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# Antioxidant and antibacterial activity of bark extracts of the wild elephant tree from the Northern Region of Madagascar: *Sclerocarya birrea*

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**Abstract**
According to observation and survey of populations, the poultice of the bark of *Sclerocarya birrea* is used in traditional use as an antivenom and the decoction of the leaves against diarrhea. Several extracts were obtained after maceration by increasing polarity of leaf and bark powders in n-hexane, ethyl acetate, dichloromethane and 80° ethanol. Phytochemical screening of the obtained extracts shows that the leaves contain alkaloids, flavonoids, coumarins and tannins. There are no alkaloids in the bark.
The antibiogram test on eight bacterial strains of these extracts revealed that only *Streptococus. pneumoniae* is sensitive to two bark extracts containing ethyl acetate and hydroethanolic. It has also been proven that these two extracts have a DPPH° radical reducing power whose $IC_{50}$ is equal to (0.585±0.004) mg/L and (1.733±0.004) mg/L respectively.

**Keywords:** Antibiogram, antioxidant, phytochemical screening, bark, *Sclerocarya birrea*

## Introduction
Man has always found in nature, to clothe himself, to feed himself and to take care of himself. Currently, three quarters of the world's population treat themselves almost exclusively with natural substances. Over the centuries, different peoples have acquired invaluable knowledge about the medicinal use of plant, animal and mineral substances. Nearly 70% of current drugs are derived from natural substances, the lead drugs having been discovered for the majority from plants or microorganisms. The active molecules are either natural, modified by semi-synthesis or completely synthesized [1]. Plants are biological materials that contain a wide variety of chemical molecules and biological activity. Currently, they constitute an important source of bioactive molecules. To valorize these molecules, first, the bioactive compounds must be separated from their original plant matrices. Often, obtaining these molecules requires many long and expensive steps, such as phytochemical screening, extraction, isolation and spectral analysis.
Nowadays, modern pharmaceutical industries rely largely on the diversity of plant secondary metabolites to find new molecules with novel biological properties. This source seems inexhaustible since only a small part of the 400,000 known plant species has been the subject of an in-depth phytochemical study, and a species can contain several different constituents. [2]
The great biodiversity of Madagascar offers a vast field of exploitation, both food, cosmetic and therapeutic. Thus, plants discover an important source for the search for new products active against many diseases [3]. Elephant tree or *Sclerocarya birrea* is one of the plants introduced to Madagascar, very interesting in the food, cosmetic and therapeutic fields. [4] Its fruits have multiple uses as they are very rich in vitamin C, around 8 times the amount found in an orange. Kernels of its nut contain lipids and proteins. The virtues of the oils obtained from its almonds are very useful in cosmetics, edibles and pharmaceuticals [5].
However, in Madagascar, especially in the DIANA region, this plant remains a wild wood. It is exploited for the manufacture of charcoal. And its fruits fell to the ground, then they are eaten by livestock like cattle, dogs. Only the leaves and bark of this tree are used in traditional use Thus, we are convinced by its traditional use against diarrhea. This is why we chose to study the antioxidant and antibacterial activity of bark extracts from the wild elephant tree from Diego Suarez and Ambilobe.

## Material and Methods

### Phytochemical screening and extraction

The leaves and bark of *Sclerocarya birrea* in Figure 1 were collected on August 23, 2020 at the French mountain ring road of Diego-Suarez. During the fruiting season, we collected the fruits of the wild marula from March 29, 2021 to the ring road of the French mountains of Diego-Suarez. They were washed in tap water in a basin and dried without mold in the open air and away from light then crushed using an electric grinder. In addition, during drying, we took the masses of the sample, the temperature and the humidity of the medium every two days using a DIGITAL brand thermometer.

**Fig 1:** Marula leaves and barks

500 grams of powders (leaves and bark) of *Sclerocarya birrea* were macerated for 48 hours with stirring, successively in hexane, ethyl acetate (AcOEt), dichloromethane (DCM) and finally hydroethanolic 80° (EtOH). And all the macerates obtained were evaporated in an oven in order to calculate the yield of each extract. The order of increasing polarity of the solvents is chosen by comparison of two parameters including the dipole moment μ and the dielectric constant ε as shown in Table 1 below. The second parameter prevails in the case of ambiguity between DCM and AcOEt with μ equal to 1.60 and 1.88 respectively. The protocol for preparing crude extracts from leaf and bark powders of the plant studied is summarized in the diagram of the maceration protocol in Figure 2.

**Table 1:** Polarity of solvents

| Solvents | Dielectric constant ε | dipole moment μ |
|---|---|---|
| n-hexane | 1.88 | 1.08 |
| Ethyl acetate | 6.02 | 1.78 |
| dichloromethane | 8.93 | 1.60 |
| Ethanol | 24.5 | 1.69 |
| Water | 78.4 | 1.85 |

(*Source:* https://blog_fr.interchim.com/solvant-polaire-vs-apolaire-comprendre-les-differences-et-les-caracteristiques/, 05/06/2024)

And the yield of each extracted extract obtained is calculated by the relation:

$$r = \frac{m_e}{m_p} \times 100$$

Where $m_e$: mass of each extract obtained
$m_p$: mass of plant powder

### Phytochemical screening

This method allows us to determine the main chemical families existing in the extracts of a plant. And the detection of natural substances contained in the plant material was carried out by the different extracts obtained [6].

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This method allows us to determine the main chemical families existing in the extracts of a plant. And the detection of natural substances contained in the plant material was carried out by the different extracts obtained [6].

### Alkaloids

0.1 g of each extract (except hexanic extract) is detected by using Mayer's reagent as developer. [7] This test results in a precipitation reaction.
The three extracts (DCM, AcOEt and EtOH extract) are divided into three tubes for each extract, including:
Tube 1 serving as the control. 0.1 g of extract to acidify with 5 mL of 2N HCl. Tube 2: by adding 0.5 mL of Mayer's reagent.
Tube 3: by adding 0.5 mL of Wagner reagent 2.

