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# Exploration of the *in vitro* antifungal activity of different organs of *Parkia biglobosa* (Jacq.) R. Br. (Fabaceae) on *Fusarim oxysporum* and *Pestalotia* sp

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## Abstract

*Parkia bliglobosa* is a wild edible plant used for its multiple nutritional and sanitary virtues. The aim of this study was to evaluate the antifungal activity of *Parkia bliglobosa* leaf, seed and bark extracts on the mycelial growth and sporulation of *Fuarium oxysporum* and *Pestalotia* sp. To achieve this, fungal strains were cultured on PDA media amended with extracts. Dose concentrations were defined in order to observe which concentrations best inhibited the fungal strains. The highest inhibition rates were obtained with the hydro-ethanolic extract of *Parkia bliglobosa* leaves at concentrations of 12 mg/ml (91.62%) and 24 mg/ml (92.45%) respectively on *Fusarium oxysporum*. As for *Pestalotia* sp, an inhibition rate of 81% was obtained with the hydro-ethanolic extract of leaves at concentrations of 12 mg/ml and 24 mg/ml. *Parkia biglobosa* therefore has antifungal activity against *Fusarium oxyporium* and *Pestalotia* sp

**Keywords:** *Fusarium oxysporum*, *Pestalotia* sp, *Parkia biglobosa*, Extract

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# Introduction

The cowpea, scientific name *Parkia biglobosa*, is one of 34 known species of the genus *Parkia*, found in Africa, South America and Asia. It is a member of the Fabaceae family (Mabetty, 2018). The natural range of cowpea in Africa covers a vast region stretching from Senegal in the west to Uganda in the east, and includes the Sudanese and Guineo-Congolese areas (Lamien, 2011). Néré is a much sought-after plant in Africa due to its multiple nutritional and therapeutic virtues. It is used for both personal consumption and marketing, and is widely known for its many anti-blennorrhagic, antineuralgic, antiseptic, diuretic, febrifuge and vermifuge properties (Aké-assi *et al*., 1991). Néré is considered a very important heritage in northern Côte d'Ivoire. It is used in the preparation of food products such as soumbala, which produces 432 calories for the body (Appia *et al*., 2023).

In terms of plant health, cowpea is effective in protecting crops against bruchid attack and, to a lesser extent, termites (Appia *et al.,* 2023). Despite its importance, it is less widely used in crop protection. In view of the above, the question arises as to whether this plant could be useful in the fight against crop diseases. The aim of the present study was to evaluate the antifungal activity of the various *Parkia biglobosa* plant organs on two phytopathogenic fungi, *Fusarium oxyporium* and *Pestalotia* sp.

# Materials and Methods

## Material

### Plant material

The plant material consisted of the various *Parkia biglobosa* plant organs (leaves, bark, seeds) harvested in Korhogo.

### Fungal material

The fungal material consisted of a strain of *Fusarium oxysporum* and a strain of *Pestalotia* sp. These strains were isolated from tomato and shea leaves respectively. The fungal strains were supplied by the laboratory of the Unité Pédagogique et de Recherche, Université Félix Houphouët Boigny, Abidjan.

## Methods

### Sample collection and conditioning

Samples of green leaves, bark and mature seeds were collected in Korhogo. Drying took place in the shade for 2 weeks. After drying, the organs were ground to a powder and placed separately in plastic bags.

### Preparation of extract

### Aqueous extract

The fine powder obtained from each plant organ was used for aqueous extraction using the Zirihi *et al*. (2003) method modified as follows: One hundred and fifty grams (150g) of each powder was poured separately into jars. To each powder was added 1.5 liters of distilled water, then homogenized for one hour using a magnetic stirrer. The resulting homogenate was wrung out in a square of white cloth, then filtered three times on absorbent cotton and twice on filter paper. The filtrate thus obtained was dried by evaporation in an oven at 60°C using plates for 72h. The powder obtained constitutes the total aqueous extract.

