

EVALUATION OF THE ANTI-INFLAMMATORY AND ANTIOXIDANT ACTIVITIES OF EXTRACTS OF *PAULLINIA PINNATA* L. AND *MEZONEURON BENTHAMIANUM* BAILL., TWO PLANTS TRADITIONALLY USED TO TREAT BREAST CANCER IN CÔTE D'IVOIRE

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How to cite this Article: Bamba Abou^{1,2*}, Aka Kouadio Ayebe Edwige Diplo³, Tchepe Flore Bernadette², Offoumou M'baï Rostand⁵, Koffi N'dri Emmanuel⁴, Kipre Gueyraud Rolland¹, Konan Ange¹, Assouan Kodjo Hugues¹, Yapi Houphouet Félix¹ (2026). Evaluation of the anti-inflammatory and antioxidant activities of extracts of *paullinia pinnata* L. And *mezoneuron benthamianum* baill., two plants traditionally used to treat breast cancer in côte d'ivoire. World Journal of Pharmaceutical and Medical Research, 12(7), 162-169.

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Article Received on 12/05/2026

Article Revised on 01/06/2026

Article Published on 01/07/2026

ABSTRACT

Breast cancer remains one of the leading causes of cancer-related mortality among women worldwide. Chronic inflammation and oxidative stress are recognized as major factors involved in tumor initiation and progression. In Côte d'Ivoire, *Paullinia pinnata* and *Mezoneuron benthamianum* are traditionally used in the management of breast cancer; however, their biological properties remain poorly documented. This study aimed to evaluate the anti-inflammatory and antioxidant activities of hydroethanolic extracts from these two medicinal plants. The anti-inflammatory activity was assessed in vitro using the inhibition of bovine serum albumin denaturation assay, while antioxidant activity was evaluated using DPPH, ABTS, and FRAP assays. The extraction yields obtained were $6.8 \pm 2.75\%$ for *P. pinnata* and $3.4 \pm 0.33\%$ for *M. benthamianum*. At 250 $\mu\text{g/mL}$, both extracts exhibited moderate anti-inflammatory activity, with inhibition percentages of 54.39% for *M. benthamianum* and 52.63% for *P. pinnata*, compared with 73.69% for sodium diclofenac. Antioxidant analyses revealed significant radical scavenging and reducing activities. In the DPPH assay, *P. pinnata* showed the strongest activity with an IC_{50} of $15.33 \pm 1.78 \mu\text{g/mL}$, whereas *M. benthamianum* exhibited superior activity in the ABTS and FRAP assays. These findings demonstrate that both plants possess significant anti-inflammatory and antioxidant properties, likely related to the presence of bioactive phytochemicals such as polyphenols and flavonoids. The study supports their potential as promising sources of natural compounds for the development of complementary therapeutic approaches against breast cancer.

KEYWORDS: antioxidant; anti-inflammatory; medicinal plants.

1. INTRODUCTION

Cancer, also known as malignant neoplasia, refers to a broad group of diseases characterized by the uncontrolled proliferation of abnormal cells capable of invading surrounding tissues and spreading to distant organs through metastasis (Okuma *et al.*, 2017). When it

develops in breast tissue, it is referred to as breast cancer. This disease generally originates in the milk ducts or breast lobules and is characterized by the abnormal growth of breast cells, leading to alterations in breast tissue such as the appearance of masses, skin or nipple

changes, and regional lymphadenopathy (Harbeck *et al.*, 2019).

Breast cancer is currently one of the most common cancers and one of the leading causes of death among women worldwide. In 2022, GLOBOCAN estimated approximately 2.3 million new cases and nearly 670,000 deaths related to this disease. In Côte d'Ivoire, it ranks first among female cancers, with 3,869 new cases and about 2,092 deaths (Sung *et al.*, 2021), making it a major public health concern.

The development of breast cancer results from a complex interaction of several biological factors, among which chronic inflammation and oxidative stress play a major role. Inflammation promotes tumor proliferation, angiogenesis, and treatment resistance, while oxidative stress, associated with excessive production of reactive oxygen species, causes cellular damage that favors tumor initiation and progression (Bray *et al.*, 2024).

