



Phytochemistry, Evaluation of Biological Activities and Cytotoxicity of Leaves and Seeds of the Plant *Lawsonia inermis*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Lawsonia inermis, with its many synonyms including its unfixed size and pyramid-shaped, grey seeds, is a plant with many therapeutic virtues in traditional and pharmacological medicine. Thus, this paper is about it and it is entitled Phytochemical study, evaluation of the biological activity and cytotoxicity of the leaves and seeds of the plant *Lawsonia inermis*.

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This showed that the seeds are richer than the leaves in terms of metabolites for all the organic solvents used, and in terms of biological activity. Antioxidant activity tests showed that the IC50s of ethanolic and acetone extracts from seeds, at 0.05 and 0.104 mg/mL respectively, were higher than those of leaf extracts. The same analysis was made with the antibacterial activity evaluated with acetone extracts on *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Enterococcus faecalis*, where resistance was noted uniquely on *Staphylococcus* with acetone leaf extract and for *Escherichia coli*. And the largest inhibition zone diameters were for acetone seed extract, with a diameter of 19 mm on *Enterococcus faecalis*. Finally, the ethanolic seed extract outperformed the ethanolic leaf extract in the study of cytotoxicity on Hep3B and Huh7 hepatocellular carcinoma and HepaRG hepatocellular carcinoma cells, even though the leaf extract showed a good selectivity index (SI) on Hep3B and Huh7 cells.

Keywords: *Lawsonia inermis*; phytochemical screening; antioxidant; antibacterial; cytotoxicity.

1. INTRODUCTION

Today, new types of disease have emerged and become epidemics or even pandemics. For example, we can cite the hemorrhagic fever disease (Ebola) with a notorious outbreak recorded in 2014-2016 in West Africa that affected large urban centers as well as rural areas. And recently the coronavirus, whose first cases were reported in Wuhan, China, on December 31, 2019. We can also mention malaria, cholera and diabetes, among many others. And let's not forget liver disease, particularly cancer linked to hepatocellular carcinoma (HCC), known as the primary liver tumor (Goossens et al., 2018). These various diseases are often linked to viruses and bacteria. As a result, the perpetual quest for a medicinal solution remains still topical. Research could therefore focus on plants. After all, medicinal plants are a source of natural substances and bioactive compounds with a wide range of biological activities (Bouterfas et al., 2014; Haddouchi et al., 2016; Allison et al., 2014).

Over the last few decades, the medical profession has become increasingly aware of the therapeutic value of plants for the effective treatment of a wide range of ailments. With this in mind, our research is based on *Lawsonia inermis*, a plant cultivated in many Senegalese villages (Diop et al., 2018). Apart from its high coloring potential (Mabika et al., 2013), this plant is rich in bioactive compounds in its various parts (Moutawalli et al., 2023; Tan et al., 2013). For it is now considered a valuable source of unique natural products for the development of drugs against various diseases (Balaei-Kahnamoei et al., 2021; Chaudhary et al., 2010; El-Haoud et al., 2018). This research is divided into three parts, after giving an overview of the plant in question.

First, a phytochemical study to identify secondary metabolites in ethanolic, acetone and hexane extracts of *Lawsonia inermis* leaves and seeds.

Then the biological study to evaluate the antioxidant activity of *Lawsonia inermis* leaf and seed extracts in different solvents (ethanol, acetone and hexane) and the antibacterial activity of acetone leaf and seed extracts on *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli* and *Pseudomonas aeruginosa*.

And finally, the cytotoxic study of ethanolic leaf and seed extracts on Hep3B and Huh7 hepatocellular carcinoma cells and HepaRG hepatoma cells. All this will enable us to compare the activity of leaves and seeds with other works, but above all with each other.

2. BOTANICAL DESCRIPTION

There are several synonyms for the expression: *Lawsonia inermis*. *L. falcata* Lour (Johnson et al., 2019), *lawsonia purpurealame*, *alcanna spinosa*, *casearia multiflora*, *lawsonia inermis speciosa* and *lawsonia inermis spinosa* (Al-Snafi, 2019). The Wolofs call it fouden, the Bambaras call it dabé débi or débé and the Sereres call it fuden or fudand, while the Diolas call it fudal or dudol (Sanogo et al., 2008). And its taxonomic classification can be broken down as follows: it belongs to the Lythraceae family (phylum: *spermatophyta*, class: *magnoliopsida*, genus: *lawsonia*, species: *Lawsonia inermis*, kingdom: *plantae*, order: *myrtale*) (Chaudhary et al., 2010; Ghédira & Goetz, 2017; Lemordant & Forestier, 1983; Muhammad & Muhammad, 2005; Nayak et al., 2007; Semwal et al., 2014).



