

# Evaluation of antibacterial activity and cytotoxicity of leaf extracts from the plant *Diospyros Mespiliformis*

## Abstract

*Diospyros mespiliformis* is a plant used in traditional medicine for the treatment of many illnesses, particularly wounds. In this context, ethanolic (E.F), hydroethanolic (H.F) and aqueous (A.F) extracts of the plant's leaves were evaluated for their antimicrobial properties. The study was carried out on four species of bacteria, two Gram-positive (*Staphylococcus aureus*, *Enterococcus faecalis*) and two Gram-negative (*Pseudomonas aeruginosa* and *Escherichia coli*). The results show that, with the exception of *Escherichia coli*, the other strains showed sensitivity to the extracts. In addition, with the exception of *Enterococcus faecalis*, the crude ethanolic and aqueous extracts were more active than the hydro-ethanolic extracts against the microbial strains. Inhibition diameters for the *Pseudomonas aeruginosa* strain were 08 mm, 00 mm and 08 mm for the ethanolic, hydro-ethanolic and aqueous extracts, respectively. For the *Enterococcus faecalis* strain, they were 08 mm, 10 mm and 09mm, respectively for the three extracts. For the *Staphylococcus aureus* strain, the characteristic inhibition diameters were 11 mm, 09 mm and 10 mm, respectively for the different extracts. The minimum inhibitory concentration (MIC) for *Pseudomonas Aeruginosa* was 9375µg/mL for both ethanolic and aqueous extracts. For the *Staphylococcus Aureus* strain, it was 586µg/mL for both ethanolic and aqueous extracts. For the *Enterococcus Faecalis* strain, it was 1171µg/mL for all three extracts. The safety of the compounds present in these extracts was studied through toxicity tests on different categories of hepatocytic liver cells, notably *Huh7*, *Hep3B* and *HepaRG*. Comparative analysis of the IC<sub>50</sub> of the extracts and controls, including the anti-cancer drug sorafenib and aflatoxin, revealed very low toxicity of the extracts towards the cells tested compared with the controls.

**Key words:** *Diospyros Mespiliformis*, phytochemical screening, antibacterial and cytotoxicity

## 1. Introduction

The discovery of antibiotics in the early 20th century revolutionized clinical therapy against bacteria [1]. In the wake of this success, their production expanded rapidly, reaching record tonnages. The Lederle scientists' report further boosted production. The Lederle scientists had

discovered accidentally that residues from antibiotic production could promote animal growth. This report promoted the use of antibiotics as dietary supplements and led to an increase in demand for these products [1, 2]. In the face of proven efficacy, a problem of supervision, of the use of treatments in therapy, and of the management of waste from the production industries, unexpectedly led to the advent of antibiotic resistance in bacteria [3-7]. This phenomenon will, inevitably, make the treatment of microbial infections more difficult, reduce the chances of controlling a declared infection and increase the risk of spreading related diseases, or even the number of deaths. So, antimicrobial resistance became a global problem. And to date, no known technique has been perfected to reverse the situation against microbial cells [4]. This situation puts on alert all the sectors on which medicine relies to maintain sanitary integrity, notably pharmacy and related disciplines such as phytochemistry [4]. As a result, research into antibacterial compounds is increasingly being undertaken along these lines. And this rush to flora is linked to the immense potential of plants to produce a very large number of molecules active against these microorganisms [4]. However, despite the potential importance of plant-derived compounds for health, their use is often not without risks [8]. In fact, certain classes of compounds or plant extracts used in certain concentrations can have undesirable effects on the organism [9-12]. So, despite their potential therapeutic activity, it is important to ensure the safety of these extracts or isolated compounds intended for medical use [13]. It is in this context that several toxicological studies are carried out in parallel with studies on the biological activities of compounds derived from plants. They are often performed on hepatocyte cells, including Huh7, Hep3B and HepaRG, which best mimic the functioning of the liver, which is the organ that suffers the most damage, as it is primarily responsible for detoxification in the body [13-15]. Our research is in line with this logic and consists of evaluating the antibacterial activity and potential toxicity of leaf extracts from the plant *Diospyros Mespiliformis*.