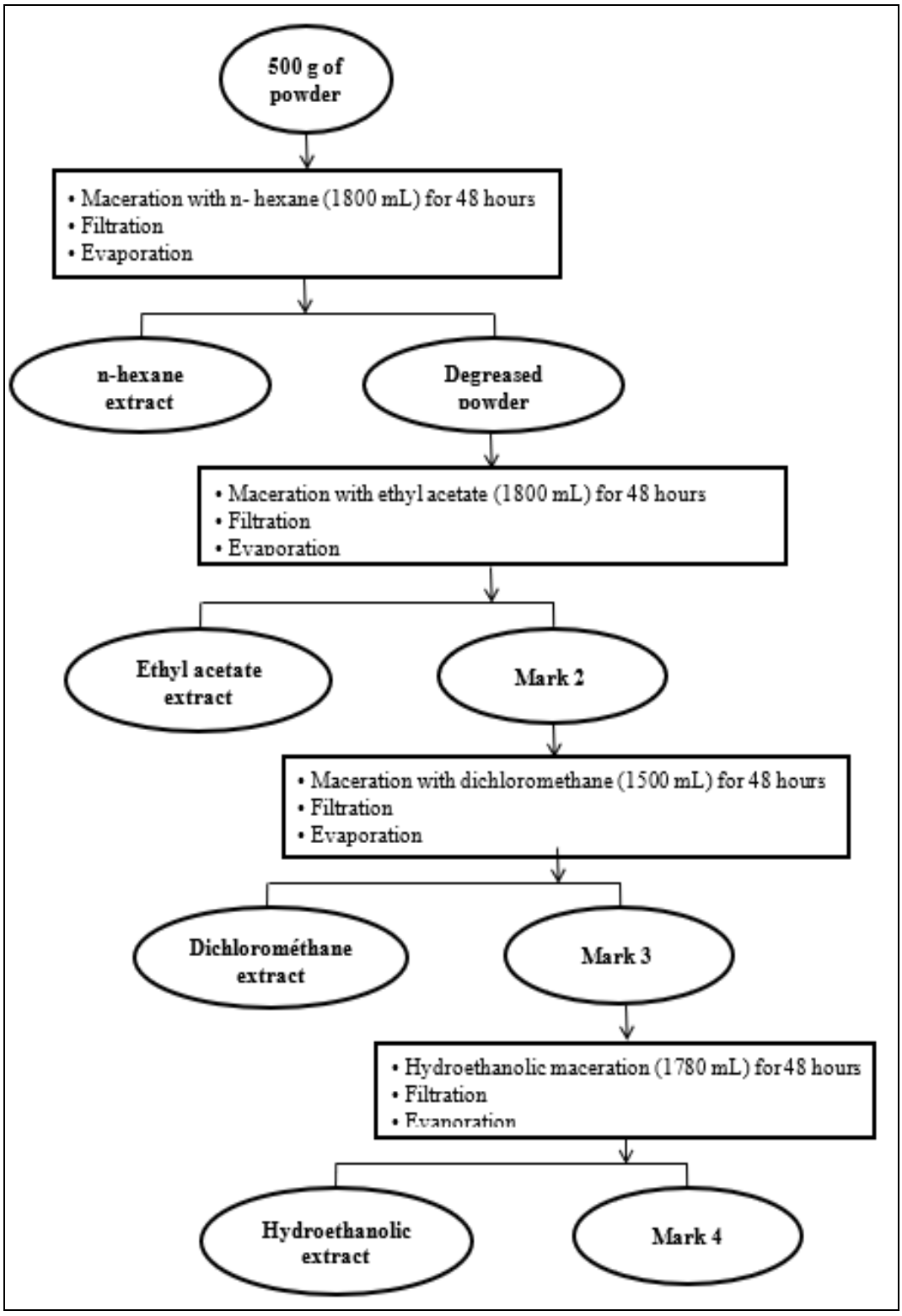


**Fig 2:** Maceration through increasing polarity

**Steroids and triterpenes**

In a solution of 0.1 g of extract, 0.5 mL of sulfuric acid was added for the Salkowski test. 1 mL of acetic anhydride and 0.5 mL of sulfuric acid were poured onto the inner wall by tilting the tube at 45°C for the Liebermann-Burchard test. [8]

**Tannins and polyphenols**

0.1 g of extract (EtOH/Water, DCM) was dissolved in 25 mL of hot $H_2O$ at 40°C for 2 min. then 1 mL of the 10% NaCl solution was added. After filtration, the solution obtained was distributed into four test tubes, the tube 1 serving as the control.

Tube no. 2: add 1mL of 1% gelatin;

Tube no. 3: add 1mL of salted gelatin (1% gelatin + 10% NaCl);

Tube no. 4: add 1mL of $FeCl_3$. [9]

**Flavonoids and Leucoantocyanins**

0.1 g of extract (hydroethanolic and dichloromethane) was solubilized and then distributed into 3 tubes, tube no. 1 serving as the control.

Tube no. 2: add 0.5 mL of concentrated $H_2SO_4$ and a spatula of Zinc.

Tube no. 3: add 0.5 mL of $H_2SO_4$ then heat to 70°C in a water bath for thirty minutes (30min) [10].

**Coumarins**
In a test tube containing 2 mL of extract (the 4 extracts), 0.5 mL of 10% NaOH was added, the mixture was then observed under UV at 366 nm. Intense fluorescence indicates the presence of coumarins. After heating in a bain-marie for 5 minutes; By adding 4 mL of distilled water, the solution becomes transparent compared to the control, and more than 3 drops of concentrated HCl causes it to lose its yellow color to make it cloudy or form a precipitate [11].

**Antimicrobial activity tests**
The antimicrobial study allows us to know the sensitivity of strains to a product and gives an exact idea of their sensitivity. That is to say, this part aims to determine the antimicrobial activities of the extracts to be tested by the sensitivity of the germs.

**Principle of diffusion on agar**
This method allows the extract to be studied to be brought into contact with the bacteria seeded in a layer. The extracts are diffused on an antibiogram disk of standard diameter (6 mm) then placed on the medium in a radial manner, its concentration decreasing as it moves away from its source, a concentration gradient is thus formed [11]. In the case where the strain tested is sensitive to the extract, a circular zone of growth inhibition called "halo of inhibition" appears around the discs. In this area, bacteria do not grow but it is not yet possible to know whether this is a bactericidal or bacteriostatic activity. The diameter of this halo directly reflects the degree of sensitivity of the strain to the extract: the more sensitive it is, the larger the diameter [12].

**Characteristics of the strains to be studied**
The strains used during the antimicrobial test of plant extracts come from the strain collection of the Environmental Microbiology Laboratory (LME)/CNRE. These germs as presented in Table 2 include 4 Gram-negative bacteria, and 3 Gram-positive bacteria and a yeast [13].

**Table 2:** Characteristics of the strains to be studied

| Germes | | Gram stain |
|---|---|---|
| Bacteria | *Bacillus cereus* ATCC 13061 | + |
| | *Staphylococcus aureus* ATCC 11632 | + |
| | *Streptococcus pneumoniae* ATCC 6301 | + |
| | *Escherichia coli* ATTC 25922 | - |
| | *Salmonella enteridis* ATTC 13076 | - |
| | *Klebsiella oxytoca* ATCC 700323 | - |
| | *Pseudomonas A* | - |
| Yeast | *Candida albicans* | |

**Inoculum preparation**
From the young colonies obtained, one or two well-isolated colonies of bacteria or yeast were suspended in 9 mL of sterile distilled water. The cell density of the inoculum was adjusted by dilution with physiological water by comparing with the 0.5 Mc Farland solution (i.e. an optical density of 0.2 at 650 nm) so as to obtain a final concentration of 106 cfu/ml after incorporation into Mueller Hinton medium [14].

**Seeding**
The flooding or sheet culture technique is applied for seeding. The entire surface of the solid Muëller Hinton medium is submerged by 9 mL of the inoculum of 106 CFU/ml of germ. The culture is incubated in an incubator at 37°C for 15 min so that the germs can adhere to the medium. Then, the excess liquid is sucked up using a sterile pipette and the medium is dried for 15 minutes in an oven at 37°C [11].

**Deposit of discs**
Sterile 6mm diameter discs are impregnated with 20 µl of extract using a sterile cone micropipette. The concentration is 20 mg/ml diluted in methanol. Then the disks are dried for 15 minutes in an oven at 37°C. After drying, the disks are placed on the culture using sterile forceps. The plates are incubated at 37°C for 24 hours. Results are read by measuring the diameter of the inhibition halo found around a disk that contains an antimicrobial as shown in Figure 3. The Table 3 below shows the references of halos which are sensitive on germs and which are necessary for the interpretation of microbiological test activities [15].