Photo 1. Leaves and seeds

3. MATERIALS AND METHODS

3.1 Harvesting *Lawsonia inermis* Leaves and Seeds

The leaves and seeds of the plant in question were harvested in one of the districts of Tivaouane known as Beyti Balla Dia, a former village now attached to the town. The seeds were collected in October and the leaves in December 2023. Grinding was carried out in the ECOT laboratory

3.2 Maceration

For the solvent's ethanol and acetone, 100 g of seed or leaf powder were macerated in 250 mL and 200 mL respectively. However, for hexane, 10 g of seed and leaf powder were used in a volume of 50 mL. Maceration procedures were virtually identical, lasting 24 hours with magnetic stirring. After filtration under a Büchner, the solvents were evaporated to obtain the noisy extracts, followed by drying in an oven. Antibacterial tests and metabolite detection were carried out with these extracts.

3.3 Chemical Screening

Phytochemical screening reveals the presence of groups of chemical families and metabolites in an extract. We highlighted alkaloids, polyphenols, sterols, terpenes and flavonoids in leaves and seeds using the protocol used by Kallo (Kallo et al., 2018) with a few modifications. As for saponin tests, we used the protocol of Bekro, limiting ourselves to the foam test (Bekro et al., 2007).

3.4 Biological Activity

Antioxidant activity tests: The protocol of Bouchenak (Bouchenak et al., 2020) was used

as a reference for extractions. For each leaf and seed category, a mass (m_1) of 1 g is used in 50 mL of solvent.

The DPPH radical was used as it is generally one of the most widely used compounds for rapid and direct assessment of antioxidant activity, due to its stability in radical form and simplicity of analysis. These tests are performed by taking 200 μ L of each dilute solution of the extracts obtained, then adding 3.8 mL of DPPH and incubating for 30 min, followed by an absorbance reading.

Antibacterial activity tests: Antimicrobial activity of the extracts was determined by the diffusion method in Mueller Hinton medium, a widely used medium for antimicrobial susceptibility testing (Sacchetti et al., 2005). Thus, the antibacterial activity of acetone extracts from the leaves and seeds of *Lawsonia inermis* was estimated in terms of the diameter of the zone of inhibition. Strains (*Staphylococcus aureus* 29213 ATCC, *Pseudomonas aeruginosa* 27853 ATCC and *Enterococcus faecalis* 29212 ATCC and *Escherichia coli* 25922 ATCC) to prepare 0.5 MacFarland microbial inoculum in tubes containing physiological water. These are then swabbed into petri dishes containing Mueller Hinton agar. Blotting discs impregnated with 30 μ L of the extracts to be tested are placed in these dishes. They are then incubated in an oven at 37°C for 24 hours. The diameters of the zones of inhibition are read. MICs are determined using initial solutions of 60 mg/mL (FAC and GAC). 100 μ L of each extract is taken to proceed to a one-half ($\frac{1}{2}$) dilution in cascade in order to obtain varied concentration ranges (C1...to C10) according to Bouharb (Bouharb et al., 2014). A precise and equal volume of each dilution is introduced into a well with 20 μ L of microbial inoculum. These are then incubated in the oven at 37°C for 24 hours. Finally, the wells

that give a clear result are correlated with the negative control (absence of microbial growth) and the wells that give a turbid result are correlated with the positive control (microbial growth), or the latter two were isolated as a control.

Viability tests: 5 g of *Lawsonia inermis* L. (leaves or seeds) were macerated in absolute ethanol (50 mL) for 24 hours. The ethanol extracts were filtered and the filtrate centrifuged (2000 rpm, 10 min) to remove any residual particles. The supernatants were evaporated in a SpeedVac Vacuum concentrator at low temperature (< 40°C). Dry extracts were dissolved in DMSO at a concentration of 100 mg/mL, vortexed and stored at -20°C prior to biological evaluation.

