WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 14, Issue 16, 1031-1050. Research Article ISSN 2277–7105

EVALUATION OF THE ANTIOXIDANT AND ANTIBACTERIAL ACTIVITY OF *PILIOSTIGMA RETICULATUM* (DC.) HOCHST LEAVES

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Article Received on
01 June 2025,

Revised on 21 July 2025,
Accepted on 10 August 2025

DOI: 10.20959/wjpr202516-37993

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ABSTRACT

This study examined the phytochemical, antioxidant, and antibacterial properties of the leaves of *Piliostigma reticulatum*, a medicinal plant commonly used in West African traditional pharmacopoeia. After extraction using hydro-ethanolic maceration, five fractions were obtained: ethanolic, hydro-ethanolic, aqueous, ethyl acetate, and dichloromethane. A qualitative phytochemical screening revealed the presence of flavonoids, tannins, alkaloids, saponins, coumarins, proteins, and reducing sugars in all fractions. Quantitative assays showed that the ethanolic fraction contained the highest concentrations of flavonoids (240.337 mg ER/g), total tannins (85.567 mg EAG/g), and alkaloids (90.161 mg EQ/g). The evaluation of antioxidant activity, conducted using DPPH and ABTS tests, revealed significant radical-scavenging activity, particularly for the ethanolic fraction, with IC50 values of 0.181 mg/ml for DPPH and 0.019 mg/ml for ABTS. Antibacterial activity tests on reference strains showed notable inhibition, especially for the aqueous and dichloromethane fractions. These results support the traditional use of *Piliostigma reticulatum* and pave the way for future research on the isolation of bioactive molecules.

MOTSCLES: *Piliostigma reticulatum* leaves, phytochemical evaluation, antioxidant and antibacterial activity.

INTRODUCTION

Infectious diseases and oxidative imbalances are major public health challenges, especially in developing countries where access to healthcare and treatments is often limited.[1] In this context, medicinal plants play a crucial role in African traditional medicine due to their richness in bioactive compounds with various pharmacological properties. In sub-Saharan Africa, approximately 80% of rural populations continue to rely on medicinal plants as their first therapeutic option.[2]

Piliostigma reticulatum (DC.) Hochst., a species from the Caesalpiniaceae family, is commonly used in West African traditional pharmacopoeia, particularly in Senegal, Mali, and Burkina Faso.[3,4,5,6] This plant is recognized for its effectiveness in treating various conditions, such as bacterial infections, wounds, digestive disorders,[7] and inflammatory diseases.[8]

Phytochemically, research has shown that the leaves of *P. reticulatum* are rich in saponins, tannins, glycosides, alkaloids, and other phenolic compounds, known for their biological activities, including antioxidant and antibacterial properties.[9] Natural antioxidants play a fundamental role in neutralizing free radicals, which are implicated in oxidative stress related to chronic diseases such as cancer, diabetes, and cardiovascular diseases.[10] Furthermore, in light of the increasing antibiotic resistance reported by the World Health Organization (WHO), the search for new natural antibacterial agents has become a global priority.[11]

Several studies have highlighted the presence of secondary metabolites such as flavonoids, tannins, saponins, and alkaloids in various parts of this plant, suggesting significant therapeutic potential.[7,8,12] However, few in-depth studies have been conducted on the scientific evaluation of its antioxidant and antibacterial properties, particularly against certain resistant strains, from the leaves.

In this study, we conducted a phytochemical investigation followed by an evaluation of the antioxidant and antibacterial activities of extracts from *Piliostigma reticulatum* leaves, aiming to valorize this local natural resource and explore its potential for the development of plant-based therapeutic products.

MATERIALS AND METHODS

Plant Preparation

The leaves of *Piliostigma reticulatum* (DC.) Hochst used in this study were collected on October 28, 2024, in the Ngoundiane forest, located 34 kilometers from the Bambey region. They were carefully washed with distilled water to remove impurities and then dried in the shade at room temperature for several days in the Organic and Therapeutic Chemistry Laboratory (LCOT) at Alioune Diop University of Bambey (UADB), located in Ngoundiane. Once dried, the samples were ground using an electric grinder to obtain a fine, homogeneous powder.

Extraction Method

A quantity of 100 g of the leaf powder was taken for hydro-ethanolic maceration (Ethanol/Water) for 24 hours under magnetic stirring, using a volume of 350 mL with a 50:50 (v/v) ratio. After filtration, the residue was subjected to a second extraction with 150 mL of solvent for 24 hours. The two filtrates were then combined and evaporated using a rotary evaporator at a temperature of 50 °C, followed by drying in an oven at the same temperature. The extraction yield was calculated using the appropriate formula:

$$Y=(Ms/TS)\times 100$$

Y: yield

Ms: mass of dry extract

TS: test sample

Fractionation

A quantity of 10 g of the extract was dissolved in 100 mL of distilled water and subjected to fractionation by decantation using solvents of increasing polarity, namely dichloromethane and ethyl acetate. The obtained organic phases were extracted four times with 100 mL of solvent. An intermediate phase formed during fractionation, which is only soluble in ethanol, was named the ethanolic fraction (F. Eth). The organic and aqueous phases were evaporated, and the different dry fractions were weighed, allowing for the determination of the masses in grams of the dichloromethane fraction (F. Dic.), ethyl acetate fraction (Ace. F), ethanolic fraction, and aqueous fraction (Aq. F).

Phytochemical Screening

Characterization tests for different molecular families were performed on the previous fractions as well as on the hydro-ethanolic fraction (He. F.), which is composed of a certain mass of the aqueous fraction (Aq F.) and ethanolic fraction (Eth. F.). Detection tests for major groups of chemical compounds were conducted according to the methods of Bekro et al. (2008) and Brou et al. (2010), as reported by Faye et al. (2022), Harborne (1984), Parekh and Chanda (2007), and Sani et al. (2024).[13,14,6,15,16,12]

Tannin Characterization: To 2 mL of crude extract, a few drops of a 2% aqueous FeCl3 solution are added. The appearance of a blue-black or green-black coloration indicates the presence of tannoids or true tannins, respectively.