### Hydroethanol extract

The hydroalcoholic extract was prepared using the same procedure as for the aqueous extract. However, the solvent used was a mixture of 70% ethanolic and 30% distilled water. The extracts obtained were weighed using a precision balance to calculate the yield. Extracts were stored in sterile plastic jars at 4°C.

## Calculating yield

Yield is the quantity of extract obtained from the plant powder. It is expressed as a percentage and is calculated according to the formula of Kouamé *et al*. (2021).

R: extraction yield
M: mass in grams of fine powder
m: mass in grams of dry extract obtained.

### *In vitro* antifungal activity of the different extracts

#### Preparation of the culture medium and incorporation of the extract into the medium

Dose preparation involved amending a PDA (Potatose Dextose Agar) medium with aqueous and hydro-ethanolic extracts to obtain concentrations of 24 mg/ml, 12 mg/ml and 6 mg/ml, 3 mg/ml. These concentrations were defined using the double dilution geometric bond method of reason ½ (Zirihi *et al*., 2003). The various quantities of extracts were incorporated into the media and then autoclaved at 121°C under 1bar pressure for 1h. Media prepared in this way were dispensed into 90 cm-diameter petri dishes under aseptic conditions around a busen nozzle. Controls received no extracts.

#### Inoculation

Inoculation was carried out with a mycelium pellet taken from the growth front of the fungus using a cookie cutter. For each concentration, four petri dishes were replicated. The cultures were then sealed with cling film and incubated in the dark at 27°C for 7 days.

#### Rate of mycelial growth inhibition

The average radial growth of the fungus was measured daily over 7 days, in parallel with that of the control. Measurements were taken in millimetres along two perpendicular axes traced on the underside of the petri dish. Radial growth was also measured over a 7-day period until the control dishes were filled. The rate of inhibition of radial mycelial growth was calculated using the formula of Leroux and Credet (1978).

T: Inhibition rate
D: Mycelial growth in control petri dishes
d: Mycelial growth in test dishes

#### Determination of 50% and 90% inhibitory concentrations

50% and 90% inhibitory concentrations were calculated using probit sigmoidal curves of the base-10 logarithm of concentration and mycelial growth inhibition rate according to the formula reviewed in Paranagama *et al*. (2003). Spore germination rates are transformed into probit values. Regression lines are established as follows:

a: regression coefficient; b: constant; x: fungicide concentration; y: probit; Log: decimal logarithm.

The equations of these lines were used to determine the $IC_{50}$ and $IC_{90}$, which are the concentrations that reduce mycelial growth by 50% and 90% respectively (Oxenham *et al*., 2005). The 50% and 90% inhibitory concentrations are expressed in ppm.

## Results

### Yield of aqueous and hydro-ethanolic extracts

The aqueous extract of *Parkia biglobosa* leaves gave a low yield of 5.5% compared with the aqueous extracts of seed and bark, which had yields of 10.66% and 7% respectively (Table 1). The yield of the hydro-ethanolic extract was higher for all organs than for the aqueous extracts. Leaves, on the other hand, recorded the lowest yield of 10%, while an 18% yield was observed with seeds. An intermediate yield of 12% was obtained with bark.

**Table 1: Yields of *Parkia biglobosa* organ extracts**

| Organs | Organs Yields (%) | |
|---|---|---|
| | Aqueous | Hydro-ethanolic |
| **Leaves** | 5,5 | 10 |
| **Seeds** | 10,66 | 21 |
| **Barks** | 7 | 15 |

## Antifungal activity of *Parkia biglobosa* extracts

### Effects of extracts on *Fusarium oxysporum* mycelial growth as a function of extract concentration

All extracts tested in vitro had a variable effect on *Fusarium oxysporum* mycelial growth (Figure 1). The results show that inhibition rates increased with increasing extract concentrations. The lowest inhibition rates were observed with the aqueous extract (Figure 2). The highest inhibition rates were observed with the hydro-ethanolic extract (Figure 3). The highest inhibition rates, 91.62% and 92.45%, were obtained with the leaf hydro-ethanolic extract at concentrations of 12 mg/ml (C3) and 24 mg/ml (C4) respectively. Also, hydro-ethanolic seed extract and hydro-ethanolic bark extract recorded high inhibition rates at C4 concentration, with values of 87.96% and 87.42% respectively.