The *HepaRG* undifferentiated cell line was purchased from Biopredic International. Cells were thawed and cultured in medium composed of Williams E medium supplemented with 10% FBS (Gibco) and L-glutamine (Gibco) for two (2) weeks. To induce differentiation, cells are then cultured in a growth medium consisting of the same medium as above, supplemented with 5 µg/mL insulin and 50 µM hydrocortisone hemisuccinate for two (2) weeks, followed by the same medium supplemented with 2% DMSO. This medium is renewed every three (3) days for two (2) weeks to obtain hepatocyte-type cells surrounded by biliary-type cells.

Hep3B and *Huh7* human hepatocellular carcinoma cells were cultured in DMEM medium (Gibco) supplemented with 10% FBS (Gibco), L-glutamine (Gibco), sodium pyruvate (Gibco) and

non-essential amino acids, then trypsinized for subculture every three (3) days.

Hep3B (ATCC) or *Huh7* (Créative Biolabs) cells were trypsinized and seeded in 96-well plates at a density of 5000 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 each test plant extract from a concentration range of 0 to 200 µg/mL for three (3) days (*Hep3B* and *Huh7* cells) or five (5) days (*HepaRG* cells). Quantification of intracellular ATP was measured by adding 50 µL of CellTiter-Glo reagent (Promega). After two (2) min of orbital shaking, 100 µL of the reaction well was transferred to an opaque plate for 10 min prior to luminescence quantification (Fluoroskan, Thermo Scientific). Inhibition of cell viability was calculated relatively to control cells treated with 0.1% DMSO. GraphPad prism 6.0 was used to calculate IC50. Experiments were repeated at least three (3) times independently to calculate standard deviations.

4. RESULTS AND DISCUSSION

4.1 Maceration

Most of the mass obtained directly from extracts are greater than those calculated from pomace, not counting those produced with hexane, since the extraction was repeated and the masses used were not the same. These masses can vary following the solvents and volumes used, what may influence yields.

Table 1. Masses of seed and leaf extracts obtained for metabolite and antibacterial activity tests

Extract mass (g)	Hexane	Acetone	Ethanol
MEF	0.4221	12.21125	16.209
MEG	0.3135	8.9212	15.9575
MEMF	1.1226	11.9596	11.1969
MEMG	1.9809	10.2351	11.759

MEF: leaf extract mas, MEMF: leaf pomace extract mass, MEG: seed extract mass, MEMG: seed pomace extract mass

4.2 Chemical Screening

The richness of *Lawsonia inermis* in secondary metabolites is no longer in question. Indeed, several molecules have been isolated from this plant (Musfiroh et al., 2024; Tuan et al., 2022). Ethanol was found to be the solvent that extracted the most metabolites. In the leaves, flavonoids were the metabolites found least frequently, as they were absent in all extracts. We can also learn from these results that, in terms of metabolites, seeds are richer than leaves. Furthermore, it has been reported that seeds are a reserve of bioactive potential (Balaei-Kahnamoei et al., 2021; Nounah et al., 2017).

Table 2. Results of screening tests carried out on seeds and leaves

Metabolite and reagent		Leaves			Seeds		
		Acetone	Ethanol	Hexane	Acetone	Ethanol	Hexane
Alkaloid	Dragendorff	-	+	+	+	+	+
	Wagner	-	+	+	+	+	+
Sterol and Terpene	Libermann	+	-	+	-	-	+
Saponin	Distilled water	+	+	-	+	+	+
Polyphenol	FeCl ₃	+	+	-	+	+	-
Condensed tannin	HCl hot	-	-	+	+	+	+
Flavonoid	Wilstater	-	-	-	+	+	-

4.3 Biological Tests

Evaluation of antioxidant activity: The observation is that yields are proportional to solvent polarity. Greater solvent polarity leads to a higher yield. Another point to note is that the leaves produced the highest yields compared with the seeds. However, during extraction, it is difficult to collect all the powder after filtration, then the yields reveal approximate values.