Alkaloid Characterization: For the test, 0.1 g of residue is dissolved in 6 mL of 60% ethanol, distributed into two test tubes. In the first tube, 2 drops of Dragendorff's reagent are added; a reddish-orange or brownish-red precipitate indicates the presence of alkaloids. In the second tube, 2 drops of Burchard's reagent are added; a brown precipitate indicates a positive test.

Coumarin Detection: A few mg of extracts is dissolved in ethanol. In two test tubes, 2 mL of each solution are introduced. In one of the tubes, 0.5 mL of 10% NaOH is added, and the tubes are heated in a water bath until boiling. After cooling, 4 mL of distilled water are added. If the solution in the tube containing the alkaline solution is clearer than that of the control tube, the reaction is positive.

Reducing Sugar Test: Reducing sugars are highlighted in plant fractions using Fehling's reagent. To 5 mL of extract, 5 mL of Fehling's solution are added, and the mixture is heated in a water bath at 70 °C for 3 minutes. The formation of a brick-red precipitate indicates a positive test.

Protein Detection: Samples are dissolved in 2 mL of 20% aqueous NaOH, then 2 to 3 drops of a 2% aqueous $CuSO_4$ solution are added. The appearance of a purple coloration, sometimes with a reddish tint, indicates a positive reaction.

Flavonoid Test: The plant extract is dissolved in 2 mL of diluted NaOH. A yellow solution that becomes pale or colorless after the addition of a few drops of hydrochloric acid indicates the presence of flavonoids.

Saponin Test: For saponins, 0.2 g of the extract is dissolved in distilled water and distributed into several test tubes, then shaken. The presence of persistent foam after moderate heating indicates saponins.

The phytochemical screening was performed on the fractions (Dic), (Ace), (He), (Eth), and (Aq) of the studied plant.

Quantification of Flavonoids, Tannins, and Alkaloids

Flavonoids: They were quantified in the ethanolic, aqueous, and hydro-ethanolic fractions of the hydro-ethanolic extract of Piliostigma reticulatum leaves using the colorimetric method with aluminum trichloride[17] with slight modifications. The principle of this assay is based on the nitration in an alkaline medium of the aromatic ring bearing the catechol group, with sodium nitrite ($NaNO_2$). Thus, 250 µL of the solution to be measured at a concentration of 1 mg/mL is mixed with 75 µL of a 5% nitrite solution. After incubation for 6 minutes at room temperature, 150 µL of a 10% aluminum chloride solution is added, followed by a second incubation of 5 minutes. After the formation of a red coloration, 500 µL of sodium hydroxide (NaOH) at 1M is added. The absorbance is measured after 30 minutes of incubation at a wavelength of 510 nm against a blank using a UV-visible spectrophotometer. The observed results were expressed in mg equivalent rutin /fraction (mg ER/g).

Tannins: Total tannins were quantified according to the Prussian method described by Graham (1992)[18], based on the formation of a blue complex with potassium ferricyanide in the presence of Fe^{3+} ions. 0.1 mL of solution is mixed with 6.9 mL of distilled water in a test tube, 1 mL of potassium ferricyanide solution at 0.008 M, and 1 mL of ferric chloride at 0.2 M in a 0.1 M HCl solution. The absorbance is measured at 700 nm after the appearance of blue coloration. The calibration curve was made from a stock solution of gallic acid, and the result is expressed in mg EAG/fraction.

Alkaloids: The quantification of alkaloids was performed according to the spectrophotometric method described by Sreevidya and Mehrotra (2003)[19], which involves precipitation with Dragendorff's reagent, followed by a bismuth complex. In practice, we first maintained a volume of 1 mL of the solution to be measured at an acidic pH (2 or 2.5) with HCl. The acidic mixture is treated with 0.4 mL of Dragendorff's reagent to precipitate the alkaloids present. After centrifugation, the precipitate is washed with an alcoholic solution, then treated with 0.4 mL of sodium sulfate solution (0.5 M) before undergoing a second

centrifugation. The final residue obtained was dissolved in 0.4 mL of concentrated nitric acid at high temperature, then this solution was diluted with 1 mL of distilled water. An equal volume of 2.5 mL of a urea solution (5 g/L) is then added to 0.5 mL of the previously diluted mixture to form a characteristic yellow complex. The absorbance of the mixture is measured at 435 nm using a spectrophotometer. A calibration curve is made from a standard solution of quinidine, and the results are expressed in milligrams equivalent of quinidine per gram of dry fraction (mg EQ/g).

Tested Microorganisms

The bacterial strains used were obtained from the National Public Health Laboratory in Thiès. The tests were conducted in the microbiology laboratory of the Mixed Research Unit for Exploitation and Diagnosis (UMRED) at the Training and Research Unit in Health at the University Ibader Thiam in Thiès (UIT). The tested strains include: *Escherichia coli ATCC* 25922, *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212.

Antibacterial Activity

The sensitivity study was performed using the agar diffusion method, in accordance with the recommendations of CASFM 2023.[20] The sensitivity of the reference strains was primarily tested against the fractions obtained by liquid-liquid separation of the crude extract. A series of dilutions was performed to obtain concentrations of 3.125, 6.25, 12.5, 25, 50, and 100 mg/mL.

Inoculum Preparation

The reference strains were first subcultured on Mueller Hinton agar and then incubated at 37 °C for 18 to 24 hours. The next day, a bacterial suspension was prepared by taking 2 to 3 colonies from pure cultures and placing them in 1 mL of sterile saline solution (0.9% NaCl), adjusted to McFarland standard 0.5 (1 to 2 x 10^8 CFU/mL). This suspension was inoculated by swabbing onto Mueller-Hinton media within the next 15 minutes.

Application of Sterile Discs Impregnated with Plant Extract

Sterile discs (6 mm) were impregnated with the plant fractions (10 µL) and then placed on the surface of the agar using tweezers, maintaining a minimum distance of 15 mm between the edge of the plate and the peripheral discs, and 30 mm between the discs. The plates were then incubated at 37 °C for 24 hours before analysis.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC was determined by the liquid microdilution method according to the protocol described by Haddouchi et al. (2016)[21], with some adaptations. The fractions were first dissolved in 1% DMSO and then diluted in Mueller-Hinton broth to obtain a range of decreasing concentrations in 96-well microtitration plates. Each well received a standardized microbial suspension at 5×10^5 CFU/mL for the bacteria. After incubation at 37 °C for 24 hours, microbial growth was visually assessed by the absence of turbidity. The MIC is defined as the lowest concentration of fraction inhibiting any visible growth. Gentamicin was used as a reference antibiotic.