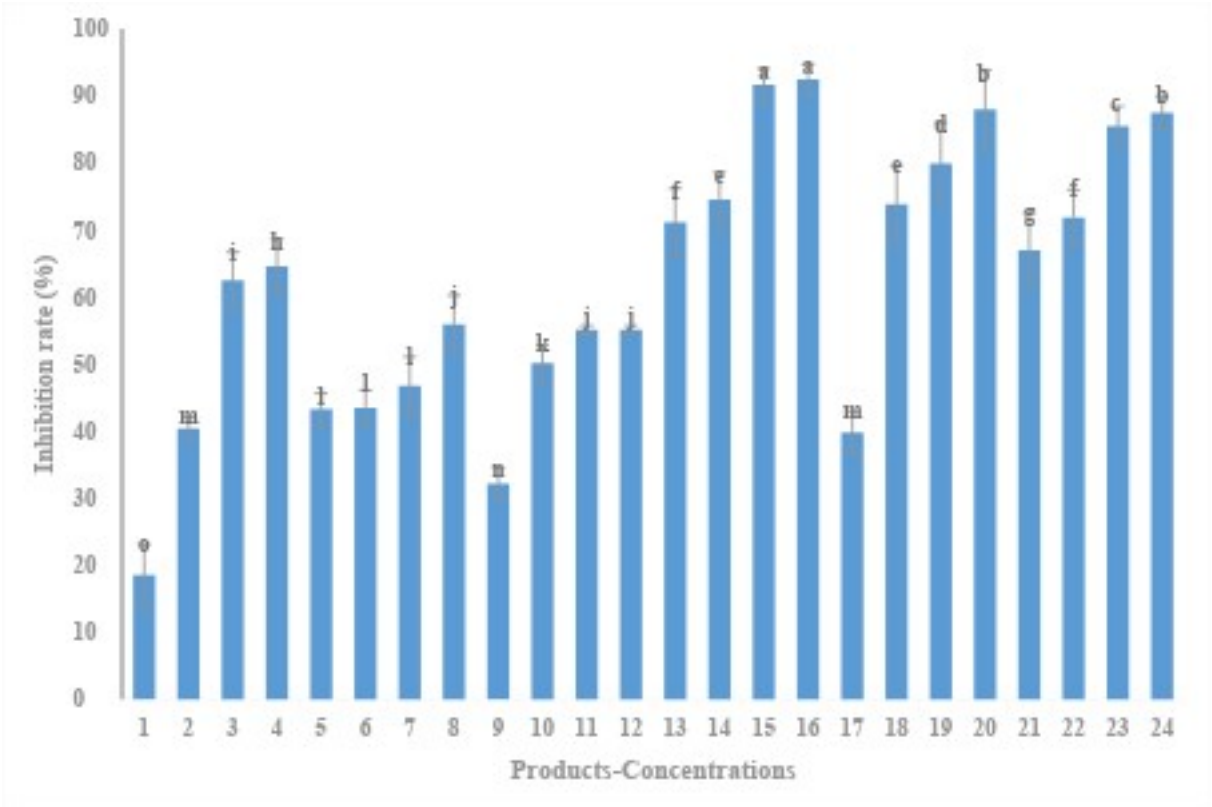
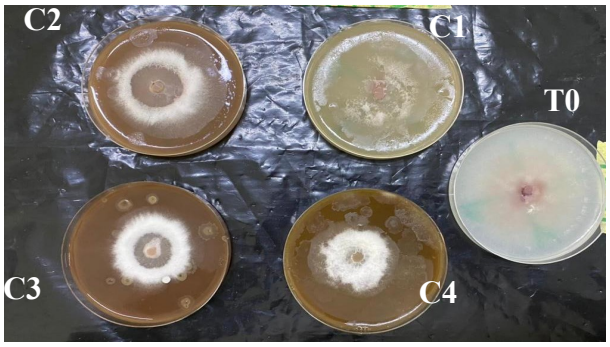
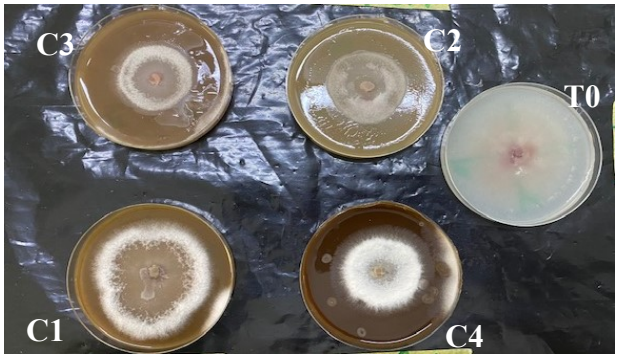



