Scientific Research Publishing

American Journal of Plant Sciences, 2026, 17(5), 601-620
https://www.scirp.org/journal/ajps
ISSN Online: 2158-2750
ISSN Print: 2158-2742

Ethnobotany and Biological Activities of the Plant *Diospyros Mespiliformis* Hochst ex A. DC

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How to cite this paper: Diouf, I.B., Karé, M., Samb, I., Carraz, M. and Gaye, M.L. (2026) Ethnobotany and Biological Activities of the Plant *Diospyros Mespiliformis* Hochst ex A. DC. *American Journal of Plant Sciences*, **17**, 601-620.
https://doi.org/10.4236/ajps.2026.175037

Received: February 8, 2026
Accepted: May 26, 2026
Published: May 29, 2026

Abstract

This study on the plant *Diospyros mespiliformis* (Ebenaceae) is part of a process to promote our natural substances. Ethnopharmacology reveals that the plant is used to combat various types of infections and mental illness. Biological activity studies conducted on the plant have confirmed some ethnopharmacological data, particularly its anti-inflammatory, antibacterial, and antioxidant activities. The results of the studies reveal that the hydroalcoholic extract of the leaves had the best anti-inflammatory activity with an IC_{50} value of 0.05281 mg/mL, followed by the extract produced from the bark of the trunk with an IC_{50} = 0.07058 mg/mL, then the extract from the roots with an IC_{50} value of 0.09429 mg/mL. For antibacterial activity, the ethanolic extract from the leaves showed the highest activity against all the strains on which it was tested. In terms of antioxidant activity, the hydroalcoholic extracts produced from the leaves and bark had the best activity with an IC_{50} < 0.00001 mg/mL, followed by the extracts from the bark and then the roots. The study also showed that hydroalcoholic extracts from different parts of the plant have more powerful antioxidant activity than vitamin C.

Keywords

Ebanaceae, *Diospyros Mespiliformis*, Phytochemistry, Antioxidant, Anti-Inflammatory, Lipoxygenase, Antibacterial, Cytotoxicity, HepaRG, Hep3B and Huh7

1. Introduction

Diospyros mespiliformis (*DM*) *Hochst. ex A. DC,* from the ancient Greek dios

(god) and pyros (fruit), means "the fruit of the gods" according to some, or "the fruit of heaven" out of modesty, according to others. The species name *D. mespiliformis*, according to Venter 1996, derives from mesos, meaning "half", and pilos, meaning "husk", alluding to the shape of the fruit. Ramadwa TE & Meddows-Taylor S., 2023, cite Pitman (1972) as stating that the reference is in fact the fruit's resemblance to a medlar [1]. The name is also followed by Hochst Ex A. DC., referring to Christian Ferdinand Friedrich Hochstetter (1787-1860), who first described it, and Alphonse Pyrame de Candolle (1806-1893) (D.C.), for his first systematic classification recognized in 1844. It is a tree 10 to 15 m tall with a sturdy, cylindrical trunk covered in black bark. Its leaves, which are alternate and elliptical, are reddish when young and dark green when mature. Its fruits are green when young and yellow when ripe. In the evolutionary classification of plants, the species is related, based on a number of characteristics, less specifically to the Ebenaceae family and more specifically to the genus Diospyros [2]. This species is characteristic of wooded savannas and, occasionally, of moist forests. It is generally found along lakes, rivers, and watercourses that dry up annually. In addition, it prefers rocky hills and sometimes colonizes termite mounds [3] [4].

The species is also characterized by a phenology marked by periods of partial leaf fall from January to March, flowering from March to May, and fruiting from April to June [2]-[4]. The species is highly regarded in traditional medicine. The numerous claims regarding its therapeutic potential have led to major scientific studies focusing on the species. These studies have confirmed most of the speculations regarding the plant's potential, but they have also shown that certain extracts of the plant are more active than some medications still used to treat certain conditions. Studies also show that certain extracts required only purification to rival or even surpass certain treatments used in the management of specific conditions. The objective of this study is to contribute to the preservation and promotion of Senegal's medicinal plants. It aims to shed light on the potential of the medicinal plant, its various therapeutic uses, its biological and physicochemical characteristics, its different active fractions, and its nutritional benefits.

2. Use in Traditional Medicine

Ethnopharmacological research reveals that various parts of *Diospyros mespiliformis* are widely used in the traditional medicine of several peoples across the continent. The plant is most commonly used for its ability to treat coughs, dysentery, diarrhea, menorrhagia, toothaches, gingivitis, fungal skin infections, ulcers, fever, pneumonia, syphilis, leprosy, and yaws [1] [2] [5]. It is also used as a wound-healing agent, purgative, laxative, stimulant, and vermifuge, as well as an antivenom and treatment for poisoning, dysentery, headaches, jaundice, arthritis, swelling, tumors, high blood pressure, diabetes, hepatitis, epilepsy, and ear infections [1] [2] [5].

Diospyros mespilformis is also used as a hemostatic agent, for abdominal pain, male sexual dysfunction, difficult childbirth, and as a psychopharmacological medication [1] [2] [5]-[10]. The traditional medicinal knowledge regarding each

part of the plant from several countries is summarized in Figure 1.