Despite significant progress, conventional treatments such as surgery, radiotherapy, and chemotherapy remain costly and are associated with numerous side effects, including immunosuppression, neurotoxicity, and deterioration in quality of life (Harbeck *et al.*, 2019; Seum *et al.*, 2024). In this context, medicinal plants represent an important source of bioactive compounds that may offer new therapeutic perspectives.



Figure 1: *Mezoneuron benthamianum*.

2.2 Aqueous Extraction

After washing, grinding, and drying the plant materials, 100 g of powdered material were weighed and dissolved in 1 L of distilled water, followed by homogenization using a blender. The resulting macerate was successively filtered once through cloth, three times through hydrophilic cotton, and once through Whatman filter paper. The filtrate was then oven-dried at 45°C for 48 h to obtain crude dry extracts. This procedure was repeated several times to obtain sufficient quantities of extracts for the different assays (Zirih *et al.*, 2003). The extraction yield was calculated using the following formula.

$$R = (M/M_0) \times 100$$

In Côte d'Ivoire, *Paullinia pinnata* and *Mezoneuron benthamianum* are two plants traditionally used in the management of breast cancer. However, their biological properties remain poorly documented. The present study therefore aims to evaluate the anti-inflammatory and antioxidant activities of their extracts in order to scientifically verify their therapeutic potential.

The general objective is to contribute to the search for new adjuvant therapeutic approaches for the management of breast cancer in Côte d'Ivoire. More specifically, the study aims to evaluate the anti-inflammatory properties and antioxidant potential of extracts from *Paullinia pinnata* and *Mezoneuron benthamianum*.

2. MATERIALS AND METHODS

2.1 Plant Material

The plant material used consisted of the bark of *Mezoneuron benthamianum* (Figure 1) and the stem of *Paullinia pinnata* (Figure 2). The plants were collected in Lakota, located in the Lôh-Djiboua region of Côte d'Ivoire. They were identified at the National Floristic Center (CNF) of Félix HOUPHOUËT-BOIGNY University in Cocody under the voucher numbers UCJ009456 for *M. benthamianum* and UCJ016410 for *P. pinnata*.



Figure 2: Tige de *Paullinia pinnata*.

R: extraction yield expressed as %, M: mass of crude dry extract (g), M_0 : mass of plant material processed (g).

2.3 In Vitro Evaluation of Anti-Inflammatory Activity

The anti-inflammatory activity of *M. benthamianum* and *P. pinnata* extracts was evaluated using the protein denaturation inhibition assay described by Williams *et al.* (2008). For this assay, three solutions (0.5 mL each) were prepared: a test solution and a standard solution, both containing 0.45 mL of 5% aqueous bovine serum albumin (BSA) solution and 0.05 mL of either plant extract or sodium diclofenac at a concentration of 250 µg/mL, respectively; and a product control solution

composed of 0.45 mL distilled water and 0.05 mL extract at 250 µg/mL. All samples were incubated at 37°C for 20 min and then heated at 57°C for 3 min. After cooling, 2.5 mL of phosphate-buffered saline (PBS) were added to each reaction mixture. Absorbance was measured at 255 nm using a SHIMADZU UV-Visible spectrophotometer. The control represented 100% protein denaturation. The percentage inhibition of protein denaturation was calculated using the following equation.

$$\text{Inhibition of protein denaturation (\%)} = \left[\frac{\text{Abs}_{\text{contrôle}} - \text{Abs}_{\text{échantillon}}}{\text{Abs}_{\text{contrôle}}} \right] \times 100$$

Abs control: absorbance of the control solution (BSA + distilled water),

Abs sample: absorbance of the sample solution (BSA + extract or diclofenac).

The obtained values were compared with those of sodium diclofenac used as the reference drug.

2.4 Evaluation of Antioxidant Activity

The *in vitro* antioxidant activity of the extracts was assessed using three complementary assays: DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS [2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)], and FRAP (Ferric Reducing Antioxidant Power).