## **2. Botanical presentation of the plant**

*Diospyros Mespiliformis* Hochst Ex A. DC, a member of the *Diospyros* genus in the Ebenaceae family, is a 10 to 15 m-high tree with a robust, cylindrical trunk and brittle, charcoal-like black bark. It is a species characteristic of savannah woodlands (dry forests) and sometimes wet forests. It is characteristic of heavy, well-drained soils [16-23]. The species is found almost everywhere on the globe. It is found in the flora of several countries, including sub-Saharan Africa and the Gulf of Guinea, southern Africa, central Africa and northern Africa in Egypt [19-40]. Its presence has been reported in the Near East (Yemen, Israel and Saudi

### 3. Materials and methods

The raw material used in our study was harvested in Ndiemane (14°18'28"N, 16°50'52"W), a village located on Senegal's Petite Côte between Mbour and Joal-Fadiouth. After harvesting, the leaves were washed thoroughly with distilled water and then dried in the shade at room temperature in our laboratory. After drying, the samples were crushed, and the powder placed in glass jars for further processing.

The extraction was carried out using the maceration process. 100 g mass of plant material powder and 500 mL solvent were mixed in a 1000 mL flask at room temperature. The mixture was left to macerate for 48 hours. The macerate was then collected and filtered on Whatman filter paper using a Büchner filter fitted with a 1000 mL volumetric flask. The filtrate obtained was reduced to a quarter of its initial volume with a rotary evaporator, then placed in the refrigerator to dry to obtain the desired crude extract. The marc was in turn recovered, dried and the process repeated with the next solvent extracting.

The chemical screening tests were carried out to qualitatively determine the various families of secondary metabolites present in extracts from the plant parts studied. These tests were inspired by chemical screening methods described in the literature[47-57].

<div> <div>Leaf Extracts</div> <div>Test</div> </div>	Alcaloïdes	Terpène	Saponine	Polyphénols	Flavonoïde	Tanin	Coumarine	Antraquinone	Quinone	Leuco anthocyanes
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Ethanol	+++	+++	+++	+++	+++	+++	+++	++	++	+++
Hydro-ethanolic	++	+	++	++	++	++	---	++	++	++
Water	++	+++	++	+++	+++	+++	---	---	---	+++

**Table 1:** Chemical screening results

### 3.4. Antimicrobial activity tests

The work consisted in testing the activity of ethanolic, hydro-ethanolic and aqueous extracts of the plant's leaves on four microbial strains. These included two Gram-positive bacteria (*Staphylococcus aureus*, *Enterococcus Faecalis*) and two Gram-negative bacteria (*Pseudomonas Aeruginosa* and *Escherichia Coli*). The activity tests were carried out in two stages: the first consisted of activity testing using the disk diffusion method, and the second was the determination of the minimum inhibitory concentration (MIC).

- **Activity determination using the disk diffusion method**

Disk diffusion activity testing was carried out as follows. After isolation of the microbial strains, a microbial inoculum of 0.5 McFarland is prepared for each strain in a test tube containing physiological water. Then, for each strain, its microbial suspension is inoculated onto a petri dish containing MH agar by swabbing. After inoculation, sterile, unimpregnated blotting paper discs are placed in the reference points of the extracts or product to be tested on the petri dish agar. Then, for each extract to be tested, a volume of 30 µL of 60 mg/mL concentration is placed on the disc reserved for it. After this step, the assembly is left to diffuse for 10 to 15 minutes on the bench, then incubated in an oven at 37°C for 24 hours. After this delay, the inhibition diameters of the various extracts on the different strains are read using a transparent ruler. The results are then recorded in millimeters.

- **Determination of minimum inhibitory concentration (MIC) of plant leaf extracts**

To determine the minimum inhibitory concentration (MIC) of extracts. First, a liquid medium (Muller Hinton (MH) broth) was prepared. 96-well microplates were numbered from 1 to 12 and 100µL of MH broth was added to each well. Then, with 100µL of plant extract of mass concentration 60mg/mL, we made a series of dilutions in half ( $\frac{1}{2}$ ) and in cascade up to well N° 10. Wells N° 11 and N°12 were used as control wells (negative control and positive control). For well N°11, an antibiotic active on the strain to be tested was added in order to obtain the sensitivity control characterized by a limpidity of the medium (negative control). For well N°12, the broth was left alone, and this well was used as a microbial growth control (positive control). After isolating the microbial strains, we prepared a 0.5 McFarland microbial inoculum for each strain in a test tube containing physiological water. Then 20µL of

each strain's microbial inoculum were distributed into each well in the space reserved for the plate test. We then incubated them in an oven at 37°C for 24 hours. The next day, the results are read using a reading mirror, comparing the appearance of the cascade-diluted wells in each line with that of the line's reserved controls. Clear wells indicate no microbial growth, and turbid wells indicate microbial growth. The last well showing no microbial growth corresponds to the MIC value.