**Table 3:** Standards used for disc method interpretation [16]

| Inhibition halo diameters | Result | Germ sensitivity |
|---|---|---|
| Ø < 8 mm | - | Insensitive or resistant |
| 8 mm< Ø < 14 mm | + | Sensitive |
| 15mm < Ø < 19 mm | ++ | Very sensitive |
| Ø > 20mm | +++ | Extremely sensitive |

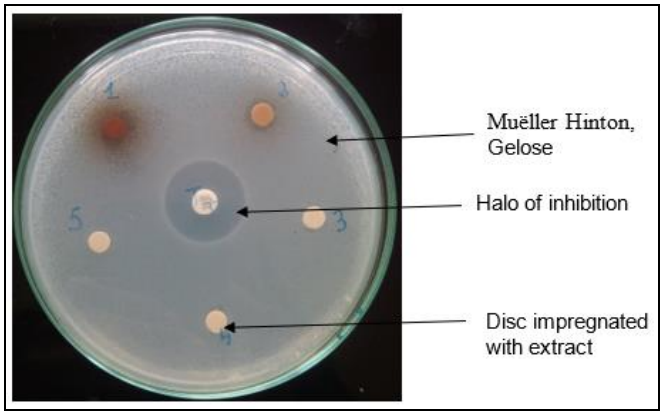


**Fig 3**: Antibiogram

**Dose effect test by spectrophotometry**
The measurement of absorbance under UV-Visible was undertaken by the Genova Bio Jenway Spectrophotometer in the presence of a DPPH° solution at 6.10-5 mol/L. This technique was applied as presented on the Chimactiv website, given the lack of equipment due to the lack of an ultrasonic bath. The solutions to be tested were prepared in pure ethanol or AcOEt which do not absorb at 515 nm depending on their polarity.
After dilution different concentrations were tested: 3; 2; 1.5; 1 and 0.5 mg.L-1. 77 µL of ethanol or AcOEt was added to 3 mL of the DPPH° solution at 6.10-5 mol/L for the control tank and 77 µL of test solution for the different concentrations in the other tanks. Each mixture was stirred by inversion, the measurement was made at 515 nm by the kinetic control for two times 9000 seconds, the measurement interval of which was 60 seconds [17-23].

**Results**
At the time of collecting this plant, GPS coordinates were taken for the exact location of the site: Longitude: South, 12°, 18 minutes, 37.7 seconds Latitude: East-West 49°, 20 minutes, 49.5 seconds With altitude of 136 meters.

**Drying**
The drying of the leaves and bark of *Sclerocarya birrea* lasted 15 and 25 days respectively. Tables 4 and 5 (Figures 4 and 5)

show the variation in temperature, humidity and mass of two samples depending on the day. Drying was stopped when the mass no longer varied.

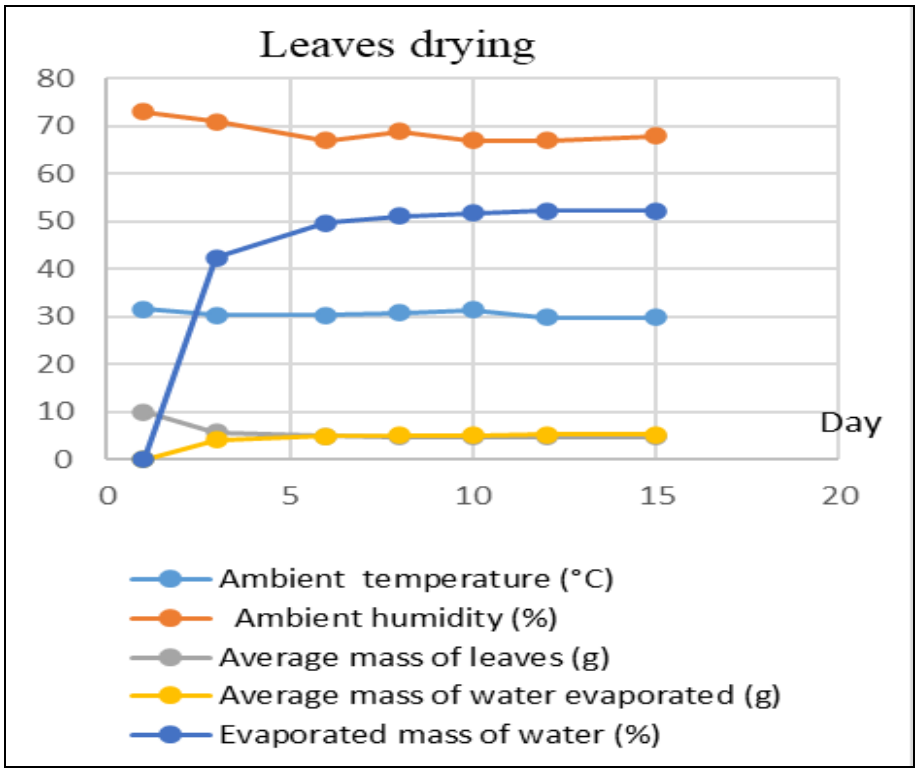


**Fig 4:** Monitoring of drying of *Sclerocarya birrea* leaves

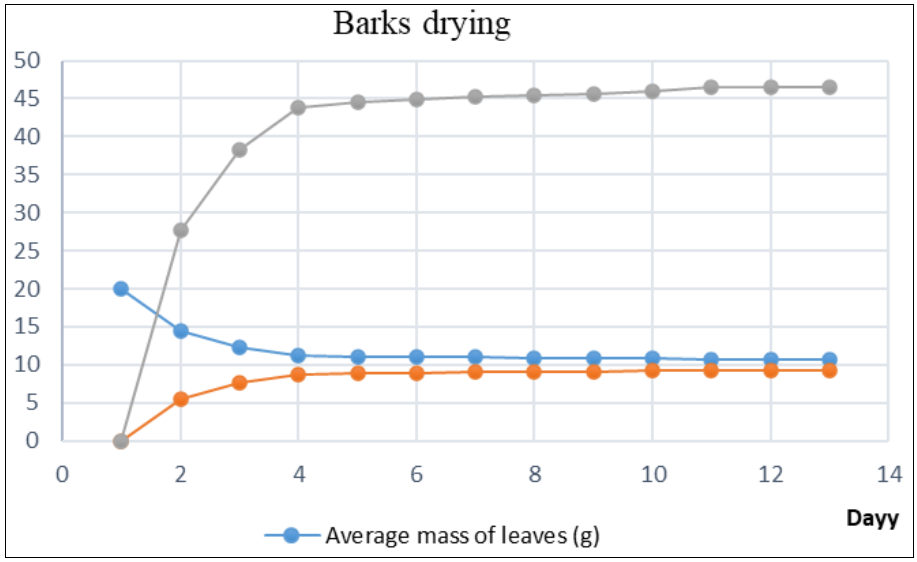


**Fig 5:** Monitoring of drying of *Sclerocarya birrea* barks

**Extraction**

The extraction results from 500 g of plant powders (leaves) of this plant are recorded in Table 6 with their yields. Maceration was carried out successively by increasing polarity of organic solvent. Indeed, the yield of the hydroethanolic extract is the highest compared to that of the ethyl acetate and hexane extracts.

**Table 4:** Monitoring of drying of *Sclerocarya birrea* leaves

| Day | Ambient temperature (°C) | Ambient humidity (%) | Average mass of leaves (g) | Average mass of water evaporated (g) | Evaporated mass of water (%) |
|---|---|---|---|---|---|
| 1 | 31.6 | 73 | 10 | 0 | 0 |
| 3 | 30.4 | 71 | 5.765 | 4.235 | 42.35 |
| 6 | 30.4 | 67 | 5.025 | 4.975 | 49.75 |
| 8 | 30.8 | 69 | 4.89 | 5.11 | 51.1 |
| 10 | 31.4 | 67 | 4.82 | 5.18 | 51.8 |
| 12 | 29.9 | 67 | 4.78 | 5.22 | 52.2 |
| 15 | 29.9 | 68 | 4.775 | 5.225 | 52.25 |