Antioxidant Activity

DPPH Method: The test with 2,2-diphenyl-1-picrylhydrazyl (DPPH) relies on the ability of antioxidant compounds to scavenge the free DPPH radical, which decreases the intensity of its purple color. In the presence of active compounds, the color changes from purple to yellow, varying according to the different concentrations of the fractions.[12] The test was performed according to the method of Molyneux (2004)[22], with slight modifications.

Preparation of DPPH Solution and Samples: The solution is prepared by dissolving 6 mg of DPPH powder in 150 mL of ethanol, then kept away from light for 12 hours. 10 mg of each fraction were dissolved in 10 mL of ethanol. These solutions, called "mother solutions" at a concentration of 1 mg/mL, underwent a series of dilutions to obtain concentrations of 1.9, 3.9, 7.8, 15.6, 31.25, 62.5, 125, 250, and 500 µg/mL ("daughter solutions"). From each "mother" and "daughter" solution, a volume of 50 µL was taken and mixed with 2 mL of DPPH solution.

Control Antioxidant: The antioxidant used in this study is ascorbic acid or vitamin C. A solution of the antioxidant at a concentration of 1 mg/mL underwent the same series of dilutions. The same volume of DPPH solution was added to the ten solutions taken (50 µL each) with different concentrations. After 30 minutes of incubation in the dark, absorbances were measured using a spectrophotometer at a wavelength of 517 nm.

Determination of Inhibition Percentages for Different Fractions: The absorbance of the blank DPPH was recorded. For each fraction, three measurements were noted. The percentages of inhibition of the various fractions were calculated according to the following formula[23]

$$\% \text{ inhibition} = [(A_{control} - A_{extract}) / A_{control}] \times 100$$

The concentrations at which the fractions neutralize 50% of free radicals (IC50) were determined according to the equations of the inhibition percentage curves plotted against concentrations on Excel.

ABTS Method: The anti-radical activity was evaluated using the discoloration test employed by Khan et al. (2012)[24], described in Dieng et al. (2017).[25] The ABTS+ radical cation solution obtained after 12 to 16 hours of incubation was diluted with ethanol. The absorbance read on the spectrophotometer at 734 nm was evaluated at 0.700 ± 0.02. Using the same range of concentrations (1.9, 3.9, 7.8, 15.6, 31.25, 62.5, 125, 250, and 500 µg/mL), after taking 50 µL from each fraction, a volume of 1.5 mL of ABTS solution was added, along with the reference antioxidant (ascorbic acid). The mixture was incubated for 10 minutes, and the different absorbances were measured at a wavelength of 734 nm.

RESULTS AND DISCUSSION

Results

1) Yield of extraction

- **Crude extract (sample**

Table 1: Test portion (leaf powder) in g Weight of dry extract in g Yield in percent.

Test (portion power) in g	Weight of dry extract in g	Yield (%)
100	14,04	14,04

- **Samples fractions**

Table 2: Quantity of the different fractions in g and their yield in percent.

Solvents	Weight (in g)	Yield (%)
Dichloromethane	0.1	1
Ethyl acetate	0.873	8.73
Ethanol	3.27	32.7
Water	6.19	61.9

2) phytochimical Screening

a. Qualitative study

Table 3: Secondary metabolite composition of *Piliostigma reticulatum* leaf fractions.

Secondary metabolites	Aqueous F.	Ethanolic F.	Hydro-ethanolic F.	Acétalic F.	Dichloromethane F.
Alkaloids	+	+	+	+	-
Tannins	+	+	+	-	-

Flavonoids	+	+	+	+	+
Saponins	+	+	+	-	-
Reducing Sugars	-	+	+	-	-
Coumarins	-	-	-	+	+
Proteins	-	-	-	-	-

b. Quantitative study

c. Case of flavonoids

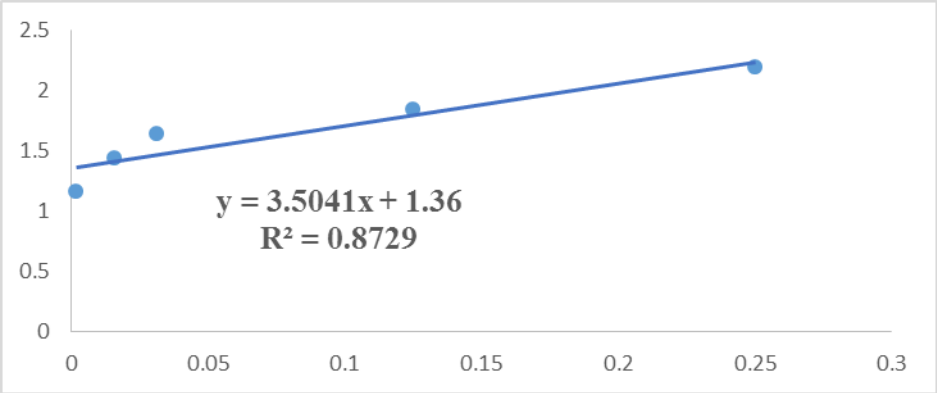


Figure 1: Average absorbance values as a function of rutin concentrations

Table 4: Amount of flavonoides contained in the sample in rutin equivalent per fraction.