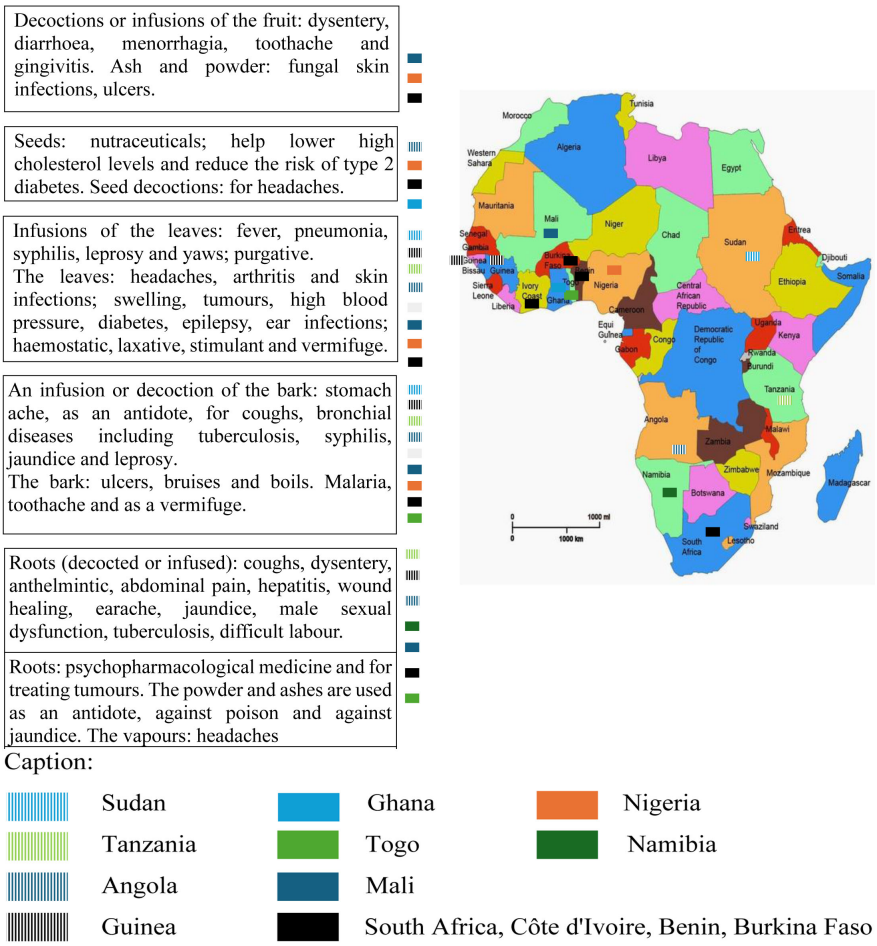

Figure 1. Traditional medical knowledge of the plant in Africa.

In Senegal, an infusion made from the plant's leaves is used to treat rheumatism [11]. A decoction made from the plant's leaves is used to treat psychosis [12] [13]. The leaves of this plant species are also used to treat skin diseases and headaches [12] [13]. The bark of the trunk and the roots, as well as the leaves of the plant, are used in the treatment of severe pneumonia, high infectious fevers, and severe syphilis [12] [13]. A decoction made from the bark of the stem or branches of the trunk, or from the bark of the underground parts, is used to treat yaws [12] [13]. The dried and powdered leaves and ripe fruits of the tree are used to treat diarrhea and menorrhagia [12] [13].

3. Study of Biological Activities

3.1. Evaluation of Antioxidant Activity

The antioxidant potential of hydroalcoholic extracts from different parts of the plant *Diospyros Mespiliformis Hochst. Ex. A. DC.* was studied using the diphenylpicrylhydrazine (DPPH) radical scavenging assay, based on the method of Bar-

kat and Imene with some modifications [14]. For this purpose, we prepared three rows of eight test tubes containing the respective samples (leaf, root, and bark) and their corresponding concentrations: 0.00001, 0.0001, 0.001, 0.01, 0.1, 0.2, 0.3, and 0.4 mg/mL in each row. Then, in each test tube, we added a volume of 0.1 mL of extract at the specific concentration as stated above, depending on the sample. Subsequently, we added a volume of 4 mL of a previously prepared DPPH solution to each test tube. The mixture was then left in the dark for 30 minutes. Absorbances were measured at a wavelength of 517 nm using an Ultrospec 7000 UV-vis dual-beam spectrophotometer (GE Healthcare, Chicago, IL, USA).

The results are expressed as the percentage of inhibition (PI). The same procedure was performed with ethanol and water extracts, with the sole difference being that the samples tested were leaves, and the various extracts were prepared in solution concentrations ranging from 0.005; 0.01; 0.015 up to 0.035 mg/mL.

The IC_{50} is obtained from the equation of the line in the graph representing the percentage inhibition (%PI) as a function of concentration (mg/mL).

3.2. Evaluation of Antibacterial Activity

The study was conducted using ethanolic, hydroalcoholic, and aqueous extracts of the plant's leaves on four microbial strains. Among these microbial strains were two Gram-positive bacteria (*Staphylococcus aureus and Enterococcus faecalis*) and two Gram-negative bacteria (*Pseudomonas aeruginosa and Escherichia coli*). The activity tests were conducted in two stages: the first involved testing for activity using the disk diffusion method, and the second was designed to determine the minimum inhibitory concentration (MIC).

- ***Assessment of Antibacterial Activity Using the Disc Diffusion Method***

The assessment of antibacterial activity using the disc diffusion method was performed as follows. After isolating the microbial strains, a 0.5 McFarland microbial inoculum was prepared for each strain in a test tube containing physiological saline. Next, for each strain, the microbial suspension was inoculated onto a Petri dish containing MH agar by swabbing. After inoculation, sterile, unimpregnated blotting paper discs were placed at designated points on the agar in the Petri dishes. Then, for each extract to be tested, a 30 µL volume of a 60 mg/mL solution was placed on the designated disc. After this step, the set is left to diffuse for 10 to 15 minutes on the lab bench and then incubated in an incubator at 37˚C for 24 hours. The next day, the inhibition zones of the extracts on the different strains are measured using a transparent ruler. The results are then recorded in millimeters.