### **3.5. Cytotoxicity test**

- **Preparation of plant extracts**

5 g *Diospyros Mespiliformis* Hochst A.DC leaves were macerated in absolute ethanol (50 mL) for 24 hours. The ethanol extract was filtered through filter paper and the filtrate centrifuged (2000 rpm, 10 min) to remove any residual particles. The supernatants were evaporated in a SpeedVac Concentrator under vacuum at low temperature (< 40°C). The dry extract was dissolved in DMSO at a concentration of 100 mg/mL, vortexed and stored at -20°C prior to biological evaluations.

- **Culture and differentiation of HepaRG cells**

The undifferentiated HepaRG cell line used in the study was purchased from Biopredic International. Frozen cells were thawed and cultured in Williams's E medium supplemented with 10% FBS (Gibco) and L-glutamine (Gibco) for 2 weeks. To induce differentiation, the previous culture medium with its additions was renewed and supplemented with 5 µg/mL insulin, hydrocortisone hemi-succinate 50 µM for 2 weeks. The differentiation medium was then renewed and supplemented with 2% DMSO. For two (2) weeks, the latter medium is renewed every three (3) days to obtain hepatocyte cells surrounded by biliary cells.

- **Monolayer culture of human hepatocarcinoma cells**

Hep3B and Huh7 human hepatocarcinoma cells were cultured in DMEM medium (Gibco) supplemented with 10% FBS (Gibco), L-glutamine (Gibco), sodium pyruvate (Gibco) and non-essential amino acids, and trypsinized for subculture every three (3) days.

- **Cell viability tests**

Hep3B (ATCC) or Huh7 (Creative Biolabs) cells were trypsinized and seeded in 96-well plates at a density of 5,000 cells per well. At the DMSO-supplementation stage, differentiated HepaRG cells (72,000 cells per well) were seeded in 96-well plates for two (2) weeks. 24 hours after seeding, hepatocellular carcinoma cells were incubated with test plant extract in a concentration range of 0 to 200 µg/mL, in 100 µL, for three (3) days (*Hep3B and Huh7 cells*) or five (5) days (*HepaRG cells*). Intracellular ATP quantification was performed by adding 50

μL CellTiter-Glo reagent (Promega). After two (2) minutes of orbital shaking, 100 μL of the reaction well were transferred to an opaque plate for 10 min prior to luminescence quantification with the instrument (Fluoroskan, Thermo Scientific). Cell viability inhibition was calculated based on control cells treated with 0.1% DMSO. GraphPad 6.0 prism was used to calculate IC50. Experiments were repeated at least three (3) times independently to calculate standard deviations.