Fractions	Concentrations of fractions in mg ER/mL	Flavonoid content in mg ER/g of fraction
EtOH f.	0.240337	240.337± 0.058
HE F.	0.091987	91.987± 0.050
Aq F.	0,131132	131.132± 0.048

- **Case of Tannins**

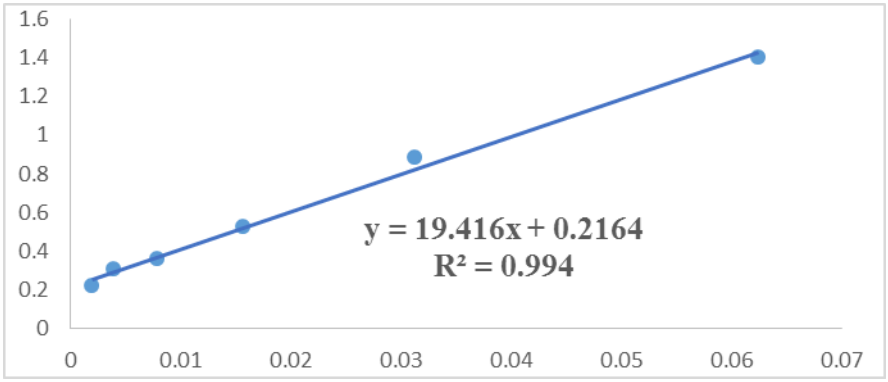


Figure 2: Average absorbance values as a function of gallic acid concentrations.

Table 5: Amount of tannins contained in the sample in gallic acid equivalent per fraction.

Fractions	Concentrations of fractions in mg EAG/mL	Tannins content in mg EAG/g of fraction
F. EtOH	0.085567	85.567± 0.002
F. HE	0.084119	84.119± 0.009
F. Aq	0.017159	17.159± 0.003

- **Case of Alkaloids**

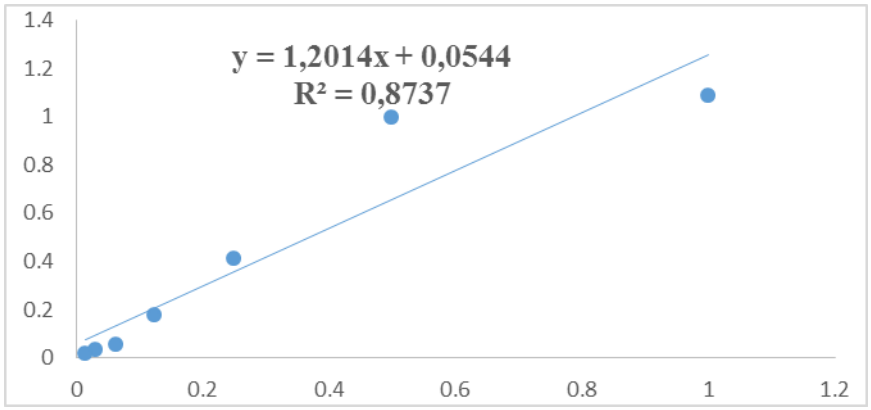


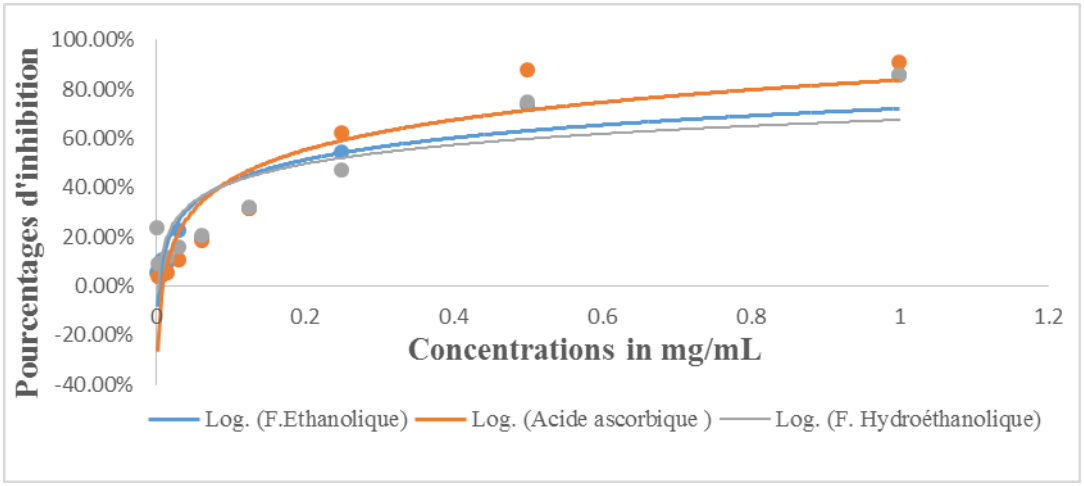
Figure 3: Average absorbance values as a function of quinidine concentrations.

Table 6: Amount of Alkaloids contained in the sample in quinidine equivalent per fraction.

Fractions	Concentrations of fractions in mg EQ/mL	Alkaloid content in mg EQ/g of fraction
F. EtOH	0.450807	90.161
F. HE	0.199711	42.772
F. Aq	0.213862	39.942

i. Antioxydant activity

TEST DPPH



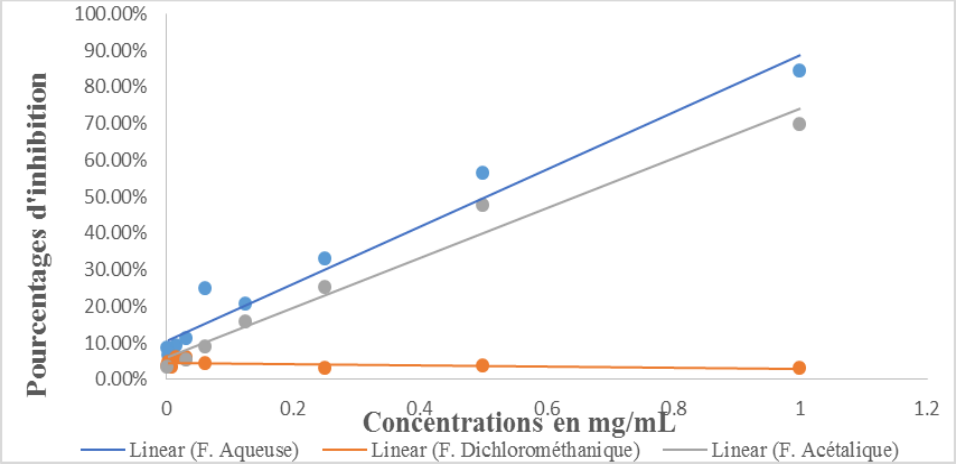
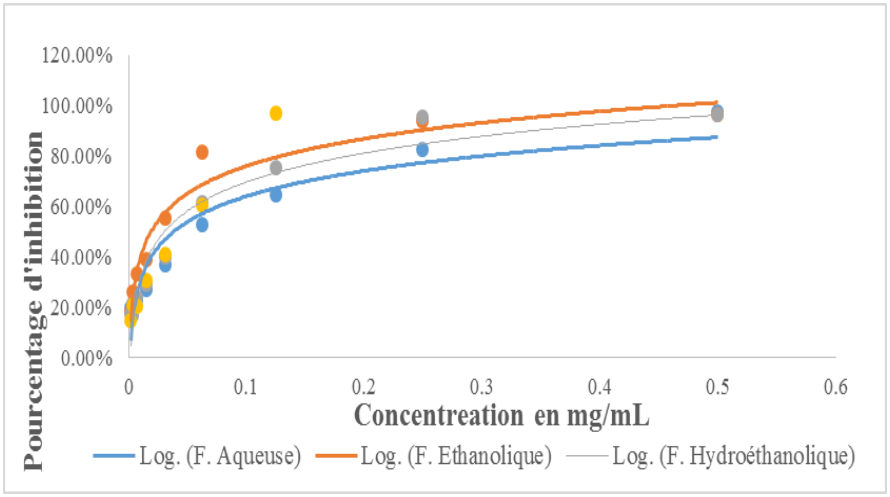