- ***Determination of the Minimum Inhibitory Concentration (MIC)***

To determine the minimum inhibitory concentration (MIC) of the extracts, 96-well microplates with wells numbered in columns from 1 to 12 were used. Then, 100 µL of MH broth was added to each well for culture. Next, for each extract, using a 100 µL volume taken from its stock solution with a mass concentration of 60 mg/mL, a series of half-volume and serial dilutions was performed from well

No.1 to well No.10 in the row designated for it, with the number of rows corresponding to the number of extracts to be tested on each strain. Wells No.11 and No.12 were used as control wells (negative control and positive control). For well No.11, an antibiotic active against the strain being tested was added to obtain a sensitivity control characterized by a clear medium (negative control). For well No.12, the broth was left alone; this well serves as the control for microbial growth (positive control).

Next, we added 20 µL of the bacterial suspension from each strain to all the wells in the designated area of the test plate, except for the control wells in column 12. We then incubated the plates in an incubator at 37˚C for 24 hours. The next day, we removed them to record the results using a reading mirror. The results were determined by comparing the appearance of the serially diluted wells in each row with those of the controls. Wells that appear clear indicate the absence of microbial growth, while those that appear cloudy indicate microbial growth. The last well in a row where there is no microbial growth is the one that provides the MIC value. This is simply the concentration value of the extract associated with that row.

3.3. Evaluation of Cytotoxicity

5 g of leaves from *Diospyros mespiliformis Hochst. Ex A. DC* were macerated in absolute ethanol (50 mL) for 24 hours. The ethanolic extract was filtered through filter paper, and the filtrate was centrifuged (2000 rpm, 10 min) to remove any particulate residue. The supernatants were evaporated in a SpeedVac low-temperature vacuum concentrator (<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 assays.

- ***Culture and Differentiation of HepaRG Cells***

The undifferentiated HepaRG cell line used in this study was purchased from Biopredic International. The frozen cells were thawed and cultured in Williams' E medium supplemented with 10% FBS (Gibco) and L-glutamine (Gibco) for 2 weeks. To induce differentiation, the previous culture medium with its supplements was replaced, and then 5 µg/mL of insulin and hydrocortisone hemisuccinate (50 µM) were added for 2 weeks. The differentiation medium is then replaced and supplemented with 2% DMSO. For two (2) weeks, this medium is replaced every three (3) days to obtain hepatocytes surrounded by bile duct cells.

- ***Culture of Human Hepatocarcinoma Cell Monolayers***

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

- ***Cell Viability Assays***

Hep3B (ATCC) or Huh7 (Creative Biolabs) cells were trypsinized and seeded into the wells of 96-well microplates at a density of 5000 cells per well. At the

DMSO supplementation stage, differentiated HepaRG cells (72,000 cells per well) were seeded into the wells of 96-well microplates for two (2) weeks. Twenty-four hours after seeding, the hepatocarcinoma cells were incubated with the extract of the test plant 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 of CellTiter-Glo reagent (Promega). After two (2) minutes of shaking, 100 µL from the reaction well was transferred to an opaque plate for 10 minutes prior to luminescence quantification using a fluorometer (Fluoroskan, Thermo Scientific). Cell viability inhibition was calculated relative to control cells treated with 0.1% DMSO. GraphPad 6.0 software was used to calculate the IC_{50}. The experiments were repeated at least three (3) times independently to calculate standard deviations.

3.4. Evaluation of Anti-Inflammatory Activity: Lipoxygenase Inhibition

The solution was prepared by adding 140 mg of linoleic acid to 5 mL of distilled water that had been deoxygenated by bubbling nitrogen through it. Next, 18 mL of Tween 80 was added to the mixture, which was vortexed for 5 minutes. A 100 µL solution of 2 M NaOH was added, and the volume was adjusted to 50 mL with deoxygenated distilled water. The linoleic acid solution was then aliquoted and stored in the freezer.

- ***Preparation of the Buffer Solution***

The optimal pH of the reaction matrix was achieved using a 0.1 M sodium borate buffer adjusted to pH 9.5 with 5 M NaOH. The borate buffer was aerated for 30 minutes prior to use.

- ***Enzyme Preparations***

The lipoxygenase solution (EC 1.13.11.12, SIGMA, 50,000 U/mg) was prepared at a concentration of 0.1 mg/mL in distilled water. After mixing, the solution was aliquoted and stored in the freezer until use. Since lipoxygenase is heat-sensitive, all experimental steps were performed in an ice bath.

- ***Procedure***

The study was conducted using hydroalcoholic extracts from various parts of the plant, including the leaves, bark, and roots. The first step involved preparing a series of concentrations ranging from 0.1 to 0.6 mg/mL for each sample, based on its dry extract. Then, in a 10 mm cuvette, 900 µL of borate buffer and 100 µL of extract or standard inhibitor (quercetin) at various concentrations were added, followed by 10 µL of lipoxygenase solution. The mixture was shaken three times and left at room temperature for 15 minutes. After this incubation, 10 µL of the prepared linoleic acid solution is added. After shaking, the absorbance kinetics are measured directly at 234 nm every 5 seconds for 5 minutes.