Percentage inhibition (PI) is calculated after obtaining the different absorbances: blank, control and extracts. Their representation as a function of concentration gives the IC₅₀. The IC₅₀ expresses the quantity of antioxidant required to reduce the concentration of the DPPH free radical by 50%. Antioxidant powder actively increased proportionally with concentration (Hsouna et al., 2011; Yusuf et al., 2012).

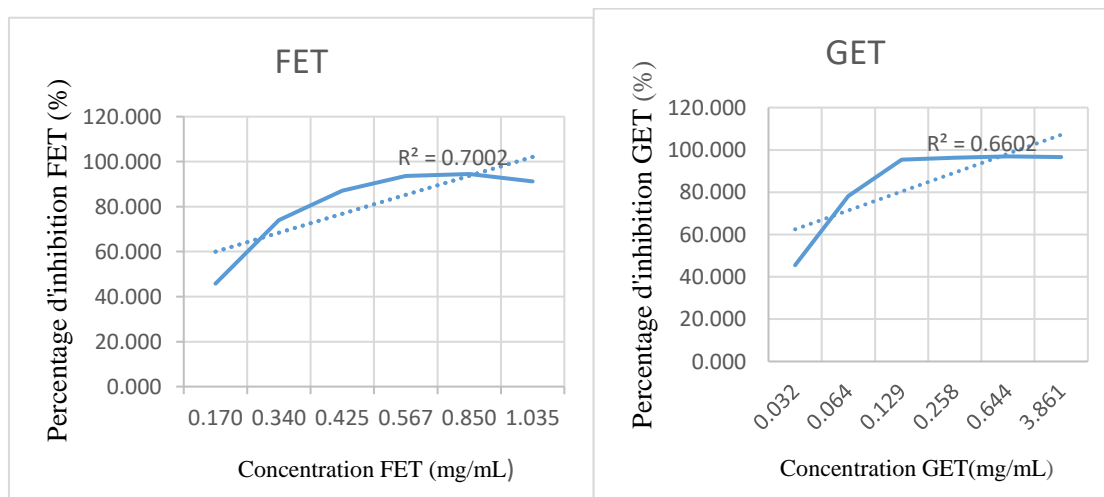
Table 3. Masses of pomace leaf and seed extracts and yields for antioxidant tests

Extracts	FET	GET	FAC	GAC	FHE	GHE
Mass in g	0.64	0.82	0.77	0.87	0.95	0.96
Yields in %	36	18	23	13	5	4

FAC: acetone leaf, GAC: acetone seed, FET: ethanol leaf, GET: ethanol seed, FHE: hexane leaf, GHE: hexane seed.

Table 4. PI of FET and GET extract of *lawsonia inermis* as a function of concentration

Concentration FET (mg/mL)	PI FET (%)	Concentration GET (mg/mL)	PI GET (%)
0,172	45,765	0,032	45,570
0,189	50	0,050	50
0,340	74,049	0,064	78,137
0,425	87,152	0,129	95,334
0,567	93,640	0,258	96,293
0,857	94,503	0,644	96,932
1,035	91,147	3,861	96,612

**Fig. 1. Percentage inhibition of *lawsonia inermis* leaf and seed extracts with the solvent ethanol as a function of their concentrations**

These graphs show the trend, the linear lines, their correlation coefficients and the shape of the curves showing the percent inhibition of leaf and seed extracts with the solvent ethanol as a function of concentration.

Table 5. PI of FAC and GAC extracts of *lawsonia inermis* as a function of concentration

Concentration FAC (mg/mL)	PI FAC (%)	Concentration GAC (mg/mL)	PI GAC (%)
0,129	42,985	0,042	26,859
0,143	50	0,085	39,467
0,258	73,442	0,104	50
0,322	83,477	0,170	85,657
0,429	90,700	0,340	93,185
0,644	89,390	0,850	95,663
1,228	75,551	1,700	95,260