**Table 5**: Monitoring of drying of *Sclerocarya birrea* barks

| Day | Ambient temperature (°C) | Ambient humidity (%) | Average mass of leaves (g) | Average mass of water evaporated (g) | Evaporated mass of water (%) |
|---|---|---|---|---|---|
| 1 | 31.6 | 73 | 20 | 0 | 0 |
| 3 | 30.4 | 71 | 14.45 | 5.55 | 27.75 |
| 5 | 30.4 | 67 | 12.34 | 7.66 | 38.3 |
| 7 | 30.8 | 69 | 11.225 | 8.775 | 43.875 |
| 9 | 31.4 | 67 | 11.085 | 8.915 | 44.575 |
| 11 | 29.9 | 67 | 11.03 | 8.97 | 44.85 |
| 13 | 29.7 | 68 | 10.96 | 9.04 | 45.2 |
| 15 | 29.9 | 66 | 10.93 | 9.07 | 45.35 |
| 17 | 29.9 | 66 | 10.865 | 9.135 | 45.675 |
| 19 | 31 | 72 | 10.795 | 9.205 | 46.025 |
| 21 | 29.3 | 73 | 10.715 | 9.285 | 46.425 |
| 23 | 29.9 | 66 | 10.695 | 9.305 | 46.525 |
| 25 | 29.7 | 66 | 10.695 | 9.305 | 46.525 |

**Table 6:** Mass of extracts obtained from leaves and barks

| Extract | Leaves | | Barks | |
|---|---|---|---|---|
| | Mass (g) | Yield (%) | Mass (g) | Yield (%) |
| n-hexane extract: $E_{Hex}$ | 10±0.1 | 2±0.020 | 15±0.1 | 3±0.021 |
| Ethyl acetate extract: $E_{AcOEt}$ | 10±0.1 | 2±0.020 | 16±0.1 | 3,2±0.021 |
| Dichloromethane extract: **$E_{DCM}$** | 4±0.1 | 0.8± 0.025 | 2±0.1 | 0.4±0.020 |
| Hydroethanolic extract: **$E_{EtOH}$** | 36±0.1 | 7.2± 0.021 | 5±0.1 | 1±0.020 |

And the yield from the dichloromethane extract is the lowest; hence this extract contains a small quantity of low polar products. As for bark maceration, the yield of ethyl acetate extract is the highest compared to that of hexane extracts and hydroethanolic extract. And the yield of the dichloromethane extract is the lowest, hence this extract contains a small quantity of poorly polar products.

**Phytochemical screening**

The phytochemical screening of this plant was carried out by detection on a TLC chromatogram and by the coloring reaction as well as by the precipitation reaction. Alkaloids

The three extracts (with ethyl acetate, dichloromethane and hydroethanolic) were detected by the Mayer and Wagner reagent therefore both by the colouring and precipitation reaction.

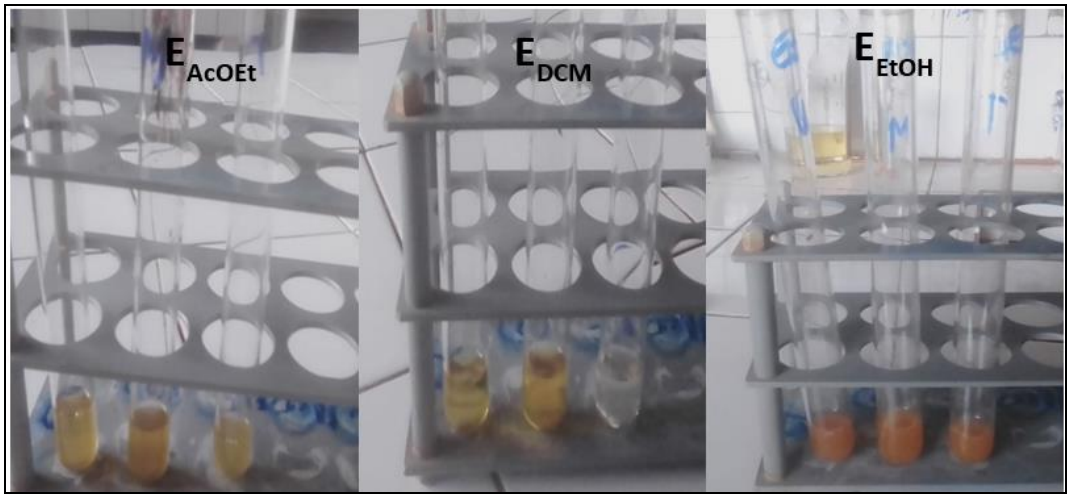


**Fig 6:** Alkaloid test on $E_{AcOEt}$, $E_{DCM}$ and $E_{EtOH}$ leaf extracts

After observing each test tube, the result was illustrated in Table 7 of the alkaloid characterization test on the three extracts.

The detection of alkaloids on the three leaf extracts (with ethyl acetate, with dichloromethane and 80% hydroethanolic) is done by the coloring and precipitation reaction as shown in Figure 6. Precipitation is a reverse phenomenon of the solubility of the reaction product, that is to say, the more the quantity of the product formed increases, the more precipitate there is.

Table 7 shows that both hydroethanolic and ethyl acetate extracts present precipitates. As for the dichloromethane extract, the solution is cloudy because there is a very low presence of alkaloid. Indeed, the leaves of *Sclerocarya birrea* contain alkaloids.

**Table 7:** Leaf phytochemical screening

| Chemical family | Tests | Reagents | Expected results | $E_{Hex}$ | $E_{AcOEt}$ | $E_{DCM}$ | $E_{EtOH}$ |
|---|---|---|---|---|---|---|---|
| Alcaloids | Mayer | $HgCl_2$/IK | Creamy, white precipitate | | ++ | + | +++ |
| | Wagner | $I_2$/IK | Orange-red precipitate | | ++ | + | ++ |
| Sterols- Triterpenes | Liebermann Burschard | 1 mL of acetic anhydride + 1 mL of concentrated $H_2SO_4$ | Red violet to pink: triterpenes<br>Blue green: steroids<br>Red: unsaturated sterols | +++ | ++ | ++ | - |
| Taninns and Polyphenols | | 1 mL of 1% gelatin | White precipitate | + | + | - | +++ |
| | | 1 mL of salted gelatin | White precipitate | +++ | ++ | - | +++ |
| | | 1 mL of 10% $FeCl_3$ | Greenish[1] or blackish-blue[2] coloring | ++ 1 | +++ | ++ 2 | +++ |
| Flavonoids and leucoanthocyanins | Wilstater | 1 g of Zn + 0,5 mL of concentrated HCl | Turning red: flavones<br>Turning purple: flavononesChange to purplish red: flavanones and flavanols | - | - | ++ | ++ |
| | Bâte-Smith | 0,5 mL of concentrated HCl | Red or reddish coloring | - | - | + | + |
| Coumarins | Observation under UV | 0.5 mL of 10% NaOH | Intense fluorescence | + | + | + | - |

(-): Absence; (+): troubled turn; (++): presence of precipitate; (+++): presence of precipitate with deposit

**Table 8 :** Bark phytochemical screening

| Chemical family | Tests | Reagents | Expected results | $E_{Hex}$ | $E_{AcOEt}$ | $E_{DCM}$ | $E_{EtOH}$ |
|---|---|---|---|---|---|---|---|
| Alcaloids | Mayer | $HgCl_2$/IK | Creamy, white precipitate | | - | - | - |
| | Wagner | $I_2$/IK | Orange-red precipitate | | - | - | - |
| Taninns and Polyphenols | | 1 mL of 1% gelatin | White precipitate | + | | +++ | +++ |
| | | 1 mL of salted gelatin | White precipitate | ++ | | +++ | +++ |
| | | 1 mL of 10% $FeCl_3$ | Greenish[1] or blackish-blue[2] coloring | +++ 1 | | +++ 2 | +++ |
| Flavonoids and leucoanthocyanins | Wilstater | 1 g of Zn + 0,5 mL of concentrated HCl | Turning red: flavones<br>Turning purple: flavononesChange to purplish red: flavanones and flavanols | | | | ++ |
| | Bâte-Smith | 0,5 mL of concentrated HCl | Red or reddish coloring | | | | ++ |