For each assay, a positive control corresponding to 0% inhibition was prepared by mixing 900 µL of borate buffer with 10 µL of lipoxygenase solution, then initiating the reaction by adding the substrate.

4. Results and Discussion

4.1. Evaluation of Antioxidant Activity

The results of the assessment of the antioxidant activity of the reference compound and the extracts studied are presented in the following tables and graphs (Tables 1-2, Figures 2-4).

- ***Results of the Inhibition Percentages of Ascorbic Acid***

Table 1. Inhibition percentages as a function of ascorbic acid concentration.

Concentration in mg/ml	Inhibition percentages (%)	Standard deviation
0.0039	8.9	0
0.0079	10.963333	0.089566859
0.01575	33.936667	0.089566859
0.0315	72.18	0

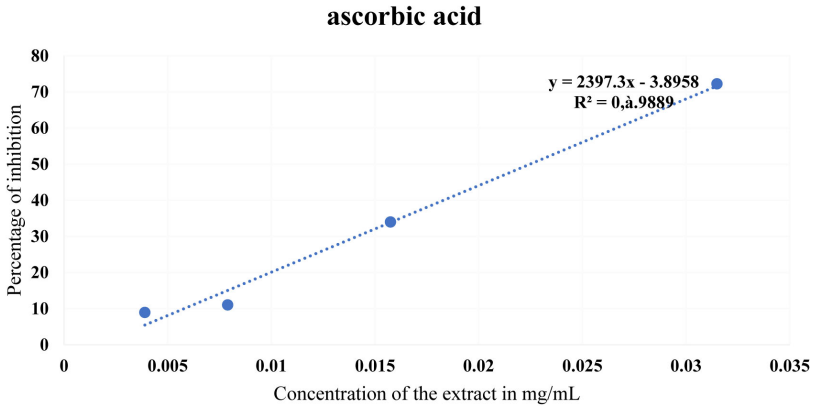

Figure 2. Graph showing the percentage inhibition of ascorbic acid.

- ***Results of the Inhibition Percentages of Hydroalcoholic Extracts***

Table 2. Inhibition percentages as a function of the concentration of extracts from the different parts.

Concentration of the extract in mg/mL	Inhibition percentages (%)		
	LEAVES	BARKS	ROOTS
0.00001	54.058314	55.49724	49.52229
0.0001	56.711321	56.17948	50.31847
0.001	58.707644	58.6985	63.21656
0.01	64.696612	63.73655	86.70382
0.1	95.220484	95.91837	96.63372
0.2	95.561878	95.58824	97.06609
0.3	95.874822	95.34814	97.12786
0.4	96.102418	95.01801	97.34404

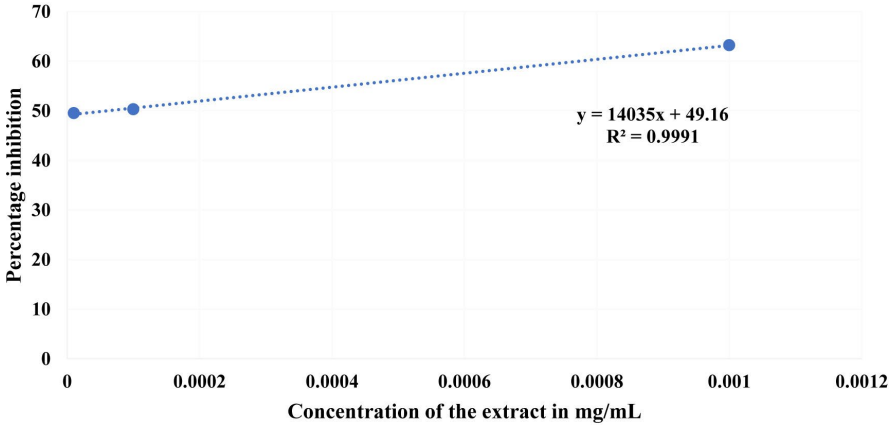

Figure 3. Graph showing the percentage inhibition of the hydro-ethanolic root extract (IC_{50}: 5.98504×10^{-5} mg/mL).

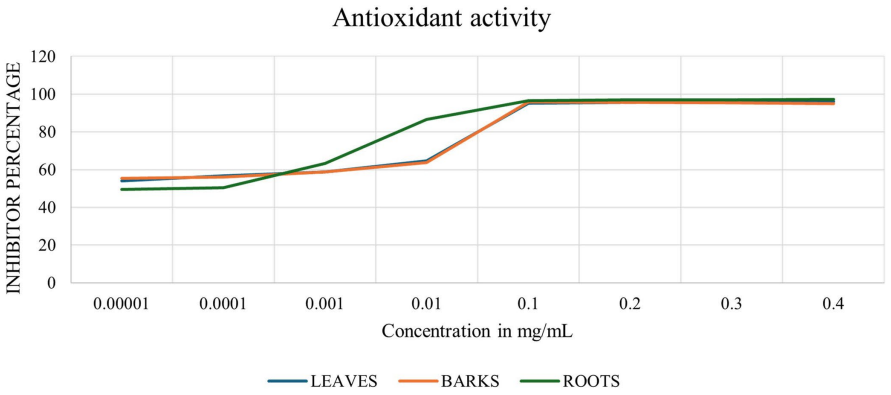

Figure 4. Comparative graph showing the percentage of inhibition in different parts of the plant

The results show that the percentage of inhibition increases with the concentration of the plant extract, indicating dose-dependent activity. This upward trend can be divided into three levels of variation, with an initial slight increase between 0.00001 and 0.0001 mg/mL, followed by a jump in activity between 0.001 and 0.2 mg/mL, and then remaining approximately constant with % IA values of 97 (root), 96 (bark), and 95 (leaf). However, starting at a concentration of approximately 0.001 mg/mL, the inhibitory activity of the root extracts is slightly higher than that of the other parts. The activity values of the bark and leaf extracts yield similar results with regard to DPPH. These values appear to be consistent with the differences in phenolic content of the extracts from these two parts. This suggests that their activities are likely influenced by total phenolic content, unlike those of the roots, where they are attributed to flavonoids.