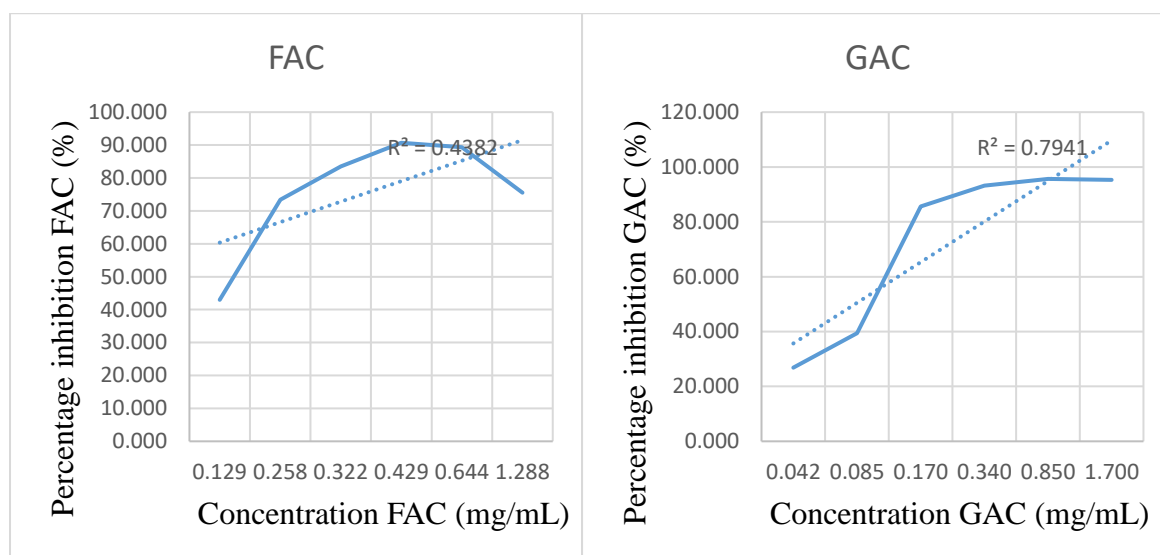


Fig. 2. PI of leaf and seed extracts of *lawsonia inermis* with the solvent acetone as a function of their concentrations

These graphs show the trend of the linear lines and their correlation coefficients, and the shape of the curves showing the percent inhibition of leaf and seed extracts with the solvent acetone as a function of concentration.

Table 6. Antioxidant capacity of extracts

Extracts	Antioxidant capacity (mg/mL)
FAC	5.33709091±24.0522847
GAC	7.046060606±41.98911049
FET	4.29181818±27.2727273
GET	1.600363636±27.4539434
FHE	0.01231212121±3.441762634
GHE	0.01167575758±2.777318603

This table shows the significant antioxidant activity of the different extracts, except for those obtained with hexane, which are very low.

The IC50s of our various extracts are shown in Table 7.

Table 7. IC50 of extracts (mg/mL)