Figure 4: percentages of inhibition of the different fractions as a function of concentrations.

Table 7: Concentrations required to trap 50% (IC50) of free radicals from the different fractions; DPPH method.

Fractions	Aqueous	Ethanol	Hydro-ethanol	Ethyl Acetate	Dichloro methane	Vitamin C
$\%CI_{50}$ en mg/mL	0.504	0.181	0.207	0.645	28.734	0.148

TEST ABTS



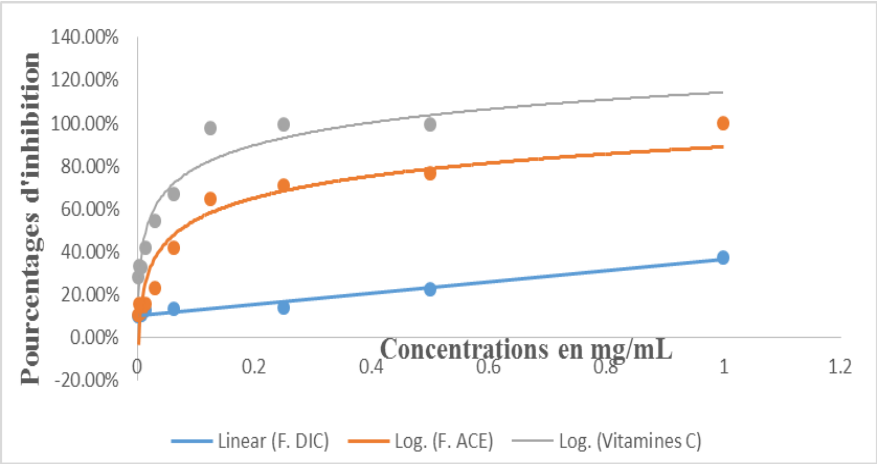


Figure 5: Percentages of inhibition of the different fractions as a function of concentrations.

Table 8: concentrations required to trap 50% (IC50) of free radicals from different fractions; ABTS method.

Fractions	Aqueous	Ethanol	Hydro-ethanol	Ethyl acetate	Dichloro methane	Vitamin C
$\%CI_{50}$ mg/mL	0.038	0.019	0.030	0.071	1.528	0.014

ii. Antibacterial Activity

Table 9: Result of the test of the different fractions on the bacterial strains.

Strains tested	Aq F.	HE F.	Et F.	Ace F.	Dic F.
S. aureus ATCC 29213	A	NA	NA	NA	A
E. coli ATCC 35218	NA				NA
E. coli ATCC 25922					
P. aeroginosa ATCC 27853					
E. faecalis ATCC 29212					

NA: Non-Active, **A** : Active

Table 10: Diameters (mm) and minimal concentrations (mg/mL) of inhibition of the aqueous fraction (Aq F.) of Piliostigma reticulatum leaves on *S. aureus ATCC 29213*.

Concentrations (mg/mL)	ID (mm)	MIC (mg/mL)
100	15	28,602
50	11	
25	8	
12,5	-	
6,25	-	
3,125	-	

Table 11: Diameters (mm) and minimal concentrations (mg/mL) of inhibition of the dichloromethane fraction (Dic F.) of Piliostigma reticulatum leaves on *S. aureus ATCC 29213.*

Concentrations (mg/mL)	ID (mm)	MIC (mg/mL)
100	14	18,080
50	12	
25	10	
12,5	7	
6,25	-	
3,125	-	

ID: Inhibition Diameter, **MIC:** Minimum Inhibitory Concentration

DISCUSSION

It is essential to determine the presence of certain molecular families, notably alkaloids, flavonoids, tannins, and saponins, as notable pharmacological effects are often attributed to them in medicinal plants. Other secondary metabolites, such as coumarins, proteins, and reducing sugars, may also play a crucial role in the pharmaceutical properties of these plants.

In our various plant fractions, tests revealed the presence of alkaloids, flavonoids, tannins, and saponins in the ethanolic (F. Eth), aqueous (F. Aq), and hydro-ethanolic (F. He) fractions. Additionally, coumarins were detected in the ethyl acetate (F. Ace) and dichloromethane (F. Dic) fractions. Reducing sugars were observed only in the ethanolic and hydro-ethanolic fractions. Compared to the results of Faye et al. (2022), our aqueous, ethyl acetate, and dichloromethane fractions are richer in these secondary metabolites. Contrary to Faye's results, we noted the presence of tannins, flavonoids, and saponins in the aqueous fraction. The ethyl acetate fraction contains alkaloids and flavonoids, which contrasts with the observations of Faye et al. (2022). For the dichloromethane fraction, our results regarding these four major molecular families are similar. Previous studies on the methanolic extract of Piliostigma reticulatum leaves also showed the presence of the initially listed metabolites, but steroids were found in the methanolic extract.[12]

The quantitative analysis of major secondary metabolites in the ethanolic, hydro-ethanolic, and aqueous fractions of Piliostigma reticulatum leaves highlights differentiated distribution profiles, linked to the polarity of the solvents and the chemical nature of the compounds.