## 4. Results and discussion

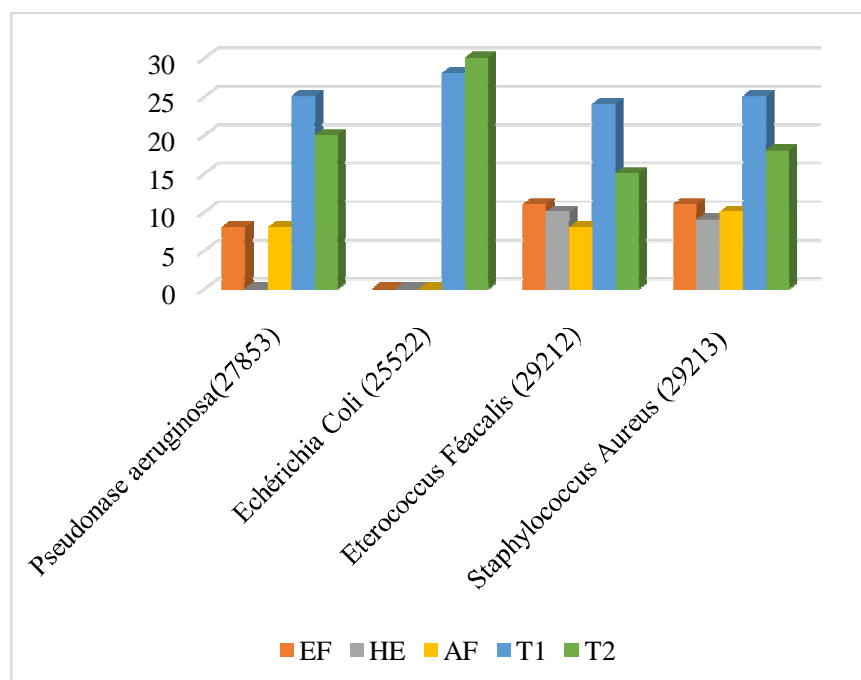
### 4.1. Antimicrobial activity tests

A clear circular zone called the inhibition zone with a specific diameter was noted around each disc. Each of these discs represented a deposit of a well-distinguished extract volume of 60 mg/ml concentration. A number of diameters characteristic of active extracts were recorded on each culture medium of a bacterial strain submitted for study. The results of inhibition diameter measurements, obtained using a transparent ruler, are shown in the following table.

**Table 2:** Results of inhibition diameter measurements of different extracts on different bacterial strains

	Inhibition diameters in (mm) by strain			
Extract	<i>Pseudonaseaeruginos</i> <i>a</i> (27853)	<i>Echerichia</i> <i>Coli</i> (25522)	<i>EterococcusFéacali</i> <i>s</i> (29212)	<i>Staphylococcus</i> <i>Aureus</i> (29213)
EF	8	0	11	11
HE	0	0	10	9
AF	8	0	8	10
T1	25	28	24	25
T2	20	30	15	18

AF: Aqueous leaf extract, EF: Ethanolic leaf extract, HE: Hydroethanolic leaf extract



**Figure 1:** Comparative sensitivity diagram for different bacterial strains to different extracts

The results showed that, with the exception of *Escherichia coli*, all the other strains showed sensitivity to the extracts. The *pseudomonas aeruginosa* strain showed the same sensitivity to both aqueous and ethanolic extracts. The characteristic inhibition diameter was 08mm. *Enterococcus faecalis* showed a sensitivity to extracts that decreased as the polarity of the extracting solvent increased. Characteristic inhibition diameters were 08 mm, 10 mm and 09 mm for ethanolic, hydroethanolic and aqueous extracts, respectively. The *Staphylococcus* strain showed greater sensitivity to the extracts obtained with the extracting solvents separately than to the extract obtained from their hydro-ethanolic mixture. Characteristic inhibition diameters were 11 mm, 09 mm and 10 mm for the ethanolic, hydro-ethanolic and aqueous extracts, respectively.

Extracts were also tested with antibiotic controls during the course of the experiment. The controls used for the *Pseudomonas* strain were ceftriaxone and amikacin. They gave respective diameters on the strain of 25 and 20 mm. For the *Enterococcus* strain, ciprofloxacin and vancomycin were used, with diameters of 24 and 15 mm, respectively. For the *Staphylococcus* strain, erythromycin and vancomycin were used, with diameters of 25 and 18 mm respectively. For the *Escherichia coli* strain, ceftriaxone and ciprofloxacin were used as controls.

The inhibition diameters obtained from these tests were compared with data provided by Abdelli *et al.* in 2017 and Ahmad *et al.* in 1999 to provide an idea of the antibacterial activity of the extracts towards microbial strains [46,58]. According to Abdelli[58], a strain is considered non-sensitive, sensitive, very sensitive and extremely sensitive to an extract when its inhibition diameter is respectively less than 8 mm, between 9 and 14 mm, between 15 and 19 mm, greater than or equal to 20 mm. According to Ahmad[8], when the inhibition diameter is between (12-18 mm), activity is low. It is moderate between (19-22 mm) and large between (23-38 mm). With regard to both classifications, our extracts appear weak, since the diameters obtained when determining the total activity of our extracts on the different strains vary between 08 and 12 mm. It should be noted, however, that this classification is usually based on the antimicrobial activity of pure compounds. As our extracts are not yet purified, their inhibitory activity cannot be estimated on this scale. However, the diameter values obtained despite the absence of prior purification remain highly satisfactory. Another classification given by Halilu and colleagues in 2008[59], linked to the inhibition diameter provided by crude plant extracts, stipulates that an inhibition zone of 10 mm diameter or more is considered significant, whereas an inhibition zone of less than 10 mm diameter is less active. This classification shows that some of our extracts with diameters greater than or equal to 10 mm on a given strain could be considered active on the strain. On the other hand, those with smaller average diameters would be considered less active. Thus, the aqueous and ethanolic extracts which had given an inhibition diameter of 08 mm on the *Pseudomonas Aeruginosa* strain will be considered less active in relation to this strain. For the *Enterococcus Faecalis* strain, the aqueous extract which gave a diameter of 08 mm is considered less active, while the ethanolic extract with a diameter of 11 mm and the hydro-ethanolic extract with an activity diameter of 10 mm are considered highly active on this strain. For the *Staphylococcus Aureus* strain, the aqueous extract with a diameter of 10 mm, the ethanolic extract with a diameter of 11 mm and the hydro-ethanolic extract with a diameter of 10 mm were found to be highly active on the strain.