CI50 FET	0,189
CI50 GET	0,050
CI50 FAC	0,143
CI50 GAC	0,104

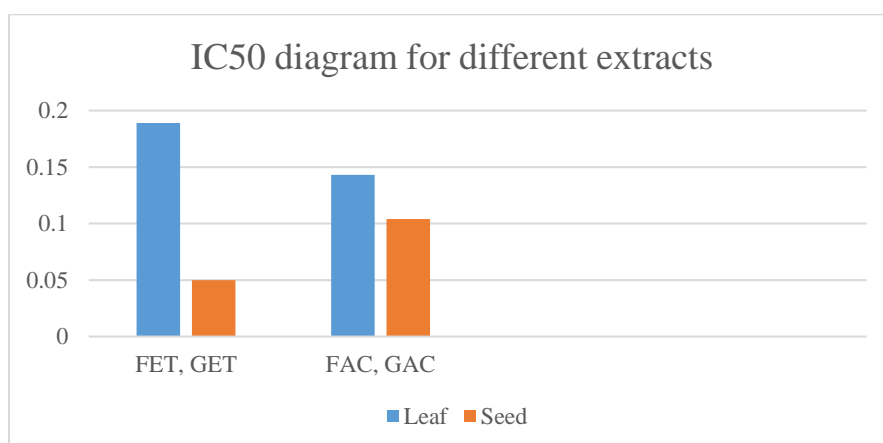


Fig. 3. IC50s for leaf and seed extracts

Our results reinforce the research reported in the literature (Elaguel et al., 2019; Guha et al., 2011; Kumar et al., 2017; Rahmani et al., 2021). They used 5 solvents of different polarities to study the free radical scavenging activity of *Lawsonia inermis* leaf extract: water, methanol/water, dichloromethane, ethyl acetate and finally 1-butanol. Added to our 3 solvents, this makes a total of 8 different solvents. Comparing the IC50s of our extracts with those of their research work, we can see that the concentrations of our leaf extracts are better at inhibiting the DPPH radical at 50% than their extracts, except for that produced by ethyl acetate, which has a very high capacity to inhibit the radical at 50%, with an IC50 value of 8.610-6. Added to this is the study carried out by H. Enneb et al., (2015), which gives us a global overview of antioxidant content in all parts of *Lawsonia inermis*. In fact, their research focused on leaves, stems and roots, and the study of our seeds covers all parts of *Lawsonia inermis*. The result: the methanoic extract of the leaves has a greater inhibitory capacity for the DPPH radical, with an IC50 of 25.73 µg/mL, which is twice as high as the activity of our seed extract with ethanol, the latter showing the best activity in our tests. From these analyses, we can draw the conclusion that, taking the whole plant, we could not assert that one part of the plant presents the best activity towards the other. Rather, this parameter could be linked to other factors (Zbadi et al., 2018). Such as environmental factors (climate, soil, season...), harvesting area, solvents or extraction.

To sum up, our research on the antioxidant activity of *Lawsonia inermis* seeds and leaves. We can say that the antioxidant capacities recorded in Table 7 gave an insight into the antioxidant activity (the capacity to inhibit the DPPH radical) of our extracts. This is because they enabled us to carry out the anti-radical tests on the extracts with the highest antioxidant capacity, i.e. extracts with acetone and ethanol solvents. Moreover, with the IC50s, we could affirm that the seed extracts provided the best activities for each solvent (ethanol and acetone). Compared with the other extracts, the ethanoic seed extract provided the best activity. Between the leaves, the acetone extract gave the best antioxidant activity. These could be justified by the fact that plants are made up of secondary metabolites, and these include a class of molecules with the power to trap free radicals, but also a class with very important antioxidant characteristics. This is the case of phenolic compounds (flavonoids and phenolic acids) (Trigui et al., 2013; Uddin et al., 2011). Consequently, our results are in agreement with the phytochemical tests we carried out with the leaves and seeds of *Lawsonia inermis*. In fact, the seeds contained more metabolites than the leaves.

Antibacterial activity: The different bioactive compounds could explain the differences observed in the antibacterial activity of extracts from the same plant species (Khantamat et al., 2021; Zohourian et al., 2012). These tests showed that the extracts had different activities against the three strains, with

Table 8. Inhibition zone diameters for extracts and control antibiotics

<i>Pseudomonas aeruginosa</i>	Extracts		Reference antibiotic	
	FAC	GAC	Ciprofloxacin (Cip)	Amikacin (Ami)
Inhibition diameter (mm)	08	12	25	20
<i>Enterococcus faecalis</i>	Extracts		Reference antibiotic	
	FAC	GAC	Ciprofloxacin (Cip)	Vancomycin (Van)
Inhibition diameter (mm)	08	19	24	15
<i>Escherichia coli</i>	Extracts		Reference antibiotic	
	FAC	GAC	Ciprofloxacin	Ceftriaxone
Inhibition diameter (mm)	//	//	30	28
<i>Staphylococcus aureus</i>	Extracts		Reference antibiotic	
	FAC	GAC	Vancomycin (Van)	Erythromycin (Ery)
Inhibition diameter (mm)	//	12	18	25