The IC_{50} (half-maximal inhibitory concentration) for roots (0.00005 mg/mL), which is higher than that for other parts (<0.00001 mg/mL), suggests that their antioxidant potency is less significant than that of the other parts. These values, when compared to the value reported by Adamu *et al*., 2020 (0.0324 mg/mL) [15], are significantly lower than that reported by the latter. This highlights the im-

portance of selecting the appropriate extraction solvent to achieve optimal activity. The IC_{50} values of our extracts, compared to those of ascorbic acid (vitamin C), were significantly lower, highlighting the potential importance of our extracts in combating free radicals.

4.2. Evaluation of Antibacterial Activity

A number of characteristic diameters of the active extracts were observed in each culture medium for the bacterial strain under study. The results of the inhibition zone measurements, obtained using a transparent ruler, are summarized in following Table 3.

Table 3. Results of measurements of the inhibition zones of the various extracts against the different bacterial strains.

	Inhibition zone diameters (mm) by strain			
Extracts	*Pseudomonas aeruginosa* (27853)	*Escherichia coli* (25522)	*Enterococcus faecalis* (29212)	*Staphylococcus aureus* (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: Ethanol leaf extract; HE: Hydro-ethanol extract; T: Controls.

With the exception of *Escherichia coli*, the other strains exhibited sensitivity to the extracts. The *Pseudomonas aeruginosa* strain exhibited the same sensitivity to both the aqueous extract and the ethanolic extract. The characteristic inhibition zone diameter was 0.8 mm. *The Enterococcus faecalis* strain exhibited sensitivity to the extracts, which decreased as the polarity of the extraction solvent increased. The characteristic inhibition diameters were 8 mm, 10 mm, and 9 mm, respectively, for the ethanolic, hydroethanolic, and aqueous extracts. The *Staphylococcus strain* exhibited greater sensitivity to the extracts obtained with the extraction solvents used separately than to the extract derived from their mixture (hydroethanolic). The characteristic inhibition diameters were 11 mm, 9 mm, and 10 mm, respectively, for the ethanolic, hydroethanolic, and aqueous extracts. The extracts were also tested against antibiotic controls during the experiment.

The control agents used for the *Pseudomonas strain* were ceftriaxone and amikacin. They yielded zone diameters of 25 and 20 mm, respectively, against the strain. For the *Enterococcus strain*, ciprofloxacin and vancomycin were used, yielding zone diameters of 24 and 15 mm, respectively. For the *Staphylococcus strain*, erythromycin and vancomycin were used, yielding diameters of 25 and 18 mm, respectively. For the Escherichia coli strain, the controls were ceftriaxone

and ciprofloxacin.

According to Tsirinirindravo & Andrianarisoa, 2009, the standards used to interpret the inhibition zones obtained from the results of antibacterial tests on plant extracts stipulate that an extract with an inhibition zone of less than 7 cm is considered inactive, active between 7 cm and 8 cm, moderately active between 8 cm and 9 cm, and highly active when greater than 9 cm [16]. This classification shows that our three extracts, which produced inhibition zones greater than 9 mm on the *Staphylococcus aureus* strain, could be considered highly active against that strain. The same applies to our aqueous and hydro-ethanolic extracts, which produced inhibition zones greater than 9 mm on the *Enterococcus faecalis* strain. In contrast, on the *Pseudomonas aeruginosa* strain, the aqueous and ethanol extracts that produced an inhibition zone diameter of 8 mm are considered active against this strain.

The results of this study were also compared with those obtained by Dangoggo *et al.* [17]. In their study, the ethanolic extract produced an inhibition zone diameter of 11 mm against *S. aureus*, 9 mm against *E. coli,* and 9 mm against *P. aeruginosa*. The antibacterial activity of the aqueous extract of the plant's leaves yielded an inhibition zone diameter of 10 mm against *S. aureus*, 9 mm against *E. coli*, and 10 mm against *P. aeruginosa*. Our results appear to be consistent with those obtained by Dangoggo *et al.* [17] in their study on the antibacterial activity of the plant's leaf ethanol extract. The only differences from our results are that the ethanolic and aqueous extracts were active against *E. coli* in Dangoggo's findings, whereas this was not the case in our study.

These results were also compared to those obtained by other researchers who studied the plant's leaves to measure their antimicrobial activity. Among these studies are those by Adzu & Salawu on *S. aureus* and *P. aeruginosa* using extracts derived from solvents such as hexane and ethyl acetate [18]. With respective diameters of 7 mm for 12 mg and 10 mm for 24 mg, these extracts exhibited less pronounced activity against the strain when compared to our extracts. Our extracts at a concentration of 60 mg yielded diameters within the range of 8 mm to 12 mm.