(-): Absence; (+): troubled turn; (++): presence of precipitate; (+++): presence of precipitate with deposit

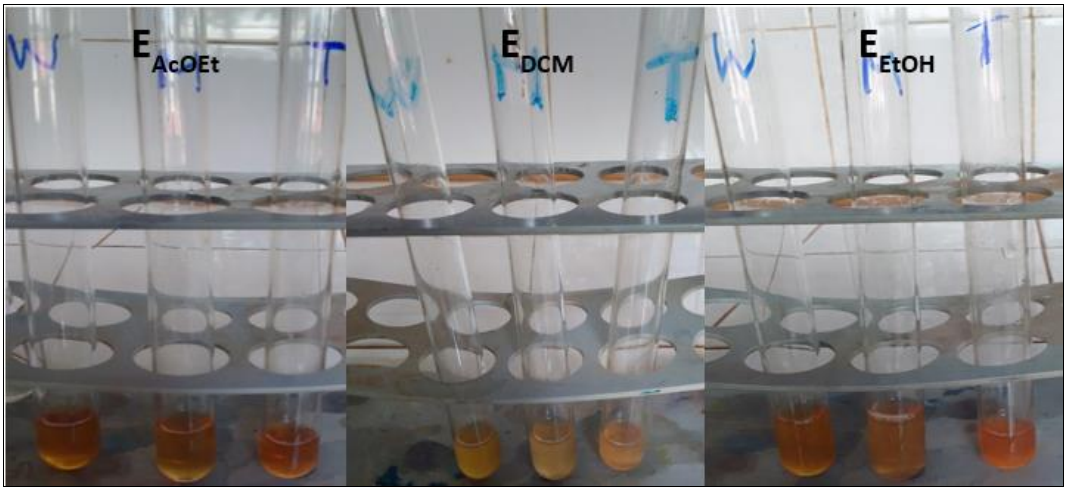


**Fig 7:** Alkaloid test on EAcOEt, EDCM and EEtOH bark extracts

There was no change observed in the different tubes after pouring the reagents as shown in Figure 7. Indeed, there are no alkaloids in the bark of the elephant tree (Table 8).

**Sterols and triterpenes:** The characterization of sterols and triterpenes from *Sclerocarya birrea* leaves was done by the chromatogram using the binary eluent system n-hexane/AcOEt (50:10). The results are shown in Figures 8 below.

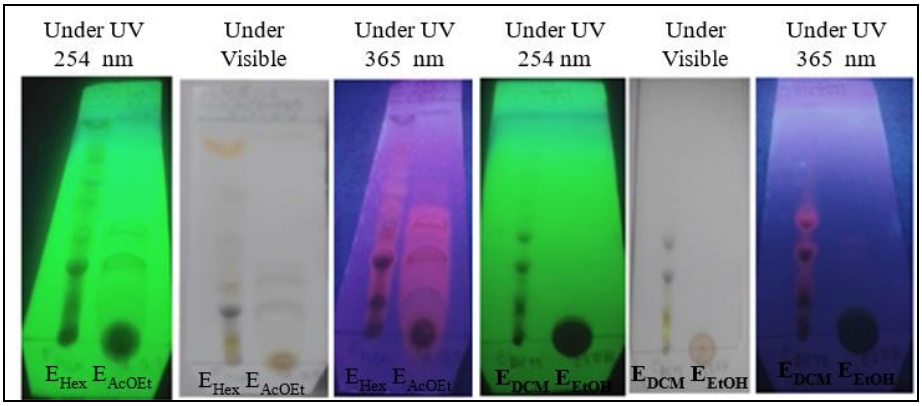


**Fig 8:** Chromatogram for detection of sterols and triterpenes ($E_{Hex}$, $E_{AcOEt}$ on the left and $E_{DCM}$, $E_{EtOH}$ on the right)

In general, sterols fluoresce under UV 365 nm in blue, yellow and green and triterpenes in blue, yellow, green and violet. So the hexane and dichloromethane extracts contain sterols and triterpenes.

**Tannins and sterols**

In this work, the tannins were characterized by the color reactions and by the precipitation of the reaction. The results are presented in Figure 9 below.

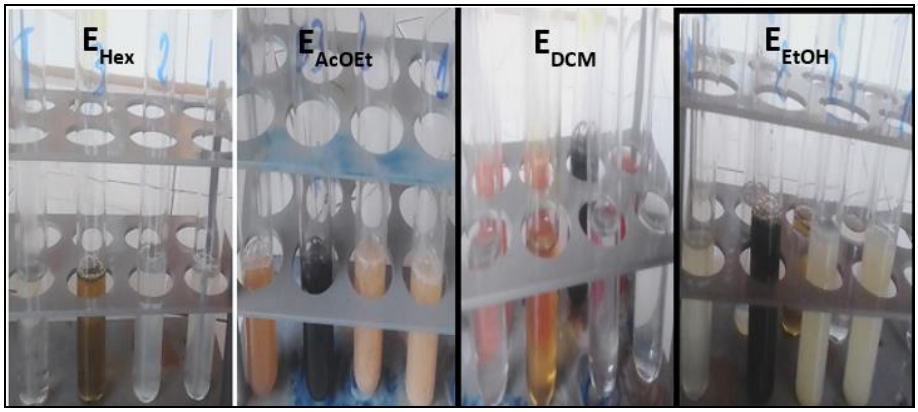


**Fig 9**: Characterization of leaf tannin extracts

The formation of the white precipitate on the three leaf extracts such as $E_{Hex}$, $E_{AcOEt}$ and $E_{EtOH}$ denotes the presence of tannins while the greenish coloring on $E_{Hex}$ and $E_{DCM}$ was reflected by the existence of gallical tannins. And the greenish black coloring of the $E_{Hex}$ tet and $E_{EtOH}$ extracts in the presence of $FeCl_3$ characterizes the catechetical tannins.

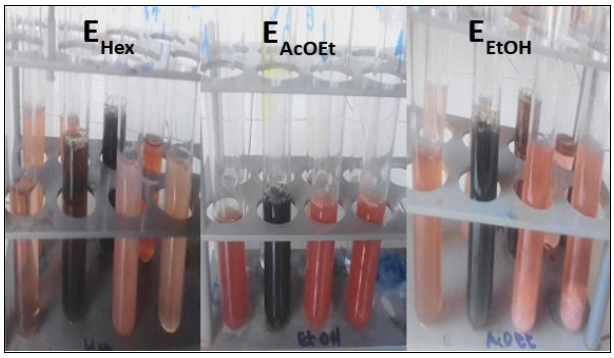


**Fig 10:** Characterisation of tannin on the barks

Likewise, the appearance of the white precipitate on these three bark extracts, namely such as $E_{Hex}$, $E_{AcOE}$ tet and $E_{EtOH}$, denotes the presence of tannins while the blackish bleu coloring on the last two was reflected by the existence of gallical annins.

And for the $E_{Hex}$ which was colored greenish, these are catechical tannins.

**Coumarins**

Coumarins were detected on a silica gel 60 F254 TLC plate developed by the n-Hex/AcOEt (50:10) binary eluent system and observed under UV at 366 nm. The chromatogram profile is given in the following Figure 11. Intense fluorescence indicates the presence of coumarins in the three extracts of the leaves: $E_{Hex}$, $E_{AcOEt}$ and $E_{DCM}$.