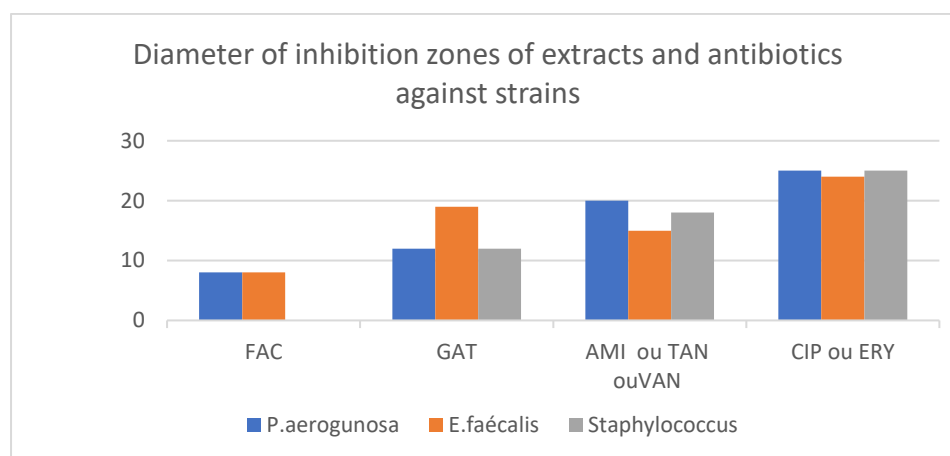


Fig. 4. Inhibition zone diameters for antibiotics and FAC and GAC extracts

the exception of FAC against *Staphylococcus*, but no activity against *Escherichia coli*. The following table shows the diameters of the zones of inhibition.

The analysis that can be made is that, for *Enterococcus* and *Pseudomonas aeruginosa*, their inhibition zone diameters are equal (8 mm) for FAC. The same applies to GACs on *Staphylococcus* and *Pseudomonas aeruginosa*, with a diameter of 12 mm. The inhibition diameter (19 mm) recorded with *Enterococcus faecalis* with GAC is greater than those recorded with all other strains. This diameter is even comparable with the control antibiotics (Ami and Van). According to one of the references on zones of inhibition, the zone diameter can be broken down as follows. Extremely sensitive $d \geq 20$ mm, very sensitive $15 \leq d \leq 19$, sensitive $9 \leq d \leq 14$ and insensitive $d \leq 9$ (Güler et al., 2023) we can conclude that the leaves have just a low sensitivity but the seeds have a medium or even very sensitive sensitivity (Lattab et al., 2017).

This confirms the antibacterial activity of GAC on *Pseudomonas aeruginosa*.

No zone of inhibition diameter was recorded with FAC on *Staphylococcus*. On the other hand, comparison of the GAC inhibition zone diameter (12 mm) with that of the gel composed of *lawsonia inermis* extract and starch glycerol (8 to 10 mm) proves GAC activity on *Staphylococcus* (Elebeedy et al., 2022). However, a double value was noted at 200 mg/mL concentration and roughly equal to 25 mg/mL concentration with the aqueous lime extract of *lawsonia inermis* on *Staphylococcus aureus*, and a much larger diameter with the ethanolic extract (28; 42 mm) (Elizabeth et al., 2022; Hasan & Ismael, 2018). This suggests that extraction parameters such as solvent polarity, extraction type and method may influence antibacterial activity.

Minimum sensitivity concentrations were then determined and reported in the Table 9.

Table 9. Minimum inhibitory concentrations (MICs) of acetone extracts and control antibiotics on the three strains

Tested strains ($\mu\text{g/mL}$)		<i>Pseudomonas aeruginosa</i>	<i>Enterococcus faecalis</i>	<i>Staphylococcus aureus</i>
C1 = 586	FAC	-	-	-
	GAC	-	-	-
C2 = 1171	FAC	-	-	-
	GAC	-	-	-
C3 = 2338	FAC	+	-	-
	GAC	-	-	-
C4 = 4688	FAC	+	-	-
	GAC	-	-	-
C5 = 9375	FAC	+	-	-
	GAC	-	-	-
C6=18750	FAC	+	-	-
	GAC	-	-	-
C7=375.10 ²	FAC	+	-	-
	GAC	+	-	-
C8 = 75.10 ³	FAC	+	+	-
	GAC	+	-	-
C9 = 15.10 ⁴	FAC	+	+	-
	GAC	+	+	-
C10 = 3.10 ⁵	FAC	+	+	-
	GAC	+	+	+
T+		+	+	+
T-		-	-	-

At C3 = 2338 $\mu\text{g/mL}$, we counted the MIC of *Pseudomonas aeruginosa* with leaf extract and C7 = 375.102 $\mu\text{g/mL}$ for seeds, for *Enterococcus faecalis* the MIC was spotted at C8 = 75.103 $\mu\text{g/mL}$ with leaves and at C9 = 15.104 $\mu\text{g/mL}$ for seeds and the MIC of *Saphylococcus* is lastly C10 = 3.105 $\mu\text{g/mL}$. Given these MICs, we can say that they are independent of the diameters of the inhibition zones. This is because the GAC extract shows the largest inhibition diameters, while the FAC extract shows the highest MICs.