The activity of the extracts on different strains could be related to several factors. First, the nature of the chemical structure of the active compounds likely to be present in these solvents [19]-[23]. Depending on the nature of the solvent, the activity varied significantly from one solvent to another. Thus, the activity could be correlated with compounds of different chemical structures appropriately distributed in the various solutions according to their polarities [24]-[29]. Among the various families of secondary metabolites known in the plant kingdom, several families and subfamilies are often hypothesized to possess antimicrobial properties. These include terpenes, flavonoids, tannins, quinones, and alkaloids. Terpenoids are known for their antimicrobial activities, particularly against E. coli [30] [31]. Flavonoids are known to possess antimicrobial, antiviral, and antifungal properties [32]-[34]. Tannins precipitate proteins in the wound, forming a pro-

tective layer over the wound. They help stop bleeding and promote wound healing [35]. Quinones exhibit antimicrobial activity against certain strains, including S. aureus, E. coli, and P. aeruginosa [36]. Alkaloids possess antimicrobial properties and are capable of acting on bacterial DNA [37] [38].

- ***Determination of the minimum inhibitory concentration***

The results were obtained by comparing the appearance of each well with that of the control wells and assigning each well the designation of the control whose appearance it matched. The results of the tests conducted on the various strains are presented in **Table 4** below.

Table 4. Results of the determination of MICs for the extracts against different microbial strains.

Extracts	Microbial	Concentration of the extract in µg/mL in each well											
Leaves	strains	3.10^5	15.10^4	75.10^3	375.10^2	18,750	9375	4688	2338	1171	586	T−	T+
AF	*S. Aureus*	−	−	−	−	−	−	−	−	−	−	−	+
	E. Faecalis	−	−	−	−	−	−	−	−	−	+	−	+
	P. Aeruginosa	−	−	−	−	−	−	+	+	+	+	−	+
HE	*S. Aureus*	+	+	+	+	+	+	+	+	+	+	−	+
	E. Faecalis	−	−	−	−	−	−	−	−	−	+	−	+
	P. Aeruginosa	/	/	/	/	/	/	/	/	/	/	/	/
EF	*S. Aureus*	−	−	−	−	−	−	−	−	−	−	−	+
	E. Faecalis	−	−	−	−	−	−	−	−	−	+	−	+
	P. Aeruginosa	−	−	−	−	−	−	−	−	−	+	−	+

P: *Pseudomonas; E*: *Enterococcus*; *S*: *Staphylococcus*; AF: Aqueous leaf extract; EF: Ethanol leaf extract; HE: Hydro-ethanol leaf extract (−) No bacterial growth; (+) Bacterial growth; T: Controls.

Determination of the MIC values for the strains against the extracts to which they were susceptible showed that the *Enterococcus faecalis* strain had an MIC of 1171 µg/mL against the ethanolic extract and 9375 µg/mL against the aqueous extract. The *Enterococcus faecalis* strain had an MIC of 1171 µg/mL against the three extracts to which it was susceptible. The *Staphylococcus* strain exhibited an MIC of 586 µg against the aqueous and ethanolic extracts and 60 mg against the hydro-ethanolic extract.

Kuete (2010) considered the antimicrobial activity of the compounds to be significant if the MIC was 10 µg/mL or less, moderate if the MIC was ≤ 100 µg/mL, and weak if the MIC was greater than 100 µg/mL [39]. When compared to this classification, it is evident that our compounds exhibited lower activity in 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. If we take into account the competitive

effect of compounds other than the active ingredient, as well as the low concentration of an active ingredient in a crude extract, the IC_{50} values could prove to be more significant after purification of the extract. The MIC values of our aqueous and ethanolic extracts were compared to the MIC values of the methanolic and aqueous extracts of the plant's leaves reported in the study by Ebbo *et al*., 2019 [40]. The results showed that our extracts had the lowest IC_{50} values, at 586 µg/mL and 1771 µg/mL for the aqueous and ethanolic extracts, respectively, compared to 625 µg/mL and 1250 µg/mL for the methanolic and aqueous extracts of the leaves in the study by Ebbo *et al*., 2019. This demonstrates the role that the extraction solvent plays in the activity of the crude extract. It also highlights the importance of selecting the appropriate extraction solvent to achieve significant activity against certain microbial strains. Another notable finding is evident in the activities and MICs of other fractions of the methanolic extract in the work by Ebbo *et al*., 2019.

The hexane and ethyl acetate fractions of the methanolic extract yielded more significant MICs than those of the methanolic extract itself. These results further suggest that our purified extracts could prove to be even more effective against these microbial strains.

4.3. Evaluation of Cytotoxicity

The results of the cytotoxicity evaluation of the reference molecules and the studied extract are shown in Table 5 below.

Table 5. IC_{50} results obtained from cytotoxicity assays of the ethanolic leaf extract on various hepatocyte cell lines.

IC_{50} (µg/ mL)	Huh7	Hép3B	HépaRG	SI HUH7	SI Hép3B
DM	73.27 ± 7.4	110.55 ± 11.4	109.8 ± 15.2	1.5	1
Sorafenib	1.49 ± 0.8	2.1 ± 1.5	NT	NT	NT
Aflatoxine B1	N. A	N. A	14.2 ± 2.8	N. A.	N. A

Not active: N. A; Not Tested: NT; Selectivity index: SI.

The extract produced an IC50 value for each of the hepatocyte cells used in the study. The IC_{50} values were 73.27, 110.55, and 109.8 µg/mL for Huh7, Hep3B, and HepaRG cells, respectively. These values are very high compared to the IC_{50} values of the control compounds. These results show that the extract concentrations required to produce 50% necrosis in the tested cells are very high compared to those of known molecules, such as sorafenib and aflatoxin B1. The extract concentrations required to compromise liver cell integrity are very high, in contrast to the very low concentrations for sorafenib and aflatoxin B1. According to the toxicity scale proposed by [41]-[43], the extract exhibited very low (non-significant) toxicity on Huh7 cells, with an IC_{50} between 50 µg/mL and 100 µg/mL. However, the extracts were non-toxic to Hep3B and HepaRG cells, with an $IC_{50} > 100$ µg/mL.