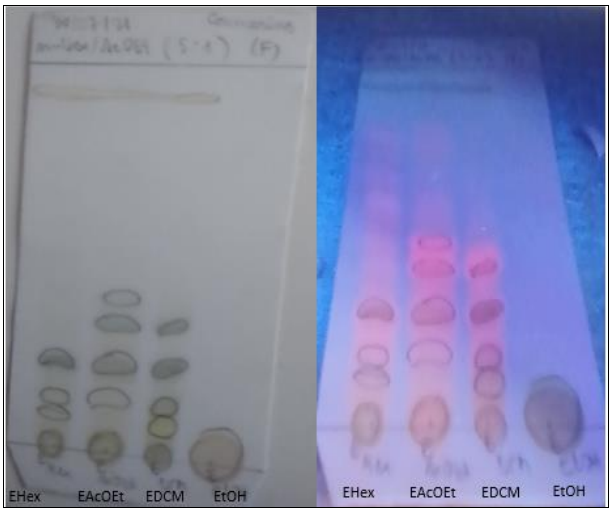


**Fig 11:** Coumarin detection chromatogram

**Flavonoids**

Flavonoids were characterized by the coloring reaction to

detect the compounds on the three extracts such as one bark extract ($E_{EtOH}$) and two leaf extracts ($E_{DCM}$ and $E_{EtOH}$), the characterization result of which was shown in the Tables 7 and 8. Thus, the presence of flavonoids in the three extracts was considered. The red coloring indicates the presence of flavonols in the three extracts. Particularly on the hydroalcollic extract of bark.

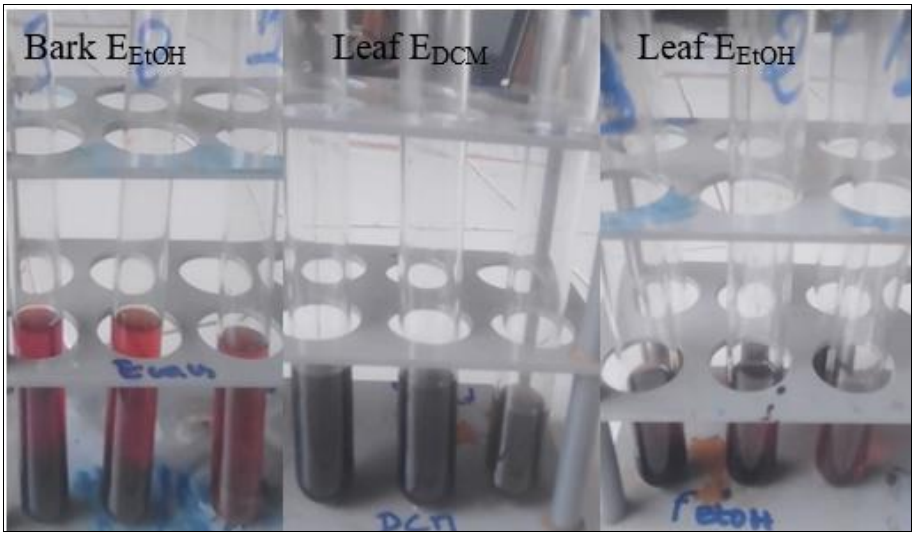


**Fig 12:** Flavonoid characterizations

**Microbiological test**

The *Streptococcus pneumoniae* ATCC 6301 strains are sensitive to $E_{AcOEt}$ and $E_{EtOH}$ extracts of the bark coded by 5 and 6.

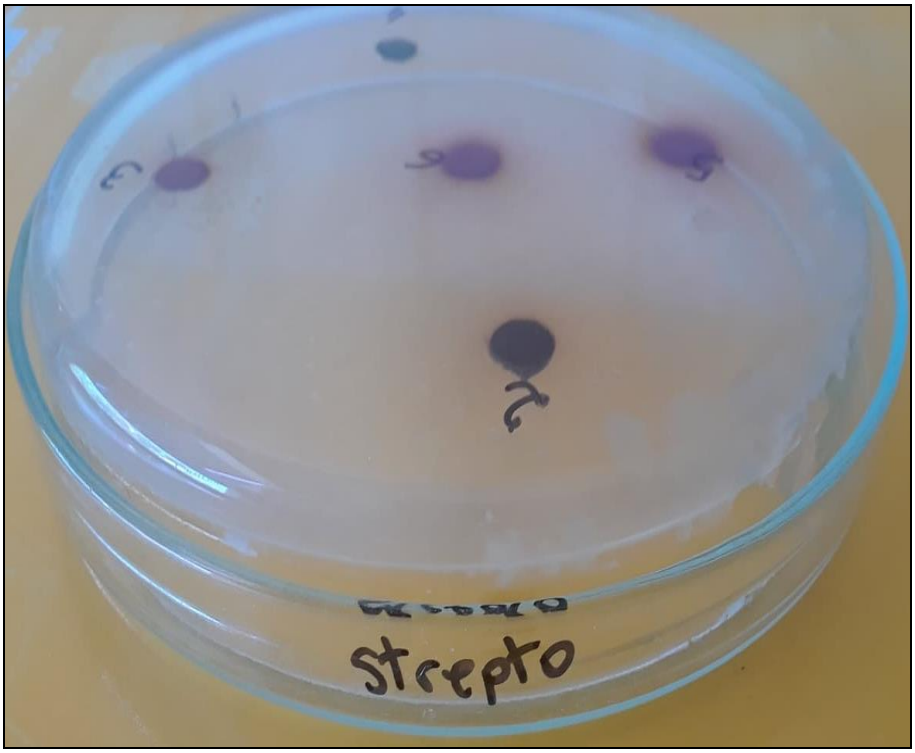


**Fig 13:** Antibiogram

The antimicrobial activity tests were carried out using the agar diffusion principle. The $E_{AcOEt}$, $E_{EtOH}$ extracts from the leaves and the $E_{AcOEt}$, $E_{EtOH}$, and $E_{DCM}$ extracts are impregnated with sterile disks using the different gram+ and gram- strains. The results of this study are shown in the following Table 9. The diameter of the inhibition halos of these extracts is greater than 8 mm, this means that these extracts have antimicrobial activity. Initially, this research project aims to detect the sensitivity of the *E. coli* microbial strain to extracts of this plant. As this strain is not sensitive, these results are promising for *S. pneumoniae* and will allow us to identify and isolate the active molecules in these extracts.

**Table 9:** Halo diameter in cm after antimicrobial testing of extracts

| Extract | *B. cereus* | *S. aureus* | *S. pneumoniae* | *P. aeruginosa* | *E. cloacae* | *S. enteridis* | *E. coli* | *C. albicans* |
|---|---|---|---|---|---|---|---|---|
| 2 | 6 | 6 | 6 | 6 | 6 | 6.5 | 6 | 6 |
| 3 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| 4 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| 5 | 7 | 6 | 11 | 6 | 6 | 7 | 6 | 6 |
| 6 | 6 | 6 | 9 | 6 | 6 | 6 | 6 | 6 |
| Nalidixic acid | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| Metilmycin | 12 | 6 | 6 | 9 | 6 | 6 | 6 | 6 |
| Spectinomycin | 6 | 6 | 7 | 6 | 6 | 6 | 6 | 6 |

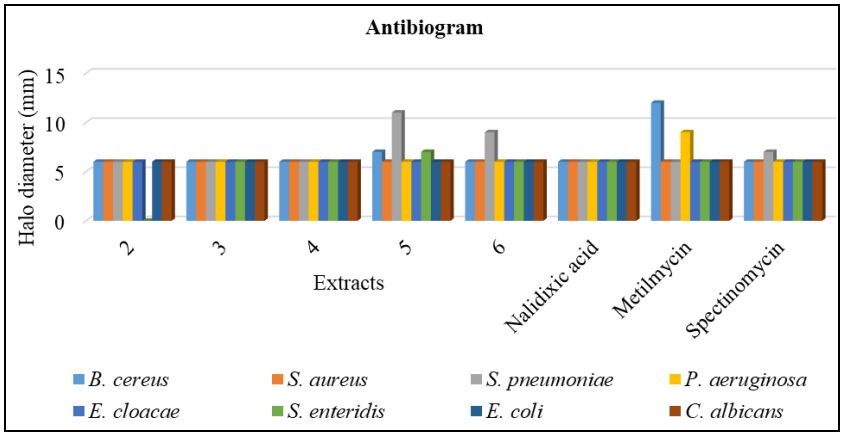


**Fig 14:** Antibacterial of leaf and bark extracts

**Antioxidant test**

The kinetics of the reduction of the DPPH radical by extracts at different concentrations evolves over time. The absorbance then decreases as a function of time and eventually stabilizes.