2.4.1 DPPH Assay

The method described by Baliyan *et al.* (2022) was used with slight modifications. DPPH is a stable free radical characterized by a violet coloration and a maximum absorbance at 517 nm. Antioxidant compounds reduce the DPPH radical, resulting in a color change from violet to pale yellow.

Different concentrations (15.625, 31.25, 62.5, 125, 250, and 500 µg/mL) were prepared by serial dilution from a stock extract solution (0.1 mg/mL). Subsequently, 2 mL of methanolic DPPH solution (100 µM) were mixed with 1.5 mL of each extract at the different concentrations. The mixtures were incubated at room temperature for 30 min in the dark. Absorbance was measured at 517 nm against a blank solution containing 2 mL of DPPH solution and 1.5 mL methanol.

Vitamin C, prepared at concentrations ranging from 0 to 500 µg/mL, was used as the reference antioxidant and tested under the same conditions. The antioxidant activity of the samples, corresponding to their DPPH radical scavenging capacity, was expressed as percentage inhibition (PI) using the following equation.

$$\text{PI (\%)} = \left(\frac{\text{Abs}_{\text{contrôle}} - \text{Abs}_{\text{échantillon}}}{\text{Abs}_{\text{contrôle}}} \right) \times 100$$

Abs control: absorbance of the DPPH solution without plant extract,

Abs sample: absorbance in the presence of the tested sample (extract or vitamin C).

A graph plotting DPPH radical inhibition against sample concentration was used to determine the IC₅₀ value, defined as the concentration required to inhibit 50% of DPPH radicals.

2.4.2 ABTS Assay

This method is based on the ABTS radical cation (ABTS^{•+}), which is stable in its free form. When an antioxidant compound is added to the ABTS^{•+} solution, the radical cation is reduced, resulting in a color change from blue-green to colorless or pale yellow depending on the degree of reduction. This reaction is associated with a decrease in absorbance at 734 nm (Wołosiak *et al.*, 2021). To generate the ABTS^{•+} radical, equal volumes of potassium persulfate solution (K₂S₂O₈, 2.6 mM) and ABTS solution (7 mM) were mixed. The resulting solution was kept in the dark for 16 h prior to use. For the antiradical assay, 2500 µL of freshly prepared ABTS radical solution were added to 100 µL of plant extracts at different concentrations (31.25, 62.5, 125, 250, 500, and 1000 µg/mL). The mixtures were incubated at room temperature for 30 min in the dark. TROLOX, prepared at concentrations ranging from 0 to 1000 µg/mL, was used as the reference standard. Absorbance was measured at 734 nm using a UV-Visible spectrophotometer.

The antioxidant activity of the extracts, expressed as percentage inhibition (PI), was calculated using the following equation.

$$\text{PI (\%)} = \left(\frac{\text{Abs}_{\text{contrôle}} - \text{Abs}_{\text{échantillon}}}{\text{Abs}_{\text{contrôle}}} \right) \times 100$$

Abs control: absorbance of the ABTS solution without plant extract,

Abs sample: absorbance in the presence of the tested sample (extract or TROLOX).

A graph representing ABTS radical inhibition as a function of sample concentration was used to determine the IC₅₀ value, corresponding to the concentration required to inhibit 50% of ABTS radicals.

2.4.3 FRAP Assay

The FRAP assay was performed according to the method described by Fernandes *et al.* (2016). This method is based on the ability of antioxidant compounds to reduce ferric ions (Fe³⁺) to ferrous ions (Fe²⁺). The FRAP reagent (10 mM) was prepared by mixing 2.5 mL of TPTZ solution (10 mM in 40 mM HCl), 2.5 mL of FeCl₃·6H₂O solution (20 mM), and 25 mL of acetate buffer (300 mM sodium acetate, pH 3.6 adjusted with acetic acid). Subsequently, 3500 µL of FRAP reagent were added to 140 µL of plant extract solution at a concentration of 500 µg/mL. After incubation for 30 min at room temperature in the dark, absorbance was measured at 593 nm. TROLOX was used as the reference standard.

3. Statistical Analysis

All experiments were performed in triplicate. The obtained data were analyzed using GraphPad Prism version 8.0 software (Microsoft, USA). Results were expressed as mean values.