The observed toxicity may be due to several factors, primarily the maximum concentration of the extracts or certain chemical elements that are tolerable by the body. It may also be due to contamination of the extracts by certain microorganisms and to certain classes of secondary metabolites known to be toxic, particularly alkaloids and quinones [44].

The differences observed between the IC_{50} values of the different cells can be investigated at several levels. First, at the cellular level, HepaRG cells are more complete in terms of CYP and/or P450 metabolic enzymes than Huh7 and Hep3B cells [45]-[48]. The absence of certain types of enzymes may explain the differences in concentration values observed in Huh7 and Hep3B.

4.4. Evaluation of Anti-Inflammatory Activity: Inhibition of Lipoxygenase

The results of the evaluation of the anti-inflammatory activity of our extracts on lipoxygenase are visible in the tables and graphs that follow (**Tables 6-9**, **Figures 5-9**).

- Part: Leaves

- ***Results***

Table 6. Results showing the inhibition percentages of the hydroalcoholic extract from the plant's leaves.

LEAVES H-E		
Concentrations in mg/mL	Inhibition percentages (%)	Standard deviations
0.01	2.68	0
0.02	7.11	0.006666667
0.04	27.95	0.004355556
0.08	86.60	0.006466667

IC_{50} = 0.05281mg/L.

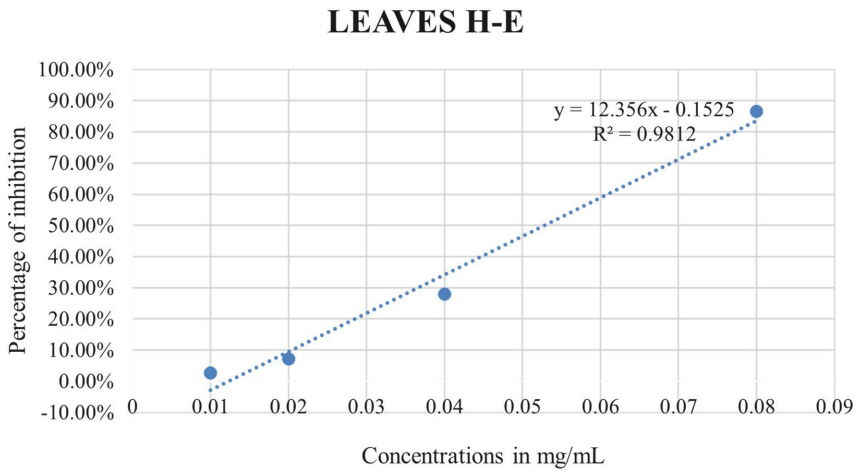

Figure 5. Graph showing the percentage inhibition of lipoxygenase as a function of the concentration of the hydroalcoholic extract from the plant's leaves.

Table 7. Results showing the inhibition percentages of the aqueous extract of the plant's leaves.

LEAVES H		
Concentrations in mg/mL	Inhibition percentages (%)	Standard deviations
0.01	1.02	0.00175
0.02	1.46	0.0035
0.04	3.52	0.0336
0.08	25.52	0.023311111

IC_{50} = 0.15394 mg/L.

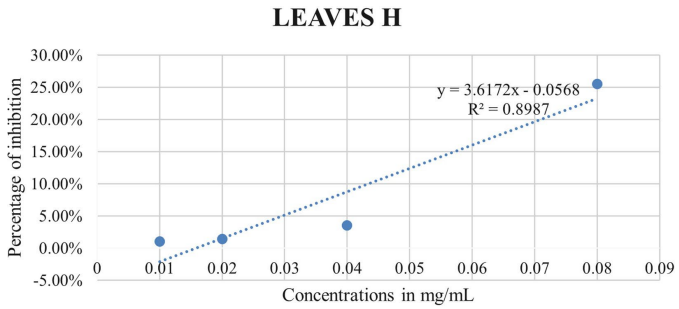

Figure 6. Graph showing the percentage inhibition of lipoxygenase as a function of the concentration of the aqueous extract from the plant's leaves.

- Section: Roots

- ***Results***

Table 8. Results showing the inhibition percentages of the hydroalcoholic extract from the plant's roots.

ROOTS		
Concentrations in mg/mL	Inhibition percentages (%)	Standard deviations
0.01	0.00	0
0.02	1.57	0
0.04	25.72	0.012644444
0.08	38.96	0.029777778

IC_{50} = 0.09429 mg/L.

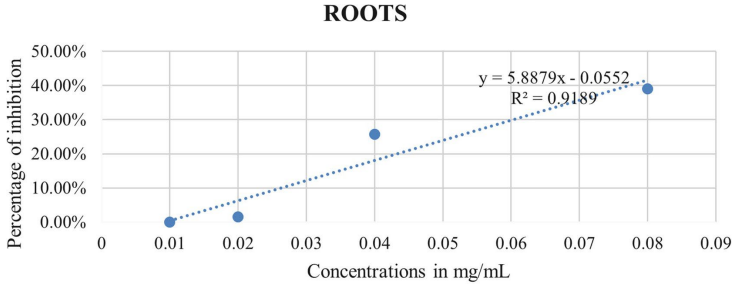

Figure 7. Graph showing the percentage inhibition of lipoxygenase as a function of the concentration of the hydroalcoholic extract from the plant's roots.

