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# *In vitro* antioxidant activity and antibacterial properties of trunk bark of *Pericopsis laxiflora* Benth. (Leguminosae)

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**ABSTRACT**

*Owing the side effects of synthetic antioxidants and the emergence of multidrug resistance, research is moving more towards plant substances ascribed various biological properties. The present study aims to evaluate the antioxidant and antibacterial properties of Pericopsis laxiflora (Benth.) a plant of the Ivorian pharmacopoeia. The antioxidant potency was evaluated using two complementary techniques, inhibition of the free radical DPPH (2.2 diphenyl picryl hydrazyl 1) and the ferric thiocyanate (FTC) test. The antibacterial activity was conducted on pathogenic strains isolated reference and clinical strains of pathological material. Susceptibility tests were performed on solid medium using the method of the wells. Macrodilution method in liquid medium was used to determine the Minimum Inhibitory Concentration and bactericidal concentrations (MIC and MBC). The results showed the existence of a considerable antioxidant activity of the extract tested with an $IC_{50}$ value of 5.71 ± 1.42 mg/mL and an inhibition of lipid peroxidation rate of 66%. Moreover, results of antibacterial activity have proved the effectiveness of our extract against all selected with inhibition values of diameters varying from 10 to 21 mm and MIC and MBC values respectively ranging from 0.39 to 12.5 mg/mL and 0.78 to 25 mg strains/mL. It follows from this work that the bivalent properties observed are related to the presence of phenolic compounds in the plant. In these conditions, the plant could be used in the treatment of several pathologies such as cellular aging and infectious diseases.*

**Keywords**: *Pericopsis laxiflora*, antioxidant activity, antibacterial activity, Côte d'Ivoire

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## INTRODUCTION

Each medicinal plant has several properties that traditional healers use to cure various diseases. For example by Ivorian traditional medicine *Pericopsis laxiflora* (Benth.) is used for the treatment of headaches, heart pains, gastroenteritis and abdominal pain **[1-2]**. Under various uses, the same plant is found throughout the dry forests and savannas of Africa Sudanese. In fact, it is used in Guinea against shigellosis and colibacillosis **[3]**. In Nigeria and Ghana, it is involved in the treatment of ulcers and malaria **[4-5]**.

Many studies have shown that the extracts from medicinal plants contains a variety of compounds such as polyphenols with various biological activities assigned **[6-7]**. Some of them have shown the important role of antimicrobial agents of plant origin in the arsenal of drugs prescribed today by clinicians **[8-9]**. Also, other studies have demonstrated the existence of bioactive compounds that may be involved in reducing risk of developing several diseases associated with oxidative stress **[10-11]**.

In the present study, we evaluated the antioxidant and antibacterial activity of the methanol extract from *Pericopsis laxiflora* (Benth.)

## MATERIALS AND METHODS

### Plant material

The plant material is made up of barks of trunk of *Pericopsis laxiflora* (Benth.) and harvested at Lataha (Korhogo), north of the Ivory Coast. The plant was identified by Professor Ake-Assi of the National Floristic Centre (NFC) of University FHB Cocody-Abidjan.

### Bacterial strains

The bacterial carrier is composed of two reference strains (*E. coli* ATCC 25922 and *S. aureus* ATCC 25923) and three clinical strains (*E. coli* ESBL, *S. aureus* Meti-S and *S. aureus* Meti-R) isolated from pathological material. The identification of the strains was performed in the laboratory of bacteriology at the Pasteur Institute of Cote d'Ivoire (IPCI) as recommended by the NCCLS **[12]**.

### Preparation of extract

This preparation is performed according to the method described by **[13]**. Indeed, crushed 50 g of plant bark was mixed with 1.5 L of methanol 96%. The resulting mixture was stirred for 48 hours at room temperature (25°C) using a magnetic stirrer type IKAMAG RCT (Staufen, Germany). Then, the mixture is filtered three times on cotton and on Whatman filter paper 3 mm. The filtrate was evaporated at reduced pressure and 40°C using a rotary evaporator Buchi 461 Watter Batch (Strasbourg, France). The resulting powder was used to make the different tests.

### Determination of total phenols

The total phenolic contents of our extract were determined by the Folin-Ciocalteu method **[14-15]**. To 0.5 mL of each plant extract of concentration 0.1 mg/mL, were added respectively 5 mL of Folin-ciocalteu diluted 1/10 in distilled water and 4 mL of sodium carbonate (1M). The whole is incubated at room temperature for 15 minutes. The optical densities (OD) are then read in a spectrophotometer at 765 nm against a blank. Gallic acid was used as standard and prepared under the same conditions as above with a solvent mixture of methanol/water (50:50, V/V) at concentrations ranging from 0 to 0.5 mg/mL. The total phenolic contents of the extracts are expressed in milligrams of gallic acid equivalents per gram of extract (mg GAE/g extract).