Viability tests:

Table 10. Results of cell viability tests with FET and GET extracts

IC ₅₀ ($\mu\text{g/mL}$)	HUH7	Hep3B	HEPA-RG	SI HUH7	SI Hep3B
FET	70.04 \pm 5.2	62.29 \pm 8.8	> 150	> 2.1	> 2.4
GET	37.60 \pm 3.9	51.64 \pm 2.1	87.29 \pm 7.5	2.3	1.7
Sorafenib	1.49 \pm 0.8	2.1 \pm 1.5	NT	NT	NT
Aflatoxin B1	NA	NA	14.2 \pm 2.8	NA	NA

FET: *lawsonia inermis* ethanol leaf extract; GET: *lawsonia inermis* ethanol seed extract; SI: selectivity index; NT: not tested; NA: not active.

For SI, a value of 2 indicates high selectivity of the extract on cancer cells. It is calculated by the ratio of the IC₅₀ value of treatments of non-tumor cells by tumor cells (Canário et al., 2020). The leaves and seeds of *lawsonia inermis* therefore show good selectivity on *Hep3B* and *Huh7* tumor cells. The SI ranged from 1.7 to 2.4.

These results also showed that among the extracts used for cell viability, for all cells (*Huh7*, *Hep3B*, *HepaRG*) the inhibition of viability was greater with the GET seed extract than with the FET leaf extract. In addition, inhibition was greater in *Huh7* and *Hep3B* cells, with IC₅₀ values of 37.60 and 51.64 \pm 2.1 $\mu\text{g/mL}$ respectively. The latter value is not far from the value given in the table summarizing the plants

found in New Caledonia that have been tested on certain cancers. This IC₅₀ value is 48.03 $\mu\text{g/mL}$ and a much smaller value was also mentioned (13.4 $\mu\text{g/mL}$). In the document from which we derived the above information, extracts were also tested on the *Huh7* hepatocellular carcinoma cell line, and most of the IC₅₀s obtained are much smaller than our results, only one of which was marked NA (not active). Apart from values above 20, the value closest to our best percentage is 19 \pm 0.5, about 2 times more inhibitory than our extracts on *Huh7* (Thieury, 2017). All the sorafenib inhibition tests on hepatocellular carcinoma cells (*Hep3B* and *Huh7*) and on *HepaRG* cells showed a major difference compared with all our extracts. This may explain why it is selected for the treatment

of liver-related diseases, in particular to reduce the tumor volume of hepatocellular carcinoma (HCC) tumors in a xenograft model (*Hep3B*) (Brossard et al., 2021). This is not the case for aflatoxin, which is not active (NA) on hepatocellular carcinoma cells (*Hep3B* and *Huh7*) and this explains why it may be involved in or even cause certain liver abnormalities. In fact, aflatoxin can cause liver cancer in humans simply by consuming cultures contaminated with it, even at low levels (Udomkun et al., 2017). Finally, with *HepaRG*, this is not the case with the previous analysis and therefore aflatoxin is not the cause of *HepaRG*-related tumors, which even explains the strong inhibition recorded $IC_{50} = 14.2 \mu\text{g/mL}$.

5. CONCLUSION

These findings have revealed several noteworthy that warrant further investigation. Seeds have consistently showed the highest efficacy among the various samples tested. As far as the metabolite test is concerned, seeds stood out in the metabolite test, producing both the highest metabolite concentration and the most significant biological effects across the same solvents. (antioxidant and antibacterial). A similar trend was observed in cytotoxicity. Further investigation into their toxicity appears mandatory to evaluate their suitability for medicinal applications, in particular for the treatment of diseases linked to the various strains we studied, with the exception of *Escherichia coli*, but also for hepatitis cancers linked to the *HepaRG* hepatoma cells and *Hep3B* and *Huh7* hepatocellular carcinoma. Despite the high selectivity values and cytotoxicity activity, the efficacy of seeds is limited compared with sorafenib. Finally, consistent with existing literature, the remarkable value of the seeds does not preclude the biochemical richness of the leaves of *lawsonia inermis*.

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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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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