *Microsporum canis* at a MIC of 2mg/ml. Griseofulvin was found not to inhibit any of these strains, thereby showing resistance of *Trichophyton rubrum*, *Trichophyton schoenleinii*, and *Microsporum canis* to this compound.

The subculture of *Trichophyton rubrum*, *Trichophyton schoenleinii* and *Microsporum canis* which did not show any growth was used to determine the minimum fungicide concentration and the MFC/MIC ratio is recorded in Table 3 for the ethanolic extract of *Daniellia oliveri* trunk bark and **Table 4** for the ethanolic extract of *Parinari curatellifolia* trunk bark

**Table 3** Summary of MIC, MFC and MFC/MIC for *Daniellia oliveri*

Parameters	Ethanolic extract of the trunk bark of <i>Daniellia oliveri</i>		
	MCF (mg/ml)	MIC (mg/ml)	MFC/MIC
<i>Trichophyton rubrum</i>	1.5	1.5	1
<i>Trichophyton choenleinii</i>	1	0.75	1.33
<i>Microsporum canis</i>	2	1.5	1.33

The MFC/MIC ratios in Tables 3 and 4 are strictly lower than 4 (MFC/MIC < 4), which shows that ethanolic extracts of *Daniellia oliveri* and *Parinari curatellifolia* trunk bark are fungicidal on the three strains *Trichophyton rubrum*, *Trichophyton schoenleinii*, *Microsporum canis* (Carbonnelle et al., 1987).

**Table 4** Summary of MIC, MFC and MFC/MIC for *Parinari curatellifolia*

Parameters	Ethanollic extract of the trunk bark of <i>Parinari curatellifolia</i>		
	MFC (mg/ml)	MIC (mg/ml)	MFC/MIC
<i>Trichophyton rubrum</i>	1.5	1.5	1
<i>Trichophyton schoenleinii</i>	0.75	0.75	1
<i>Microsporum canis</i>	1.5	1.5	1.5

#### 4. Discussion

During the extraction the yields ranged from 11.2 to 35.7% according to the plant species and the best yield was the ethanollic extract of *Daniellia oliveri* trunk bark with 35.7%. These results are in line with those of Peni et al (2010) who obtained a yield of 10.47% from the methanolic extract (alcohol) of the bark of *Parinari curatellifolia*. This similarity could be explained by the use of the same organs and polar solvents that allow the secondary metabolites contained in these organs to be dissolved more easily.

The phytochemical analysis showed the presence of the following main chemical groups: flavonoids, sterol terpenoids, tannins, quinones, alkaloids, heterosides, anthocyanins and saponosides, which are almost similar in the ethanollic extracts of the trunk barks of *Daniellia oliveri* and *Parinari curatellifolia*. The trunk barks of these two plants contain almost the same compounds characterized by some variations in the concentrations of the metabolites in the trunk bark of both plants. The compounds alkaloids, saponosides, sterols and terpenoids were found to be present in slightly higher quantities in the trunk bark of *Parinari curatellifolia* than in that of *Daniellia oliveri* and gall tannins were not found in the trunk bark of either plant. These results are similar to those of de Peni et al (2010) who revealed the presence of these secondary metabolites in the methanolic extracts of the trunk barks of these two plants. In the same way, the phytochemical analysis made by Tittikpina et al, (2013) in Togo, on the hydroethanol extracts of the leaves, trunk barks, root barks and roots of *Daniellia oliveri* revealed the richness of these different extracts in alkaloids, sterols, terpenoids, gall tannins, catechic tannins, free quinones, anthraquinones, heterosides, anthocyanins and saponosides except for the gall tannins

Keta et al., (2019) have obtained from ethanollic extracts of fresh leaves of *Daniellia oliveri*, other organs but with the same solvent (ethanol) which elucidated the presence of these secondary metabolites. All these identified phytochemicals are likely to exhibit antimicrobial properties (Emmanuel et al., 2010).

The antifungal activity illustrated on fungi (dermatophytes) in this study might be the result of the combined action of the different secondary metabolites contained in the barks of the two plants in the study. The results of the study show that the ethanollic extract of the bark of the trunks of *Daniellia oliverie* inhibits the growth of *Trichophyton schoenleinii* at a MIC of 0.75 mg/ml and of 1.5 mg/ml for *Trichophyton rubrum* and *Microsporum canis*. Emmanuel et al., (2010) showed that the antifungal activity of *Daniellia oliveri* is explained by the presence of secondary metabolites present in the plant during phytochemical screening. Therefore, they report that the revelation of metabolites (alkaloids, saponosides, tannins, flavonoids) by phytochemical screening is the origin of the antifungal activity and that the variation of the inhibitory effects is a function of the quantity and quality of bioactive compounds present in the plant. Keta et al., (2019) studied the dermatophytes namely: *Trichophyton rubrum* and *Microsporum audouinii*, ethanollic and aqueous extracts of leaves inhibited the growth of these strains at MICs of 3.125mg/ml and 200 mg/ml respectively. Their study showed that the ethanollic extract was more active than the aqueous extract and compared to our study with ethanollic bark from *Daniellia oliveri* trunks, we obtained a MIC of 1.5 mg/ml which is lower than 3.125 mg/ml. This difference could be explained by the concentration of secondary metabolites depending on the organs. The ethanollic extract of trunk bark was more active than that of the leaves according to the MICs obtained on the strains tested. This is also the case for the ethanollic extract of the trunk bark of *Parinari curatellifolia* illustrated in this study, whose antifungal activity on the isolated strains is revealed by minimum inhibitory concentrations (MIC) of 0.75 mg/ml on *Trichophyton Schoenleinii* and 1.5 mg/ml on *Microsporum canis* and *Trichophyton rubrum*. The antidermatophyte activity of ethanollic extracts of *Daniellia oliveri* and *Parinari curatellifolia* trunks shown in this study would be due to their high content of different secondary metabolites resulting from the phytochemical analysis linking structure to activity as described by Peni et al., (2010); Tittikpina et al., (2013); Keta et al., (2019) who stated that these plants with these bioactive compounds, possess antibacterial, antifungal, and anti-inflammatory properties.

## 5. Conclusion

The ethanolic extracts of the bark of the trunks of *Daniellia oliveri* and *Parinari curatellifolia*, which are rich in secondary metabolites, may be responsible for the anti-dermatophyte activity revealed by these two plants in the Chadian pharmacopoeia. These plants could be used as a potential source for the development of phytomedicines to tackle infectious diseases, particularly dermatophytosis.

## Compliance with ethical standards

### Acknowledgments

For this study, the authors would like to thank the Department of Dermatology of the Center Hospitalier Universitaire de Référence Nationale (CHURN) for the isolation of strains of dermatophytes, the Herbarium of the Institut de Recherche en Elevage pour le Développement (HIRED) for the nesting plants and traditional healers for their guidance on the choice of these two plants.

### Disclosure of conflict of interest

No conflict of interest.

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