**Figure 1: Dose-response effects of *Parkia biglobosa* extract concentrations on *Fusarium oxysporum* mycelium growth**

C1: 3 mg/ml; C2: 6 mg/ml; C3: 12 mg/ml; C4: 24 mg/ml
Bars marked with the same letters indicate that there were no significant differences between inhibition diameters at the 5% threshold according to the Newman-Keuls test.




A : Leaves

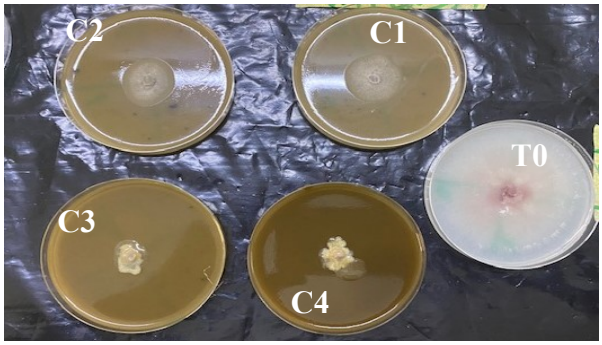



B : Barks

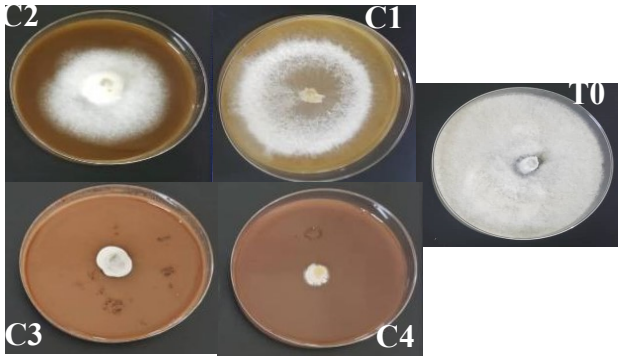

C : Seeds

**Figure 2: Dose-response effects of aqueous extracts of *Parkia biglobosa* organs on the growth of *Fusarium oxysporum* mycelium**

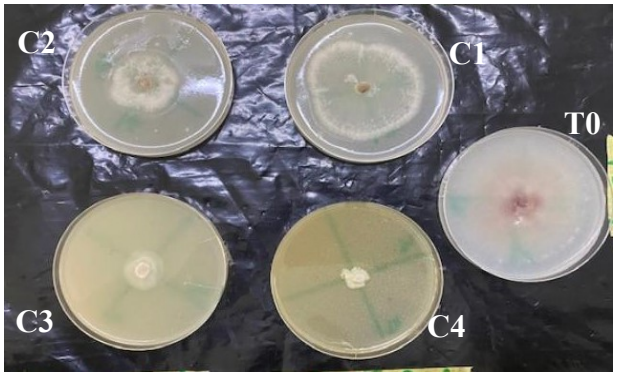
C1 : 3 mg/ml ; C2 : 6 mg/ml ; C3 : 12 mg/ml ; C4 : 24 mg/ml; T0: control without extract