The results of this study were also compared with those obtained by Dangoggoet *al.* in 2012 [37]. In their work, ethanolic extract gave an inhibition diameter of 12 mm on *Staphylococcus aureus*, 9 mm on *Echerichia Coli* and *Pseudomonas aeruginosa*. The antibacterial activity of the aqueous extract of plant leaves gave an inhibition diameter of 16 mm on *Staphylococcus aureus*, 0 mm on *Echerichia Coli* and 14 mm on *Pseudomonas aeruginosa*. Our results appear to be in line with those obtained by Dangoggoet *al.* in 2012 in their study of the antibacterial activity of the ethanolic extract of the plant's leaves. The only difference with the results of



our work remains the fact that the ethanolic extract was active on *Echerichia coli* in Danggogo's results. This was not the case in our study. And concerning the aqueous extract, the diameters obtained in our work were 5 mm smaller than those found by Danggogoet *al.* in 2012 [37] on *Staphylococcus aureus* and *Pseudomonas aeruginosa*. These results were also compared with those obtained by other researchers who have worked on the plant's leaves to measure their antimicrobial power. Among the works taken for comparison are those of Adzuet *al.* [60] on *Staphylococcus aureus* and *Pseudomonas aeruginosa* with extracts derived from solvents such as hexane and ethyl acetate. With respective diameters of 07 mm for 12 mg/mL and 10 mm for 24 mg/mL, these extracts had less marked activity against the strain when compared with our extracts. Our 60 mg/mL extracts gave diameters in the range (08 mm and 12 mm).

The activity of the extracts on the different strains could be correlated to several factors. Firstly, despite any synergy of action, to the nature of the chemical structure of the responsible compounds likely to be encountered in these solvents [61-65]. Depending on the nature of the solvent, activity varied greatly from one solvent to another. Thus, activity could be correlated to compounds of different chemical structures suitably distributed in the different solutions according to their polarities [66-71]. Among the various families of secondary metabolites known in the plant world, several families and sub-families are often speculated to be antimicrobial. These include terpenes, flavonoids, tannins, quinones et alkaloids [72-83].

Terpenoids are known for their antimicrobial activities, particularly against *Echerichia coli*. [84,85] Flavonoids are known to have antimicrobial, antiviral and antifungal properties [86-89]. Tannins precipitate wound proteins, forming a protective layer over the wound. They help stop bleeding and promote wound healing [90]. Quinones have antimicrobial activity against certain strains, notably *Staphylococcus aureus*, *Echerichia coli* and *Pseudomonas aeruginosa*. [91,92]. Alkaloids have antimicrobial powers, with the ability to act on bacterial DNA [90, 93-94].

The position of the first well where microbial growth or proliferation is used to determine the minimum inhibitory concentration. Results were obtained by comparing the appearance of each well with the appearance of the control wells and assigning to each well the sign of the control whose appearance matched. The results of the tests carried out on the different strains are shown in the table below.

**Table 3:** Results of extract MIC determinations on different microbial strains

Leaf extracts	Strains microbial	Extract concentration in µg/ml for each well
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		3.10 <sup>5</sup>	15.10 <sup>4</sup>	75.10 <sup>3</sup>	375.10	18750	9375	4688	2338	1171	586	T-	T+
AF	<i>S. Aureus</i>	-	-	-	-	-	-	-	-	-	-	-	+
	<i>E. Faecalis</i>	-	-	-	-	-	-	-	-	-	+	-	+
	<i>P. Aeruginosa</i>	-	-	-	-	-	-	+	+	+	+	-	+
HE	<i>S. Aureus</i>	+	+	+	+	+	+	+	+	+	+	-	+
	<i>E. Faecalis</i>	-	-	-	-	-	-	-	-	-	+	-	+
	<i>P. Aeruginosa</i>	/	/	/	/	/	/	/	/	/	/	/	/
EF	<i>S. Aureus</i>	-	-	-	-	-	-	-	-	-	-	-	+
	<i>E. Faecalis</i>	-	-	-	-	-	-	-	-	-	+	-	+
	<i>P. Aeruginosa</i>	-	-	-	-	-	-	-	-	-	+	-	+