- Part: Bark

- ***Results***

Table 9. Results showing the percentage inhibition of the hydroalcoholic extract from the plant's bark.

BARKS		
Concentrations in mg/mL	Inhibition percentages (%)	Standard deviations
0.01	0.00	0
0.02	6.54	0.0586
0.04	9.23	0.012444444
0.08	63.74	0.023266667

IC_{50} = 0.07058 mg/mL.

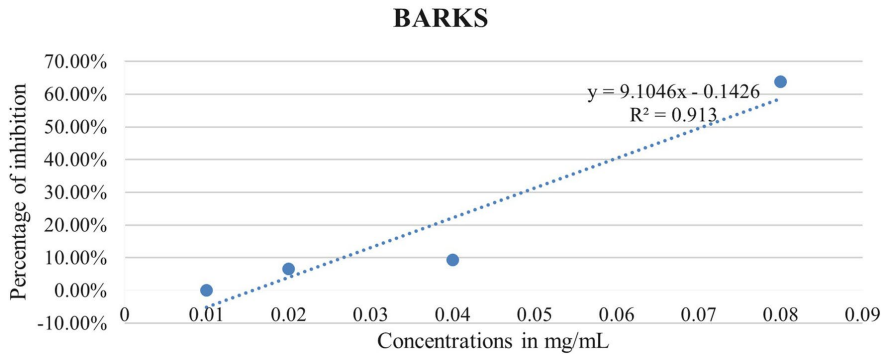

Figure 8. Graph showing the percentage inhibition of lipoxygenase as a function of the concentration of the hydroalcoholic extract of the plant's bark.

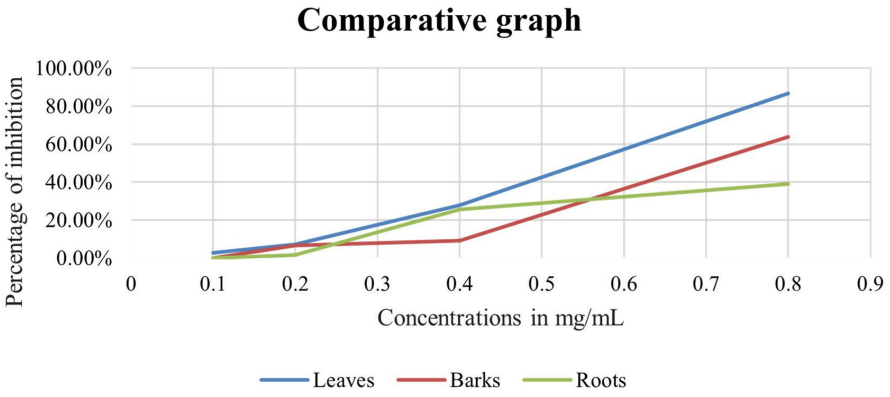

Figure 9. Comparative graph showing the percentage inhibition of lipoxygenase as a function of the concentration of hydroalcoholic extracts from different parts of the plant.

The results show that our different extracts had IC_{50} values higher than that of the inhibition standard, quercetin (IC_{50} = 0.007 mg/mL), used in the study. Among the extracts from the different parts of the plant, the leaf extract provided the best enzyme inhibition, with an IC_{50} value of 0.05281 mg/mL, which is higher than the reference value for quercetin. It was followed by the hydroalcoholic ex-

tract of the trunks, which had an IC_{50} value of 0.07058 mg/mL. The roots ranked third with an IC_{50} value of 0.09429 mg/mL. To justify the choice of solvent used in the study, the inhibition capacity of the aqueous extract of the most active part, namely the leaves, was verified in parallel and yielded an IC_{50} of 0.15394 mg/mL. This value reinforces the accepted idea of the superior potential of hydroalcoholic extracts compared to aqueous extracts of the same plant part for lipoxygenase inhibition.

An extract is considered highly active and promising when its IC_{50} is less than or equal to 23 µg/mL. It is considered moderate or good when its IC_{50} is between 23 and 53 µg/mL, less or weak between 53 and 83 µg/mL, and of little significance when it is greater than 83 µg/mL [49]-[52].

According to this classification, the leaf extract with an IC_{50} of 0.05281 mg/mL exhibits good lipoxygenase inhibitory activity. The hydroalcoholic extract of the trunk bark, with an IC_{50} of 0.07058 mg/mL, exhibits less or weak activity. Roots with an IC_{50} of 0.09429 mg/mL exhibit very weak, if not insignificant, inhibitory activity.

Anti-inflammatory properties are often attributed to the presence or detection of a significant amount of phenolic compounds in plant extracts [49] [51]. Chemical screening tests performed on the different extracts revealed a high concentration of compounds from this family in our extracts. The presence of these phenolic compounds could be responsible for their demonstrated anti-inflammatory properties. The IC_{50} values of our extracts were higher than the IC_{50} of 15.27 µg/mL for the aqueous decoction of the leaves obtained by M. Nitiema *et al*., 2023 [53]. This difference could be correlated with the extraction method used in the study

5. Conclusion

Diospyros mespiliformis is a plant highly valued in traditional medicine. Its promising medicinal applications have led to several chemical and biological studies. These studies, conducted on crude extracts, have confirmed its medicinal properties, such as its antioxidant, antibacterial, and anti-inflammatory activity, as well as its non-toxicity for medicinal use. Despite the number of biological studies conducted on the plant and the very satisfactory results obtained, its therapeutic potential remains largely unexplored and requires further research from a chemical perspective.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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