A : Leaves




B : Barks




C : Seeds

**Figure 3: Dose-response effects of hydroethanolic extracts of *Parkia biglobosa* organs on the growth of *Fusarium oxysporum* mycelium**

C1 : 3 mg/ml ; C2 : 6 mg/ml ; C3 : 12 mg/ml ; C4 : 24 mg/ml; T0: control without extract

### Effect of *Parkia biglobosa* extracts on *Pestalotia* sp mycelium growth as a function of concentration

The different extracts had varying effects depending on the type of extract and the concentrations tested (Figure 4). Aqueous extracts of leaf, seed and bark showed the lowest inhibition rates (Figure 5). The results show that with these extracts, as the concentration increases, the inhibition rate decreases. These extracts also had a stimulatory effect on mycelium growth. With the aqueous seed extract, rates of -1.4% and -5.55% were recorded at concentrations C3 and C4 respectively. On the other hand, the hydro-ethanolic extracts of leaves and bark had the highest inhibition rates (Figure 6). A rate of 81.00% was obtained with the leaf hydro-ethanolic extract at concentrations C3 and C4. For the bark hydro-ethanolic extract, the results showed inhibition rates of 80.02% (C3) and 80.19% (C4) respectively.

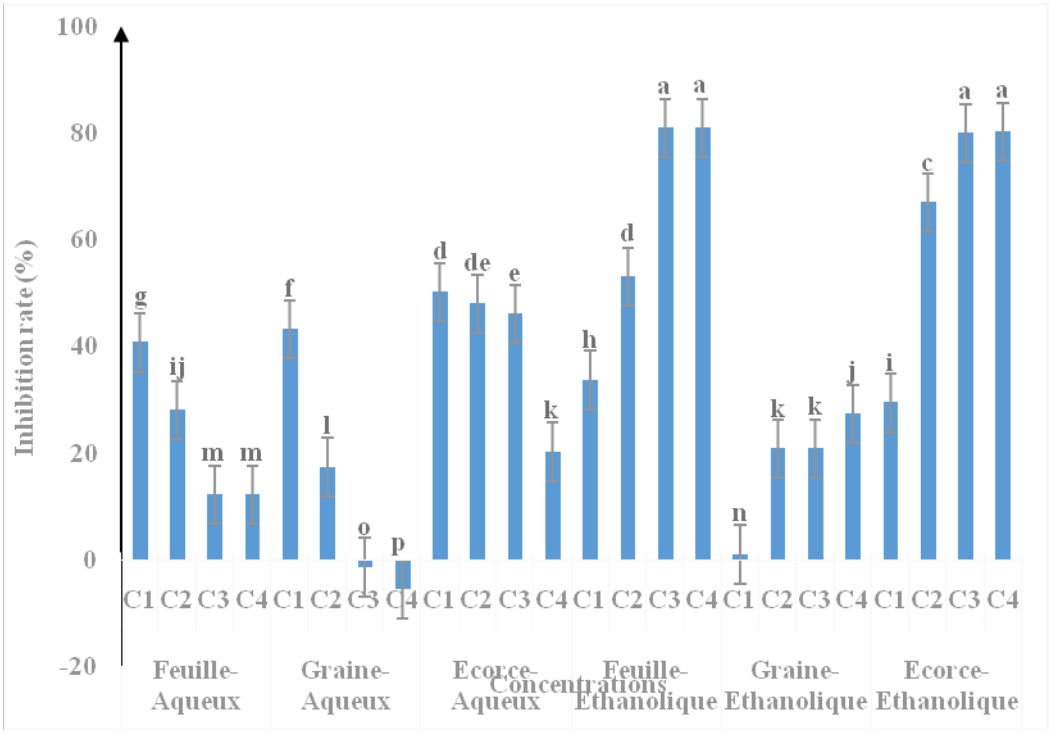
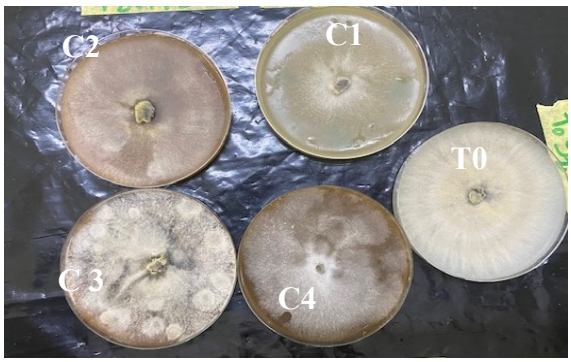