*Pseudomonas Aeruginosa*; *Enterococcus Faecalis*; *Staphylococcus Aureus*

AF: Aqueous leaf extract; EF: Ethanolic leaf extract; HE: Hydroethanolic leaf extract

Legend: (-) no bacterial growth; (+) no bacterial growth

The *Pseudomonas Aeruginosa* strain had an identical MIC against the extracts to which it was sensitive. The approximate value was 9375 µg/mL. *Enterococcus faecalis* had a MIC value of 1171 µg/mL against the three extracts to which it was sensitive. The *Staphylococcus* strain showed a MIC value of 586µg/ml under the aqueous and ethanolic extracts, and 60mg/ml under the hydro-ethanolic extract. Kuete *et al.*[95]in 2010 considered the antimicrobial activities of compounds to be significant if the MIC is 10µg/mL or less, moderate if the MIC ≤ 100µg/mL and weak if the MIC is greater than 100µg/mL. A comparison with this classification shows that our compounds were less active for certain extracts. This can be explained by the fact that crude plant extracts are in the form of mixtures. However, it should be noted that this classification most often refers to pure compounds. This is shown by the MIC value found by Hawas *et al.* [89]during tests of the antimicrobial activity of flavonoids isolated from *D. mespiliformis* leaves against four human pathogenic bacteria. Among them, flavonol O-rhamnoside had significant activity against *S. aureus*, with a MIC of 9.77 µg/mL, while Lajubuet *et al.* [91]tested the antibacterial activity of diosquinone and plumbagin isolated from *D. mespiliformis* roots. The MICs of diosquinone ranged from 3 to 30 µg/mL for *S. aureus* NCTC 6571 and *S. aureus* E3T, while they were 15 to 16 µg/mL for *E. coli* KL16 and *P. aeruginosa* NCTC 6750. If we take into account the competitive effect of other

compounds other than the principle, as well as the low quantity of an active principle in a crude extract, the MICs could prove more significant after purification of the extract. The MIC values of our aqueous and ethanolic extracts were compared with the MIC values of methanolic and aqueous extracts of plant leaves in the work of Ebbo *et al.* [96]. The results showed that our extracts had the lowest MIC values, with 586 µg/mL and 1771 µg/mL respectively for the aqueous and ethanolic extracts, compared with 625 µg/mL and 1250 µg/mL for the methanolic and aqueous leaf extracts in the work of Ebbo *et al.* [96]. The efficiency of the activity extends inversely with variation in concentration. Our extracts obtained with water and ethanol are more active than the aqueous and methanolic extracts in the work of Ebbo *et al.* [96]. This demonstrates the role played by the extraction solvent in the activity of the noise extract. It also highlights the importance of the choice of extraction solvent in obtaining significant activity on certain microbial strains. The activities and MICs of other fractions of the methanolic extract in the work of Ebbo *et al.* [96]. The hexane and ethyl acetate fractions of the methanolic extract gave more significant MICs than those of the methanolic extract. These still suggest that our purified extracts could prove all the more significant on these microbial strains.

#### 4.2.Cytotoxicity test

**Table 4:** IC<sub>50</sub> results from cytotoxicity testing of ethanolic leaf extract on various hepatocyte cells

CI <sub>50</sub> (µg/ mL)	<i>HUH7</i>	<i>HEP3B</i>	<i>HEPA-RG</i>	<i>SI HUH7</i>	<i>SI HEP3B</i>
<b>DM</b>	73.27 ±7.4	110.55 ±11.4	109.8 ±15.2	1.5	1
<i>Sorafenib</i>	1.49 ±0.8	2.1 ±1.5	NT	NT	NT
<i>Aflatoxine B1</i>	NA	NA	14.2 ± 2.8	NA	NA

The extract gave an IC<sub>50</sub> for each of the hepatocyte cells used in the study. The IC<sub>50</sub>s were 73.27, 110.55 and 109.8 µg/ mL, respectively, for *HUH7*, *Hep3B* and *HEPA-RG cells*. Compared with the IC<sub>50</sub>s of the control compounds, these values are very high. These results show that the extract concentrations required to produce 50% necrosis in the cells tested are very high compared with those of known molecules, notably Sorafenib and Aflatoxin B1. The values in terms of extract concentration required to damage liver cell integrity are very high, in contrast to Sorafenib, Aflatoxin B1, for which they are very low. The toxicity of the extract is therefore very low compared to Sorafenib and Aflatoxin B1.