### Determination of total flavonoids

The technique used for the determination of the levels of total flavonoids extracted from ours plants the colorimetric method of aluminum trichloride described by **[16]** modified by **[17]**. Thus, 0.1 mL of 5 mg/mL of each extract are collected, to which are successively added 1.5 mL of methanol, 0.1 mL of 10% aluminum trichloride, 0.1 mL of potassium acetate (1M) and 2.5 mL of distilled water. After incubation at room temperature for 30 minutes, the optical densities were measured in a spectrophotometer at 415 nm. A methanolic solution of quercetin with concentrations ranging from 0 to 100 mg/mL is used as a standard. The contents of flavonoids extracts are expressed in milligrams of quercetin equivalent per gram of extract (mg QE/g of extract).

### Measurement of anti-radical power

The measurement of the antiradical activity of plant extracts was performed by testing the 2, 2- diphenyl-1-picrylhydrazyl (DPPH) according to the method of **[18]**. From a stock solution of each plant extract to 0.1 mg/mL, a concentration range is prepared by successive doubling dilution of 1.56 mg/mL to 100 mg/mL. Then, each extract concentration, the same volume of a methanolic solution of DPPH is added. After 30 minutes of incubation at room temperature (37°C) and protected from light, the absorbance is read in a spectrophotometer at 517 nm against a blank sample (0 mg/mL of extract). Vitamin C (100 mg/mL) which is the reference material is prepared in the same conditions. The percentage inhibition of DPPH radicals is calculated by the following formula:

Inhibition (%) = [(Abs blank - Abs sample / Abs blank)] x 100.

Abs Blank is the absorbance of the control reaction (containing all reagents except the test compound) and Abs sample, the absorbance of the test compound.

From a curve representing the percentage of inhibition of DPPH radicals versus concentrations of the extracts and vitamin C, concentrations of extract and vitamin C that reduces 50% of DPPH radicals ($IC_{50}$) are determined and compared.

**Measurement of inhibition of lipid peroxidation by FTC (ferric thiocyanate) method**

This method measures the level of peroxides formed by measuring the complex formed by ferrous ion **[19]**. Thus, in protected foil bottles are separately dissolved 4 mg of plant extract in 4 ml of ethanol (99.5%). In these mixtures, were added 4.1 mL of linoleic acid (2.5% in ethanol at 99.5%), 8.0 mL of phosphate buffer (20 mM, pH 7, 0) and 3.9 mL of distilled water to a final volume of 20 mL. Quercetin (reference molecule), prepared under the same conditions is used as a positive control. The bottles containing mixtures were incubated in a water bath at 45°C. During incubation, every day (24 h), 0.1 mL of the mixture is collected in test tubes. To this amount are successively added 9.7 mL of ethanol (75%), 0.1 mL of ammonium thiocyanate (30% in distilled water) and 0.1 mL of iron II chloride ($FeCl_2$) to 20 mM of hydrochloric acid (HCl 3.5%). After 3 minutes of incubation at room temperature, the absorbance of the resultant red color is measured using a spectrophotometer at 500 nm. The assays are performed for 7 days until the absorbance of the control reaches its maximum value.

**Antibacterial assay**

Antibacterial activity of extract was performed on Mueller Hinton agar (Biorad, France) using the well method **[20-21]**. Thus, as in the conventional embodiment of an antibiogram, each well or hole of 6 mm in diameter was filled with 80 µL of extract concentration of 200 mg/mL, taking care to separate two holes of at least 20 mm. A control well was produced for each bacterial strain with 80 µL mixture of DMSO/sterile distilled water solution (V/V) **[22]**. After a pre-release of 45 minutes at room temperature in the hood, the whole is incubated in an incubator at 37°C for 18 to 24 hours. After this time, the action of the extract is assessed by measuring a growth inhibition zone (lack of colonies) around the well. In parallel, Oxacillin (5µg) and Cefoxitin (30µg) were used as positive controls. These antibiotics were selected on the basis of their rather wide spectrum of action and their frequent use in hospitals in the treatment of infections caused by most germs in our study. So as to reduce the risk of errors, the experiment was performed three times.

**Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration of (CMB)**

By following the recommendations of the French Society of Microbiology, each bacterial inoculum was prepared so as to obtain an estimated suspension under standard conditions at approximately $10^6$ cells/mL. Then, the dilution macrométhode in liquid medium was used **[23]**. By this method, the MIC of extracts corresponds to the concentration of the first tube in which there is no growth visible to the naked eye of the bacteria tested after incubation for 18 to 24 hours at 37°C. To determine the MBC 0.1 mL of the contents of the tubes having a concentration greater than or equal to the MIC is seeded on the surface of a fresh Mueller-Hinton agar flow in Petri dishes. This MBC is the smallest concentration which leaves survive at most 0.01% of the initial suspension of seed within 24 hours. Furthermore, the report MBC/MIC of the extract was calculated to apprecite its antibacterial power.