Flavonoids

The ethanolic fraction exhibits an exceptionally high total flavonoid content (240.337 ± 0.058 mg ER/g) of dry extract, followed by the aqueous fraction (131.132 ± 0.048 mg ER/g) and the hydro-ethanolic fraction (91.987 ± 0.050 mg ER/g). These values indicate a high richness in moderately polar phenolic compounds, more soluble in absolute ethanol. These flavonoids, likely in aglycone or weakly glycosylated form, are known for their antioxidant, anti-inflammatory, and antimicrobial activities.[26,27] These results are consistent with those reported by Boualam et al. (2021) and Ajayi et al. (2019), who also observed a significant concentration of flavonoids in the methanolic extracts of Piliostigma reticulatum leaves, suggesting better extraction of phenolic metabolites in organic solvents of intermediate polarity.

Tannins

Total tannins are also well represented in the ethanolic (85.567 ± 0.002 mg EAG/g) and hydro-ethanolic (84.119 ± 0.009 mg EAG/g) fractions, while the aqueous fraction contains much less (17.158 ± 0.003 mg EAG/g). This distribution can be explained by the better affinity of condensed tannins for polar organic solvents. These compounds have demonstrated astringent, antimicrobial, and antioxidant properties, acting through chelation of metal ions and enzymatic inhibition.[28,29,30] The proximity of values between the two ethanol-rich fractions suggests an equivalent distribution of intermediate polarity tannins in these phases. The low tannin content in the aqueous fraction suggests that the most water-soluble tannins were partially retained in the previous phases or are less represented in the parts of the extract soluble only in water. This profile is consistent with previous studies showing that tannin extraction is more effective in ethanol-water mixtures than in water alone.[3,31]

Alkaloids

Total alkaloids are found at a maximum concentration in the ethanolic fraction (90.161 mg EQ/g), compared to 42.772 mg EQ/g in the hydro-ethanolic fraction and 39.942 mg EQ/g in the aqueous fraction. These data reveal a predominance of non-polar alkaloids, more soluble in absolute ethanol. Even at low doses, these compounds are biologically active (antibacterial, analgesic, antiparasitic), and their high concentration in the ethanolic fraction suggests particular interest for bio-guided studies. This is consistent with the results reported

by Nacoulma (1996) and Harborne (1998),[31, 32] who associate alkaloid richness with a wide spectrum of pharmacological activities.

These results demonstrate that liquid-liquid fractionation with an increasing polarity gradient allows for the distribution of bioactive compounds according to their chemical nature. The ethanolic fraction, concentrating both flavonoids, tannins, and alkaloids at significant levels, appears to be the most promising for pharmacological valorization[33], particularly in the fields of anti-oxidation, which aligns with the results obtained from DPPH and ABTS tests performed on this fraction as well as the other two. The antioxidant study conducted on the fractions from Piliostigma reticulatum reveals that the ethanolic fraction exhibits the best antioxidant activity, with a lower IC_{50}, followed by the hydro-ethanolic fraction, and then the aqueous fraction. This hierarchy of antioxidant activity is perfectly correlated with the flavonoid contents measured in the same fractions.

Fraction	Flavonoids (mg ER/g)	IC_{50} (antioxidant activity)
Ethanolic	240.337 ± 0.058	Lowest IC_{50} → best activity
Hydro-ethanolic	91.987 ± 0.050	Intermediate IC_{50}
Aqueous	131.132 ± 0.048	Highest IC_{50} → weaker activity

Flavonoids are powerful free radical scavengers due to their hydroxyl groups and conjugated aromatic rings. The high concentration of flavonoids in the ethanolic fraction logically justifies its high antioxidant capacity,[34] as it offers more molecules capable of neutralizing DPPH radicals. The presence of condensed tannins, also recognized for their reducing power, in similar proportions in the ethanolic and hydro-ethanolic fractions reinforces the antioxidant effect, although flavonoids remain the main contributors here. The aqueous fraction, while containing a moderate flavonoid content, displays weaker antioxidant activity. This suggests that the nature of the flavonoids extracted in water may be less favorable (e.g., more glycosylated or less reactive flavonoids),[35] or that their availability is reduced. The positive correlation between flavonoid contents and antioxidant activity confirms that these metabolites play a central role in the antioxidant potential of *Piliostigma reticulatum*. These results reinforce the idea that the ethanolic fraction is the most promising for therapeutic valorization, particularly in applications related to oxidative stress, inflammation, and cellular aging.

The presence of reducing sugars in the ethanolic and hydro-ethanolic fractions, as well as that of coumarins in the ethyl acetate and dichloromethane fractions, constitutes new parameters in the phytochemical composition of *Piliostigma reticulatum*. In contrast, no proteins were detected in the various fractions studied.

Antimicrobial activity was observed in the dichloromethane and aqueous fractions. Although the ethanolic and hydro-ethanolic fractions are the richest in flavonoids, they showed no antibacterial activity against Staphylococcus aureus. In contrast, the dichloromethane fraction, containing lipophilic flavonoids and coumarins, as well as the aqueous fraction, rich in bioactive water-soluble compounds, both inhibited this bacterium. These results suggest a specificity of action related to the chemical nature of the flavonoids and the presence of coumarins, known for their targeted antimicrobial properties.[36] The antibacterial activity observed in the dichloromethane fraction contrasts with the results of Faye et al. (2022) and constitutes new data for research on this plant.

CONCLUSION

The phytochemical investigation of the leaves of *Piliostigma reticulatum* has yielded significant results. In addition to alkaloids, flavonoids, and tannins, we sought the presence of reducing sugars, coumarins, and proteins in the leaves of this plant. Phytochemical screening showed the presence of alkaloids, flavonoids, tannins, and saponins in the ethanolic (F. Eth), aqueous (F. Aq), and hydro-ethanolic (F. He) fractions. However, reducing sugars were only observed in the ethanolic and hydro-ethanolic fractions. Tests for coumarins and proteins were negative in these three fractions. Furthermore, the presence of coumarins, flavonoids, and alkaloids constitutes new and crucial data, given the differences with the results of other researchers such as Ousmane Faye in 2022. The antibacterial activity observed for the dichloromethane fraction against Staphylococcus aureus is also a new result that requires a broader and more in-depth approach in the medical field.