The toxicity noted may be due to several factors, firstly to the limited concentration of use of the extracts or of certain chemical elements tolerable at certain concentrations by the organism. It may also be due to the contamination of extracts by certain microorganisms et also to certain classes of secondary metabolites reputed to be toxic, notably alkaloids and quinones [97,98].

The differences noted between the IC50s of the different cells can be sought at several levels. Firstly, at cellular level, *HEPA-RG* cells are more complete in terms of CYP and/or P450 metabolic enzymes than *HUH7* and *HEP3B cells*[99-102]. The absence of certain types of enzymes may explain the differences in concentration values noted in *HUH7* and *HEPA3B*.

## **5. Conclusion**

The study of the antimicrobial activity of plant extracts have been very interesting, with characteristic inhibition diameters and highly significant MICs. However, there are many disparities between the results, so purification of certain extracts could remove any doubts concerning certain hypotheses formulated. The safety of the plant's leaf extracts was also verified by studying the damaging effects they can have on the liver. However, the concentrations capable of causing hepatocyte cell necrosis were found to be very low. So, the toxicity of the extract remains very low compared with aflatoxin and sorafenib. The crude extract, which is usually distributed in the form of a mixture, may contain compounds that can affect the state of certain cells in the body. Thus, purification or isolation of the compounds responsible for the biological activities noted could make the use of plant extracts by humans for medicinal purposes even safer.

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## **Disclaimer (Artificial intelligence)**

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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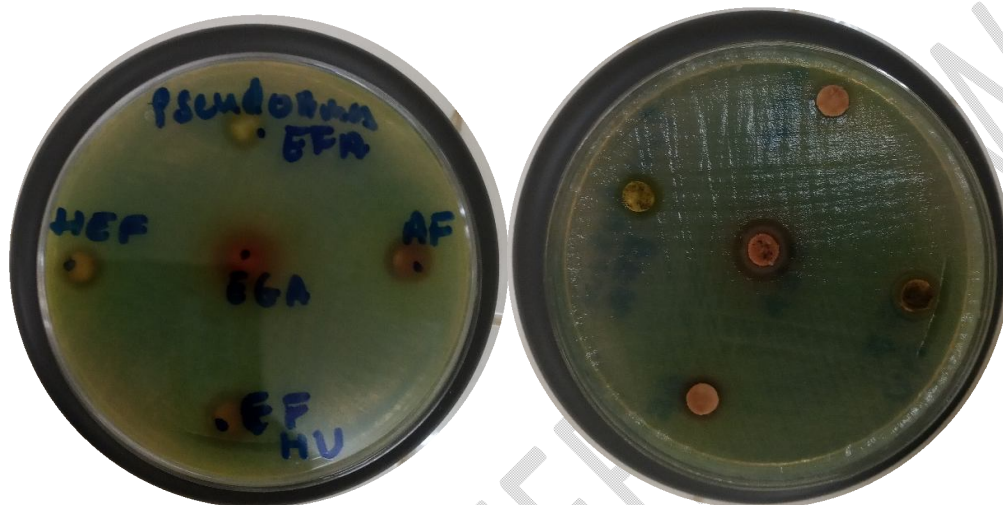
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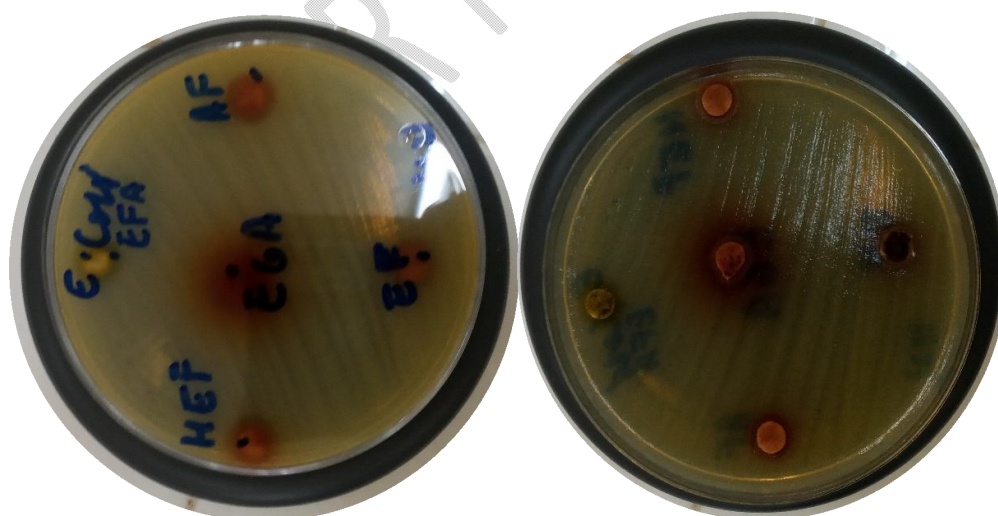
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## ANNEXES