**Screening Tri phytochimique**

Phytochemical tests for tannins, flavonoids, alkaloids, sterols and polyterpenes, saponins, cardiac glycosides and reducing compounds were performed according to the methods described by **[24-25]**.

**Statistical analysis**

The statistical analysis was performed using one-way analysis of variance (ANOVA) of the multiple test of comparison of Tukey-Kramer. The level of significance was determined in comparison with the control group. $p<0.05$ was considered significant. All values are expressed as mean ± SEM.

## RESULTS AND DISCUSSION

Assay results of the phenolic compounds contained in our extract are reported in Table 1.

**Table 1: Levels of phenolic compounds**

| Phenolic compounds | |
|---|---|
| **Total Phenols** | **Total Flavonoids** |
| 160.38 ± 5.81 (mg GAE/g of extract) | 72 ± 2.42(mg QE/g of extract) |

The analysis of these results reveals a significant proportion of total phenols (160.38 ± 5.81 mg GAE/g of extract) and total flavonoids (72.12 ± 2. 42 mg QE/g extract). The presence of these compounds appear to be in adequacy with the antioxidant activity obtained. Indeed, many studies, including those of **[26-10]** reported an excellent correlation between the amounts of these compounds and various anti-radical activity of plant extracts.

Our results are also consistent with those obtained by **[27-11]**, because they have shown that plants having good antioxidant activity contain high contents of phenolics. However, on the basis of $IC_{50}$ determined, we deduce by referring to studies by **[28]** that the anti-radical activity of the methanol extract of *P. laxiflora* (5.71 ± 1.42 µg/mL) was significantly lower ($p \leq 0.001$) than that of vitamin C (4.28 ± 1.42 µg/mL) (Figure 1). For according to these authors, plus the value of the $IC_{50}$ of a compound is high more its antioxidant activity is low.

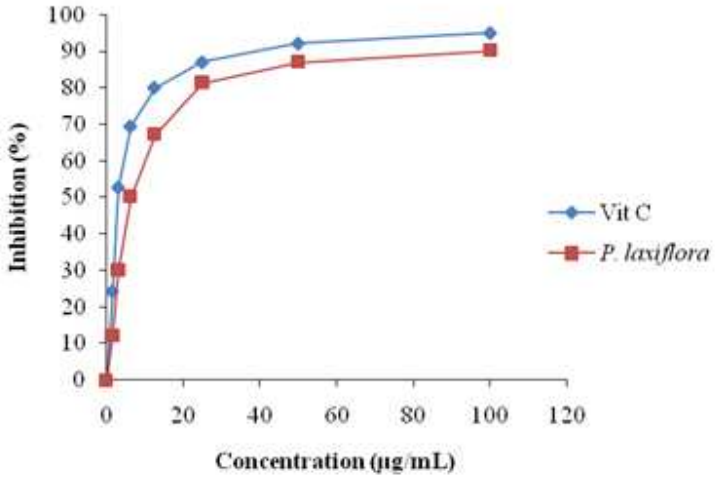


**Figure 1: Anti-radical activity of *Pericopsis laxiflora* extract and vitamin C**

Furthermore, analysis of the results of antioxidant activity recorded by the FTC method shows that the extract tested has good antioxidant activity (66% inhibition of lipid peroxidation) compared to that obtained with vitamin C (67% inhibition of lipid peroxidation) (Figure 2).

The results of the evaluation of antibacterial activity of extract tested are summarized in the Table 2. According to the work of **[29]**, an extract is considered active if it causes a zone of inhibition greater or equal to 10 mm. In these conditions we can affirm that our extract is active on all tested germs. The greatest activity of the extract was observed on the *S. aureus* strains which are Gram-positive bacteria (19 to 21 mm in diameter) and the greatest resistance against strains of *E. coli* which are Gram-negative bacteria (10 to 12 mm in diameter). However, no zone of inhibition was observed with the usual antibiotics on *S. aureus* Meti-R and the two *E. coli* strains (0 mm).