4. RESULTS AND DISCUSSION

1.1 Extraction Yield

The extraction of bioactive compounds from the bark of

Mezoneuron benthamianum and the stems of *Paullinia pinnata* yielded extraction rates of $3.4 \pm 0.33\%$ and $6.8 \pm 2.75\%$ dry extracts, respectively. Statistical analysis revealed that the extraction yield of *P. pinnata* was significantly higher than that of *M. benthamianum* ($p < 0.05$), indicating a marked difference in the extraction efficiency of secondary metabolites between the two species. The results showed that the hydroethanolic extract yield of *Paullinia pinnata* was approximately twice that obtained for *Mezoneuron benthamianum*, suggesting a higher abundance of extractable compounds under the experimental conditions used.

This variation in extraction yield may be explained by several factors. On one hand, the use of a hydroethanolic solvent system provides an efficient extraction medium due to the complementary polarity of water and ethanol, thereby promoting the solubilization of a broad spectrum of secondary metabolites, particularly polar and semi-polar compounds such as polyphenols, flavonoids, alkaloids, and tannins (Tourabi *et al.*, 2023). On the other hand, the nature of the plant organs used also plays a determining role in extraction efficiency. Indeed, the stems of *P. pinnata* may contain a higher proportion of compounds soluble in hydroalcoholic solvents, whereas the bark of *M. benthamianum*, generally richer in structural compounds such as lignin and complex polysaccharides, may exhibit lower extractability of bioactive metabolites.

Furthermore, the observed differences may also be attributed to intrinsic and extrinsic factors. Intrinsically, the genetic characteristics specific to each species influence the biosynthesis, accumulation, and distribution of secondary metabolites within plant organs. Extrinsically, environmental conditions such as soil composition, water availability, sunlight exposure, temperature, and seasonal variations may significantly modulate the production of secondary metabolites (Bruno *et al.*, 2013). The physiological stage of the plant at the time of harvesting also constitutes an important factor, since the concentration of bioactive compounds may fluctuate during plant development.

These results differ from those reported by Chechet *et al.* (2018), who obtained extraction yields reaching up to 14% for certain medicinal plants. This disparity could be explained by differences related to the plant material used, extraction conditions, solvent polarity, and the phytochemical specificities of the investigated species. These observations are consistent with the findings of Qaderi *et al.* (2023), who demonstrated that ecological, physiological, and methodological factors significantly influence both the quality and quantity of secondary metabolites extracted from medicinal plants.

Thus, the significant difference in extraction yield observed between *Paullinia pinnata* and *Mezoneuron benthamianum* may result from the complex interaction between the nature of the plant material, the intrinsic

phytochemical characteristics of the species, and the extraction conditions, conferring *P. pinnata* with a higher extraction potential under the studied experimental conditions. However, a high extraction yield does not necessarily imply superior biological activity, which depends primarily on the nature and concentration of bioactive compounds present in the extract.

1.2 Anti-Inflammatory Activity

The anti-inflammatory activity results of *M. benthamianum* and *P. pinnata* extracts, expressed as percentages of inhibition of bovine serum albumin (BSA) denaturation, showed that both extracts possess in vitro anti-inflammatory activity at a concentration of 250 $\mu\text{g/mL}$. Statistical analysis revealed that *M. benthamianum* extract exhibited an inhibition percentage of 54.39%, slightly higher than that of *P. pinnata* (52.63%). However, this difference between the two extracts was not statistically significant ($p > 0.05$), indicating globally comparable anti-inflammatory activity between the two species. In contrast, their activities were significantly lower than that of sodium diclofenac (73.69%; $p < 0.05$), used as the reference anti-inflammatory drug. These results indicate that although both extracts possess notable inhibitory capacity, their effectiveness remains lower than that of the pharmacological standard.