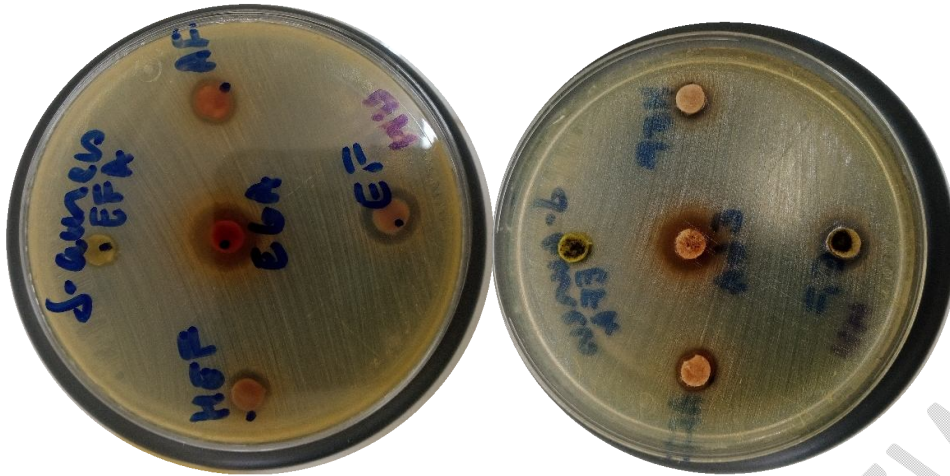


*P. aeruginosa*

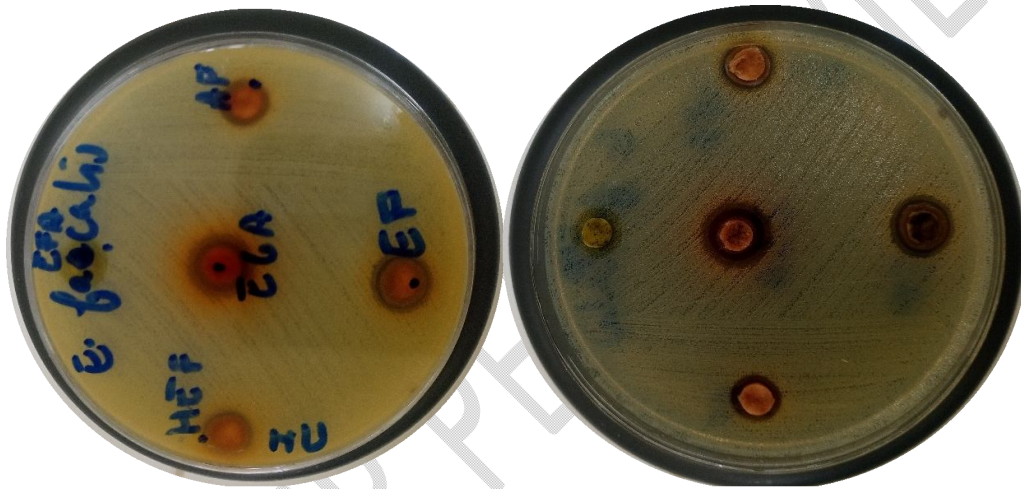


*E. coli*





*Staphylococcus Aureus*



*Entococcus Féacalis*



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