**Table 10:** Dose effect on $E_{AcOEt}$ and $E_{EtOH}$ bark extracts

| Mass concentration (mg/L) | Residual DPPH (%) to within 2% | |
|---|---|---|
| | $E_{AcOEt}$ | $E_{EtOH}$ |
| 3.0±0.1 | 16,00 | 53,60 |
| 2.0±0.1 | 10,67 | 44,60 |
| 1.5±0.1 | 15,47 | 26,52 |
| 1.0±0.1 | 6,93 | 25,18 |
| 0.5±0.1 | 43,73 | 85,61 |
| 0 | 100,00 | 100,00 |

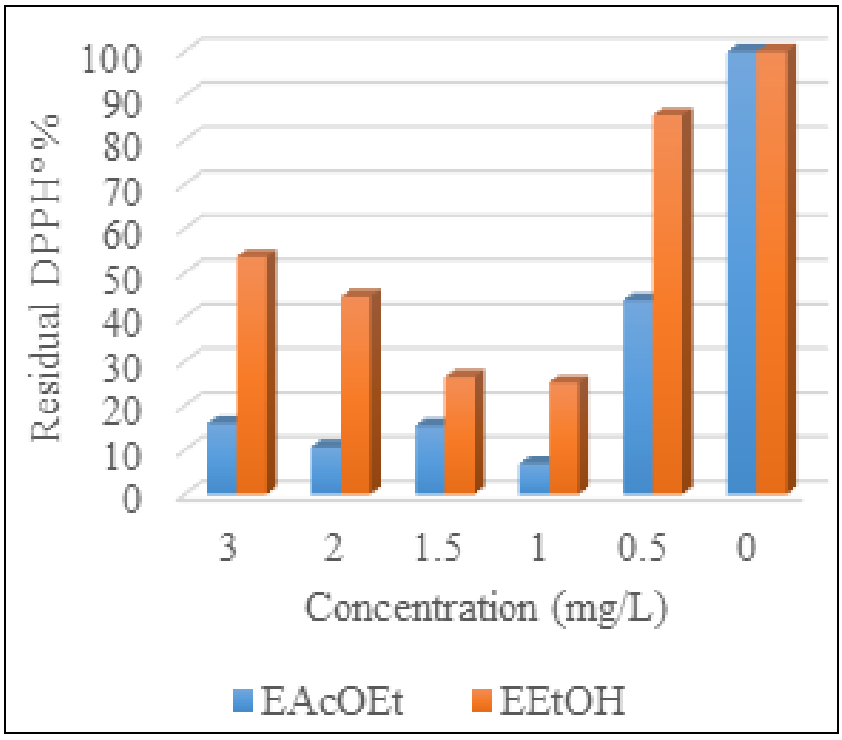


**Fig 15:** Variation of residual DPPH depending on mass concentration

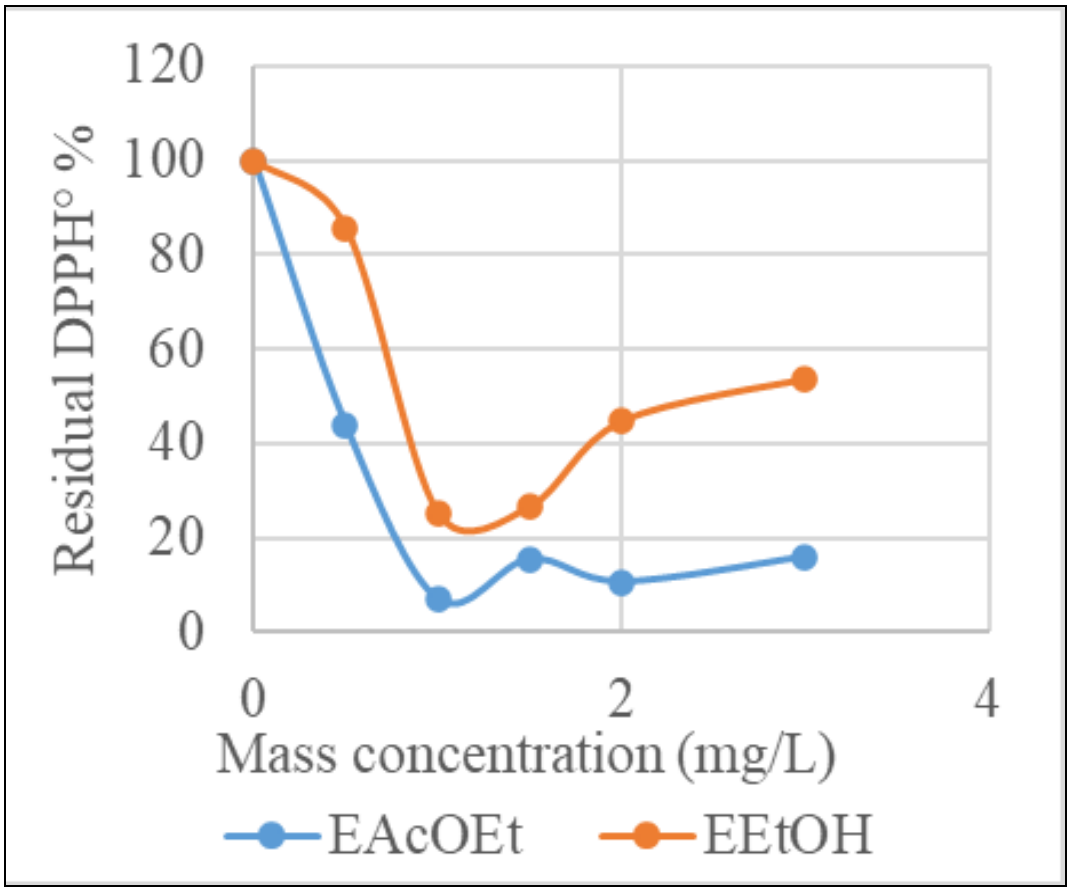


**Fig 16:** Dose effect curve on $E_{AcOEt}$ and $E_{EtOH}$ bark extracts

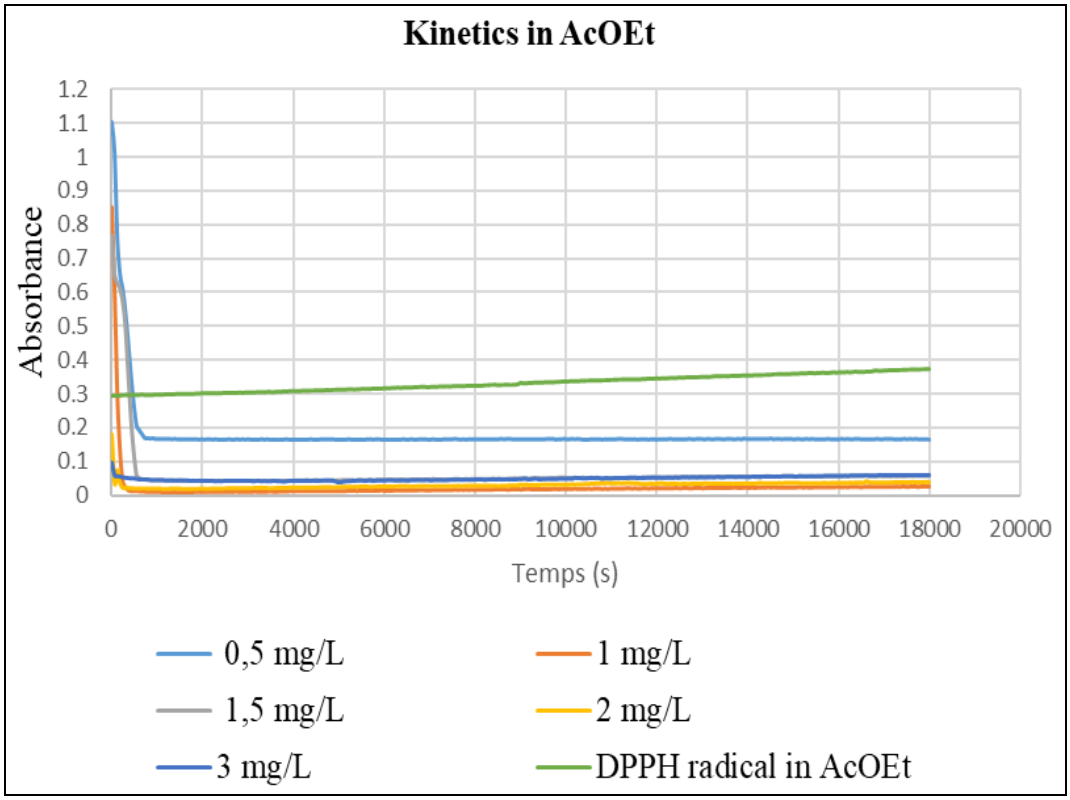


**Fig 17:** Kinetics of the reduction of the DPPH radical by the $E_{AcOEt}$ bark extract

After 18,000 seconds these values can be used to calculate the percentage of residual DPPH° radical (Figures 17 and 18). These values are recorded in Table 10. These extracts reduce the DPPH° radical, the correspondence between their dose and the effect provided is highlighted by the histogram in Figure 15 and the curves in Figure 16. By using the kalythos command in the R software, the inhibitory concentration IC50 are respectively (0.585±0.004) mg/L and (1.733±0.004) mg/L for the $E_{AcOEt}$ and $E_{EtOH}$ bark extracts.

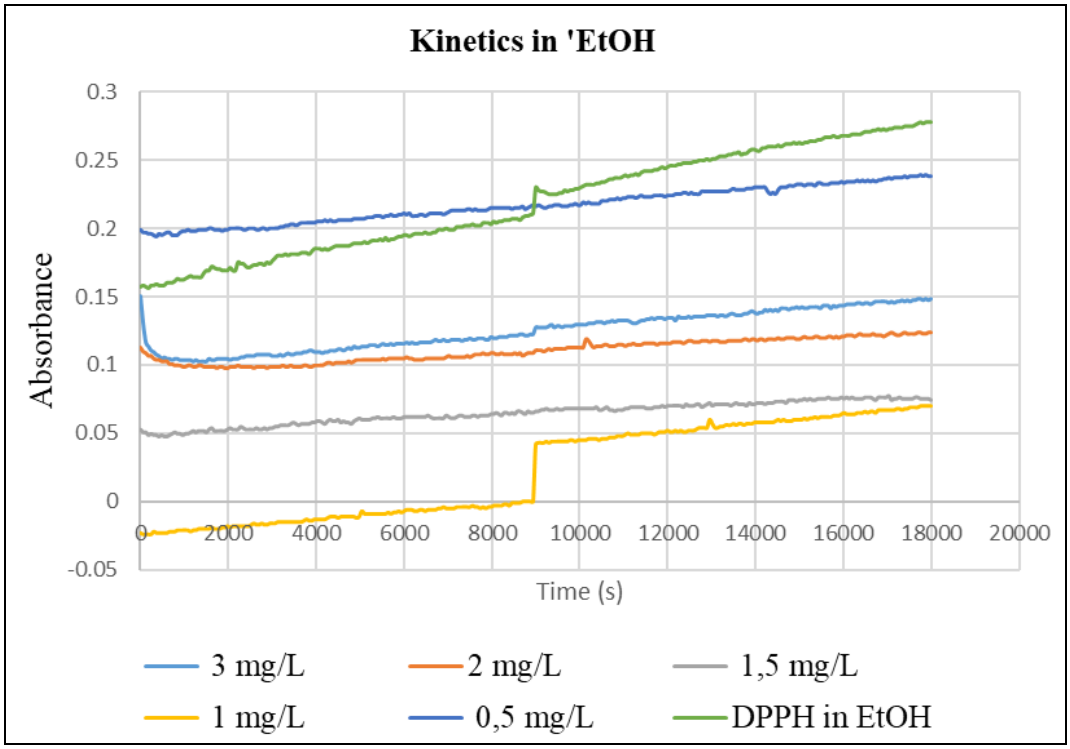


**Fig 18:** Kinetics of the reduction of the DPPH radical by the $E_{EtOHt}$ bark extract

## Discussion

As the leaf of *Sclerocarya birrea* is known for its traditional use to cure diarrhea, the studies were oriented to identify the chemical families present in it, initially with comparison of the chemical families which were found in the barks. During screening the precipitation reaction is considered. However, if the alkaloids were of tiny quantity where the concentration of the reaction product was lower than its solubility, there would be no precipitate but only the turn yellow. It has been found that there are no alkaloids in the bark unlike in the leaves. Secondly, the antibiogram on eight strains including seven bacteria and one yeast was able to inform us that only the two bark extracts $E_{AcOEt}$ and $E_{EtOH}$ have antibacterial activity against *Streptococcus pneumoniae* (Table 9 and Figure 14). The antioxidant test was conducted in the sense that the choice was based on antibacterial activity. On the power to reduce the DPPH° radical, the effect-dose curves in Figure 16 show a similar evolution between these two extracts. Although the absorbance value does not reach the prescribed value on the Chimactiv site, the percentage of residual DPPH° radical remains the same regardless of this value. The more their mass concentration increases, the more the percentage of residual DPPH° radical increases.

Consequently there is an antagonistic effect which increases with the dose of extracts. The IC50s are low at (0.585±0.004) mg/L for EAcOEt and (1.733±0.004) mg/L for EEtOH. These values suggest that there is no risk at large quantities. Furthermore, moderation in consumption would be more effective in reducing free radicals.

## Conclusion

At the end of this work, we can say that the bibliographic studies have provided us with several methods, such as antimicrobial biological tests and the antioxidant test. The phytochemical screening of leaf powder was carried out by two methods, on the one hand the extracts of this plant which were detected by a chromatogram revealed under UV or visible, and on the other hand, they were characterized by the coloring or precipitation of a specific reaction. During this screening, the presence of alkaloids,

flavonoids, coumarins and tannins on the leaves was noted. In the bark we find very abundant tannins. In addition, the detection of glycosides and quinones was not noted in these extracts of *Sclerocarya birrea*. The extraction of leaf powder (500.0±0.1g) was carried out by successive macerations with hexane, ethyl acetate, dichloromethane and 80% hydroethanolic. The extracts obtained were evaluated for antimicrobial activity using the different gram + and gram - strains. In short, the *Streptococcus pneumoniae* strain was sensitive to ethyl acetate and hydroethanolic extracts from the bark. These extracts also have DPPH° radical reducing power, they have antioxidant activity.

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