The results obtained from tests conducted on extracts of *Piliostigma reticulatum* leaves highlight significant antioxidant and antibacterial potential, particularly for the ethanolic and hydro-ethanolic fractions. This activity could be correlated with the richness in secondary metabolites such as flavonoids, tannins, and alkaloids identified in the extracts.

REFERENCES

1. World Health Organization. (2004). *The global burden of disease: 2004 update*. Geneva: WHO Press.
2. Fakih, Muhammad, Candra Perbawati, et Monalisa. « Relevance of WHO traditional medicine strategy (2014-2023) with traditional health care policy in the perspective of national law and international law ». *Asian Journal of Legal Studies* 1, n° 1 (2022); 25‑34.
3. Ouédraogo, Y., Kiendrebeogo, M., & Traoré, A. S. (2020). Évaluation des polyphénols et des tanins de quelques plantes médicinales du Burkina Faso. Journal of Scientific and Industrial Research, 11(2): 45–51.
4. Dosso, K., B. B. N'guessan, A. P. Bidie, B. N. Gnangoran, S. Méite, D. N'guessan, A. P. Yapo, et E. E. Ehilé. « Antidiarrhoeal Activity of an Ethanol Extract of the Stem Bark of Piliostigma Reticulatum (Caesalpiniaceae) in Rats ». *African Journal of Traditional, Complementary and Alternative Medicines* 9, n° 2 (2012); 242‑49. https://doi.org/10.4314/ajtcam.v9i2.9.
5. Paul, Bazongo, Madjelia Cangre Ebou DAO, Salifou Kabré, et Ouédraogo Lassané. « Usages de Piliostigma reticulatum (dc.) hochst. et contribution à la sécurité alimentaire des populations rurales en zone nord-soudanienne au Burkina Faso » 41 (4 juillet 2024); 151‑63.
6. Faye, Elhadji Ousmane, Rokhaya Gueye, Abdoulaye Diop, Idrissa Wagane Faye, Pape Issakha Dieye, Harouna Tirera, Thierno Mouhamed Wane, Kady Diatta Badji, Mbaye Dieng, et Assane Dieng. « Caractérisation phytochimique et étude de l'activité antimicrobienne d'extraits de feuilles de trois plantes de la flore sénégalaise: Detarium senegalense, Detarium microcarpum et Piliostigma reticulatum ». *International Journal of Biological and Chemical Sciences*, 2022; 16(1): 286‑99.
7. Ajayi, Victoria F., Olalekan S. Ojerinde, Awase Yatar, Awhobiwom D. Agba, et Mary O. Uguru. « Antidiabetic Effect of Methanolic Extract of Piliostigma Reticulatum Leaf in Streptozotocin-Induced Diabetic Rats ». *Journal of Pharmacy & Bioresources* 16, n° 2 (8 novembre 2019); 158‑64. https://doi.org/10.4314/jpb.v16i2.10.
8. Boualam, K., B. Ndiaye, H. Harhar, M. Tabyaoui, N. Ayessou, et K. Taghzouti. « Study of the Phytochemical Composition, the Antioxidant and the Anti-Inflammatory Effects of Two Sub-Saharan Plants: Piliostigma Reticulatum and Piliostigma Thonningii ». Édité

par Abdeslam Jaafari. *Advances in Pharmacological and Pharmaceutical Sciences* 2021 (4 mai 2021); 1‑8. https://doi.org/10.1155/2021/5549478.

9. Awe, S., et P. F. Omojasola. « A comparative study of the antibacterial activity of Piliostigma reticulatum bark extract with some antibiotics ». *Ethnobotanical leaflets* 2009, n° 9 (2009); 11.
10. Bonnefont-Rousselot, D., J. P. Bastard, M. C. Jaudon, et J. Delattre. « Consequences of the Diabetic Status on the Oxidant/Antioxidant Balance ». *Diabetes & Metabolism* 26, n° 3 (mai 2000); 163‑76.
11. F, Nascimento, Juliana Locatelli, Freitas C, et Giuliana Silva. « Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria ». *Brazilian Journal of Microbiology* 31 (1 octobre 2000). https://doi.org/10.1590/S1517-83822000000400003.
12. Sani, A. A., A. K. Dalla Dalla, S. B. Idris, N. Suleiman, et A. H. Jibril. « Antioxidants and Vitamins Content of Methanol Extract of Piliostigma Reticulatum Leaves ». *Journal of Agriculture and Environment* 20, n° 1 (4 septembre 2024): 141‑49. https://doi.org/10.4314/jagrenv.v20i1.14.
13. Bekro, Y-A, Ja Mamyrbekova, Bb Boua, Fh Tra Bi, et Ee Ehile. « Étude ethnobotanique et screening phytochimique de *Caesalpinia benthamiana* (Baill.) Herend. et Zarucchi (Caesalpiniaceae) ». *Sciences & Nature* 4, n° 2 (25 septembre 2008): 217‑25. https://doi.org/10.4314/scinat.v4i2.42146.
14. Brou KG, Mamyrbekova-Bekro JA, Dogbo DO, Gogbeu SJ. 2010. Composition phytochimique qualitative des extraits bruts hydrométhanoliques des feuilles de 6 cultivars de Manihot esculenta crantz de Côte d'Ivoire. European Journal of Scientific Research, 45(2): 200-211.
15. Harborne, Jeffrey B. Harborne J. B. *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*. 2nd edition. Dordrecht: Springer, 1984.
16. Parekh, Jigna, et Sumitra Chanda. « In vitro Antimicrobial Activity and Phytochemical Analysis of Some Indian Medicinal Plants ». *Turkish Journal of Biology* 31, n° 1 (1 janvier 2007; 53‑58. https://doi.org/-.
17. Zhishen, Jia, Tang Mengcheng, et Wu Jianming. « The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals ». *Food Chemistry* 64, n° 4 (1 mars 1999); 555‑59. https://doi.org/10.1016/S0308-8146(98)00102-2.