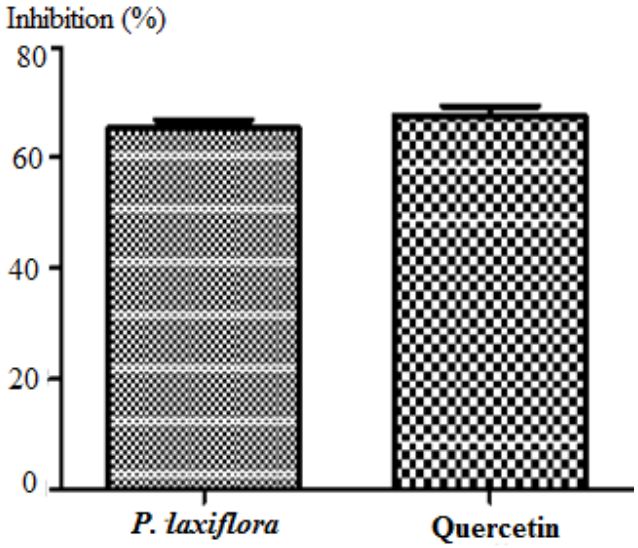


**Figure 2: Antioxidant activity of *Pericopsis laxiflora* extract and quercetin**

**TABLE 2: Values of inhibition diameter of methanolic extract and usual antibiotics**

| | Values of inhibition diameters (mm) | | |
|---|---|---|---|
| **Bacterial straims** | Extract (200 mg/mL) | Oxacillin (5µg) | Cefoxitin (30µg) |
| *E. coli* ATCC 25922 | 10 ±0.1 | 0 | 0 |
| *E. coli* BLSE | 12±0.1 | 0 | 0 |
| *S. aureus* ATCC 25923 | 19±0.2 | 25±0.2 | 25±0.1 |
| *S. aureus* Meti-S | 21±0.1 | 31±0.3 | 27±0.2 |
| *S. aureus* Meti-R | 20±0.2 | 0 | 0 |

The same observation is done on the basis of antibacterial parameters determined (Table 3). In fact, the Gram negative bacteria showed larger MIC values (12.5 mg/mL) and MBC (25 mg/mL) in contrast to Gram positive bacteria for which the lowest values were recorded: 0, 39 to 0.78 mg/mL for the MIC and 0.78 to 1.56 mg/mL to the MBC.

**Table 3I: Antibacterial parameters of methanolic extract**

| **Bacterial straims** | **MIC** (mg/mL) | **MBC** (mg/mL) | **MBC/MIC** |
|---|---|---|---|
| *E. coli* ATCC | 12.50 | 25.00 | 2 |
| *E. coli* BLSE | 12.50 | 12.50 | 1 |
| *S. aureus* ATCC | 0.78 | 1.56 | 2 |
| *S. aureus* Meti-S | 0.39 | 0.78 | 2 |
| *S. aureus* Meti-R | 0.78 | 1.56 | 2 |

An analysis of these results shows that Gram-negative bacteria are more resistant than Gram-positive face our extract. Many studies, including those of **[30-31]** reported the resistance of Gram-negative bacteria as compared to gram-positive bacteria. According to some, this resistance is linked to the presence of a layer of lipopolysaccharide (LPS), which function as an effective barrier against most biocidal agents **[32-33]**. For others, this resistance is related to the nature of the extract and/or bacteria tested **[34-35-36]**.

In all cases, whether gram-positive or gram negative, it was observed that the inhibitory activity of our extract is lower than that recorded with conventional antibiotics. However, his power is bactericidal against all bacteria tested if we refer to the work of **[37]**. According to these authors the effect of a substance is considered bactericidal if the ratio MBC/MIC is less than or equal to 4, in the opposite case it is said bacteriostatic. Several research maintained that the antioxidant and antibacterial activities of plant extracts were likely related to phenolic compounds, particularly phenols, flavonoids and tannins **[38-7]**. Or the phytochemical screening performed on the methanol extract of *P. laxiflora* revealed the presence of all these compounds in significant proportion (Table 4). So if we

stick to these authors, the results of this study could help confirm the phenolic compounds as antioxidants and antimicrobial agents.

**Table 4II: Different groups of compounds identified in methanolic extract**

| | |
|---|---|
| Polyphenols | +++ |
| Flavonoids | ++ |
| Alkaloids | +++ |
| Tannins | ++ |
| Sterols and Polyterpens | + |
| Reducing compounds | +++ |
| Cardiac glycosides | ++ |
| Saponins | - |

***Key**: (-): Absent; (+) : Present in low concentration ; (++) : Present in moderate concentration ; (+++) : Present in high concentration.*

## CONCLUSION

This work showed that the methanol extract of *P. laxiflora* contains various phenolic compounds including phenols, flavonoids and tannins. And, all of our results indicates that these polyphenols are responsible for the antioxidant and antibacterial activity of the extract tested. Proving these bivalent properties of polyphenols, this study validates in part the traditional use of *P. laxiflora* in the treatment of various diseases such as cellular aging and gastritis enteritis.

These preliminary results are interesting and we think continue investigations on other diseases caused by oxidative stress such as cancer and cardiovascular disease.

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