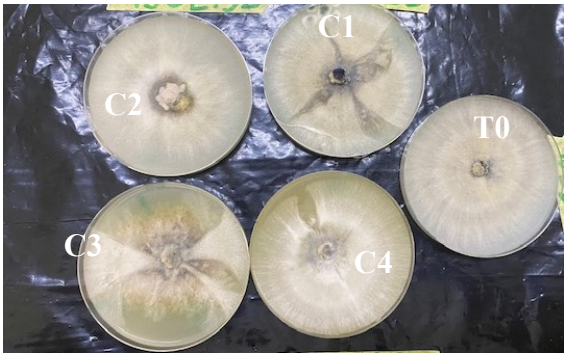

**Figure 4: Dose-response effects of *Parkia biglobosa* extract concentrations on *Pestalotia* sp mycelium growth**

C1: 3 mg/ml; C2: 6 mg/ml; C3: 12 mg/ml; C4: 24 mg/ml
Bars marked with the same letters indicate that there were no significant differences between inhibition diameters at the 5% threshold according to the Newman-Keuls test.




**A :** Leaves

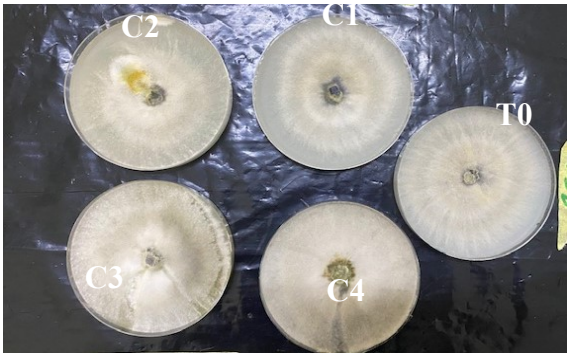

**C:**Barks



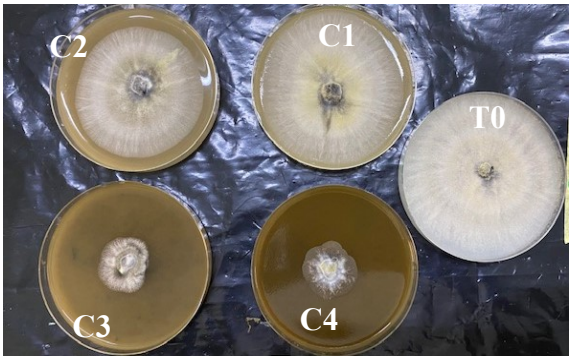

**B :** Seeds

**Figure 5: Dose-response effects of aqueous extracts of *Parkia biglobosa* organs on the growth of *Pestalotia* sp mycelium**

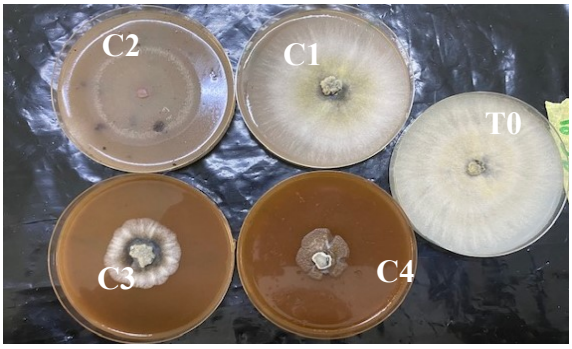
C1 : 3 mg/ml ; C2 : 6 mg/ml ; C3 : 12 mg/ml ;C4 : 24 mg/ml




A :Leaves




B : Barks




C : Seeds

**Figure 6: Dose-response effects of hydro-ethanolic extracts of *Parkia biglobosa* organs on the growth of *Pestalotia*sp mycelium**