18. Graham, Horace. « Stabilization of the Prussian Blue Color in the Determination of Polyphenols ». *Journal of Agricultural and Food Chemistry - J AGR FOOD CHEM* 40 (1 mai 1992). https://doi.org/10.1021/jf00017a018.
19. Sreevidya, Narasimhan, et Shanta Mehrotra. « Spectrophotometric Method for Estimation of Alkaloids Precipitable with Dragendorff's Reagent in Plant Materials ». *Journal of AOAC International* 86, n° 6 (2003); 1124‑27.
20. Comité de l'antibiogramme de la Société Française de Microbiologie. Recommandations 2023; V.1.0 Juin.
21. Haddouchi, Farah, Khadidja Zerhouni, Adel Sidi-Yekhelef, et Tarik Mohammed Chaouche. « Évaluation de l'activité antimicrobienne de différents extraits d'Helichrysum stoechas subsp. rupestre Evaluation of antimicrobial activity of different extracts ofHelichrysum stoechas subsp. rupestre ». *Bulletin de la Société Royale des Sciences de Liège*, 1 janvier 2016; https://doi.org/10.25518/0037-9565.5894.
22. Molyneux, Philip. « The Use of the Stable Free Radical Diphenylpicryl- Hydrazyl (DPPH) for Estimating Antioxidant Activity » 26, n° 2 (2004).
23. Zerbo, Alexandre, Jean Koudou, Noufou Ouédraogo, Rasmata Ouedraogo, et Innocent P. Guissou. « Antioxidant and antibacterial activities of Piliostigma reticulatum (DC.) Hochst extracts ». *African Journal of Biotechnology* 9, n° 33 (2010); https://www.ajol.info/index.php/ajb/article/view/92086.
24. Khan, R.A., Khan, M.R., Sahreen, S. (2012). ***Assessment of flavonoids*** *contents and in vitro antioxidant activity of Launaea procumbens*. **Romanian** Biotechnological Letters**,** 17**(3): 7227–7236.**
25. Dieng, Serigne Ibra Mbacke, Alioune Dior Fall, Kady Diatta-Badji, Abdou Sarr, Madieye Sene, Moussa Sene, Amadou Mbaye, William Diatta, et Emmanuel Bassene. « Evaluation de l'activité antioxydante des extraits hydro-ethanoliques des feuilles et écorces de *Piliostigma thonningii* Schumach. » *International Journal of Biological and Chemical Sciences* 11, n° 2 (19 juillet 2017); 768. https://doi.org/10.4314/ijbcs.v11i2.19.
26. Pone, Boniface Kamdem, et Elizabeth Igne Ferreira. « Therapeutic Potential of Flavonoid Derivatives for Certain Neglected Tropical Diseases ». *Current Drug Targets* 23, n° 7 (2022); 680‑82. https://doi.org/10.2174/1389450123666220309093827.
27. Boniface, Pone Kamdem, et Elizabeth Igne Ferreira. « Flavonoids as Efficient Scaffolds: Recent Trends for Malaria, Leishmaniasis, Chagas Disease, and Dengue ». *Phytotherapy Research: PTR* 33, n° 10 (octobre 2019); 2473‑2517. https://doi.org/10.1002/ptr.6383.

28. Smeriglio, Antonella, Davide Barreca, Ersilia Bellocco, et Domenico Trombetta. « Proanthocyanidins and Hydrolysable Tannins: Occurrence, Dietary Intake and Pharmacological Effects ». *British Journal of Pharmacology* 174, n° 11 (2017); 1244‑62. https://doi.org/10.1111/bph.13630.
29. Naumann, Harley D., Luis O. Tedeschi, Wayne E. Zeller, et Nichole F. Huntley. « The Role of Condensed Tannins in Ruminant Animal Production: Advances, Limitations and Future Directions ». *Revista Brasileira de Zootecnia* 46 (décembre 2017); 929‑49. https://doi.org/10.1590/S1806-92902017001200009.
30. Zhang, Liangliang, He Zhang, Lihua Tang, Xinyu Hu, et Man Xu. « Isolation, Characterization, Antioxidant Activity, Metal-Chelating Activity, and Protein-Precipitating Capacity of Condensed Tannins from Plum (Prunus Salicina) Fruit ». *Antioxidants* 11, n° 4 (avril 2022); 714. https://doi.org/10.3390/antiox11040714.
31. Nacoulma, O. G. « Plantes médicinales et pratiques médicales traditionnelles au Burkina Faso: cas du plateau central ». *Fac. Sci. Tech. Univ. Ouagadougou* 320 (1996); 42‑53.
32. Harborne, A. J. *Phytochemical methods a guide to modern techniques of plant analysis*. springer science & business media, 1998.
33. Belemlilga, Bonewendé Mohamed, Tiami Jonas Coulibaly, Abdoul Gilchrist Laurent Boly, Boukaré Kabore, Dibi Jacques Konan, Alimata Bance, Dofini René Magnini, et al. « Activité anthelminthique in vitro sur les vers adultes Hamonchus contortus et propriétés antioxydantes de l'extrait hydroalcoolique des fruits de Piliostigma reticulatum (DC.) Hochst (Fabaceae) ». *Sciences de la Santé* 47, n° 2(1) (2024); 238‑56.
34. Daglia, Maria. « Polyphenols as antimicrobial agents ». *Current Opinion in Biotechnology*, Food biotechnology - Plant biotechnology, 23, n° 2 (1 avril 2012); 174‑81. https://doi.org/10.1016/j.copbio.2011.08.007.
35. Xiao, Zhengcao, Liangliang He, Xiaohui Hou, Jianping Wei, Xiaoyu Ma, Zihan Gao, Yahong Yuan, Jianbo Xiao, Pengmin Li, et Tianli Yue. « Relationships between Structure and Antioxidant Capacity and Activity of Glycosylated Flavonols ». *Foods* 10, n° 4 (14 avril 2021); 849. https://doi.org/10.3390/foods10040849.
36. Venugopala, K. N., V. Rashmi, et B. Odhav. « Review on Natural Coumarin Lead Compounds for Their Pharmacological Activity ». *BioMed Research International* 2013, n° 1 (2013); 963248. https://doi.org/10.1155/2013/963248.