C1: 3 mg/ml ; C2: 6 mg/ml ; C3: 12 mg/ml ;C4: 24 mg/ml

### Inhibitory concentration at 50% and 90%

The IC50 and IC90 concentrations induced by the hydro-ethanolic leaf extract on the *Fusarium oxysporum* strain were the lowest. The results are 67.66 ppm (IC50) and 18516.69 ppm (IC90). For the *Pestalotia* sp strain, the hydro-ethanolic seed extract recorded the highest 90% inhibitory concentration (IC90) (Table 2).

**Table 2: Inhibitory concentrations at 50% and 90% of mycelial growth**

| Extracts | *Fusarium oxysporum* | | *Pestalotia* sp | |
|---|---|---|---|---|
| | $IC_{50}$(ppm) | $IC_{90}$(ppm) | $IC_{50}$(ppm) | $IC_{90}$(ppm) |
| Aqueous leaf extract | 13040,70 | 33514,60 | 6965,58 | 62282,78 |
| Aqueous seed extract | 15449,19 | 78865,82 | 28015,62 | 26878,90 |
| Aqueous bark extract | 13497,46 | 61489,79 | 5096,74 | 22657,54 |
| Hydro-ethanolic leaf extract | 67,66 | 18516,69 | 5362,24 | 24647,96 |
| Hydro-ethanolic seed extract | 19903,47 | 22157,04 | 45490,93 | 87681,58 |
| Hydro-ethanolic extract of bark | 17724,56 | 23792,262 | 4208,17 | 21377,48 |

## Discussion

The two types of extracts (aqueous and hydro-ethanolic) had different yields. The yield of aqueous extracts was low compared to the yield of hydro-ethanolic extracts. This difference in yields is due to the solvent. Extraction involves mass transfer of solutes to the solvent. The yield of hydro-ethanolic extraction is higher because the solvent extracts both water-soluble and alcohol-soluble molecules, whereas in the case of aqueous extraction the solvent extracts only water-soluble molecules, as described by Anonymous (2019). In terms of antifungal activity, the best inhibition rates were obtained with hydro-ethanolic extracts of leaves, seeds and bark, which inhibited 91%, 72% and 75% respectively at a concentration of 24mg/ml. The inhibition of the pathogen by the extracts is thought to be due to the fact that the extracts possess natural organic compounds with antimicrobial activities recognized as reported in other plants. These results are in line with those of Saraka *et al.* (2019), who showed that *Mallotusoppositifolius* leaf extract had antifungal activity on *Fusarium* sp. Similar studies by Tiendrebeogo (2011) showed the efficacy of extracts of *Eclipta alba*, *Cymbopogon citratus*, *Agave sisalana* and *Lippia multiflora* against seminicular fungi of rice. At concentrations C3 and C4, the aqueous seed extract stimulated mycelial growth of *Pestalotia* sp. The stimulation of mycelial growth could be explained by the fact that extracts often act as sources of nutrients for pathogens, as already reported by Tiendrebeogo *et al*. (2011). Indeed, the latter showed that *Cymbopogon citratus* extracts stimulate mycelial growth in *Bipolaris oryzae*. The stimulation of mycelial growth could also be due to the ability of these fungal species to adapt to the extract (Jarchelou *et al*., 2013; Lecomte, 2016). Determination of inhibitory concentrations at 50% and 90% verified the sensitivity of fungal strains to the extracts. The difference in concentrations can be explained by the fact that strains can be sensitive or resistant to the same fungicide family. The level of resistance varies according to the active ingredients within that family (N'guessan *et al*., 2016).

## Conclusion

This study showed that hydroethanolic extracts of *Parkia biglobosa* seeds, leaves and bark had better antifungal activity on *Fusarium oxysporum* than on *Pestalotia* sp. Aqueous extracts were less effective on *Pestalotia* sp. than on *Fusarium oxysporum*.

## Conflict of interest statement

Authors declare that they have no conflict of interest.

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