The anti-inflammatory activity of the extracts was evaluated through their ability to inhibit protein denaturation, a mechanism commonly used to assess in vitro anti-inflammatory potential. Protein denaturation corresponds to the alteration of secondary and tertiary protein structures under the influence of physical or chemical factors, particularly heat, resulting in loss of biological function (Hasan *et al.*, 2023). This phenomenon is involved in inflammatory processes because denatured proteins may act as autoantigens and amplify inflammatory responses. Therefore, the ability of an extract to inhibit this process reflects its capacity to stabilize proteins and attenuate inflammatory reactions in vitro (Mba *et al.*, 2022).

The results obtained in this study indicate that both extracts exhibit moderate anti-inflammatory activity, with inhibition percentages above 50%, suggesting the probable presence of bioactive secondary metabolites capable of interfering with protein denaturation mechanisms. The slightly higher activity observed with *M. benthamianum* may be related to a higher concentration or better synergistic interaction of certain anti-inflammatory compounds present in this extract. Nevertheless, the absence of a statistically significant difference between the two extracts suggests that their anti-inflammatory potentials are globally comparable under the studied experimental conditions.

This activity may be attributed to the presence of phytochemical compounds such as polyphenols, flavonoids, tannins, saponins, and alkaloids, which are

known for their ability to inhibit protein denaturation, stabilize cellular membranes, and modulate the production of pro-inflammatory mediators (Kumar *et al.*, 2017). In particular, polyphenols and flavonoids possess antioxidant properties capable of neutralizing reactive oxygen species involved in the amplification of inflammatory processes, thereby contributing to the protection of protein structures against oxidative damage.

The findings of this study corroborate those reported by Tseuguem *et al.* (2019), who demonstrated notable anti-inflammatory activity of aqueous and methanolic extracts of *Paullinia pinnata*, as well as those of Mbagwu *et al.* (2007), who reported anti-inflammatory properties of aqueous extracts of *Mezoneuron benthamianum* both in vitro and in vivo. These studies confirm that these plant species constitute potential sources of bioactive molecules with anti-inflammatory properties.

However, the activity of the extracts remained significantly lower than that of sodium diclofenac, which may be explained by the fact that diclofenac is a pure compound with a specific mechanism of action, particularly through cyclooxygenase (COX) inhibition, whereas plant extracts are complex mixtures in which active compounds are present at variable concentrations and may exhibit synergistic or antagonistic interactions. Thus, the obtained results demonstrate that *Mezoneuron benthamianum* and *Paullinia pinnata* possess significant in vitro anti-inflammatory activity, although moderate when compared with sodium diclofenac. The absence of a statistically significant difference between the two extracts suggests similar anti-inflammatory potentials, making these plants promising candidates for the search for natural anti-inflammatory compounds.

1.3 Antioxidant Activity

The in vitro antioxidant activity of *Mezoneuron benthamianum* and *Paullinia pinnata* extracts was evaluated using three complementary assays: DPPH, ABTS, and FRAP, which are commonly used to assess the antioxidant potential of plant extracts. Statistical analysis of the results revealed significant differences between the extracts depending on the assay used ($p < 0.05$), indicating variability in antioxidant behavior according to the reaction mechanism involved. Indeed, these assays evaluate different antioxidant mechanisms, particularly free radical scavenging and electron transfer capacity, thereby providing a more comprehensive assessment of the antioxidant potential of plant extracts (Munteanu and Apetrei, 2021).

1.3.1 DPPH Radical Scavenging Activity

The DPPH assay results showed that the percentage inhibition of radicals increased with extract concentration, indicating dose-dependent antiradical activity. The regression curve obtained allowed determination of the inhibitory concentration 50 (IC_{50}), corresponding to the concentration required to inhibit 50% of free radicals (Figure 3). The obtained IC_{50} values were $4.33 \pm 0.5 \mu\text{g/mL}$ for vitamin C, $15.33 \pm 1.78 \mu\text{g/mL}$ for *P. pinnata*, and $23.67 \pm 0.36 \mu\text{g/mL}$ for *M. benthamianum*. Statistical analysis revealed that the IC_{50} of *P. pinnata* was significantly lower than that of *M. benthamianum* ($p < 0.05$), indicating significantly higher antiradical activity. However, both extracts exhibited IC_{50} values significantly higher than that of vitamin C ($p < 0.05$), demonstrating antioxidant activity lower than that of the reference standard.

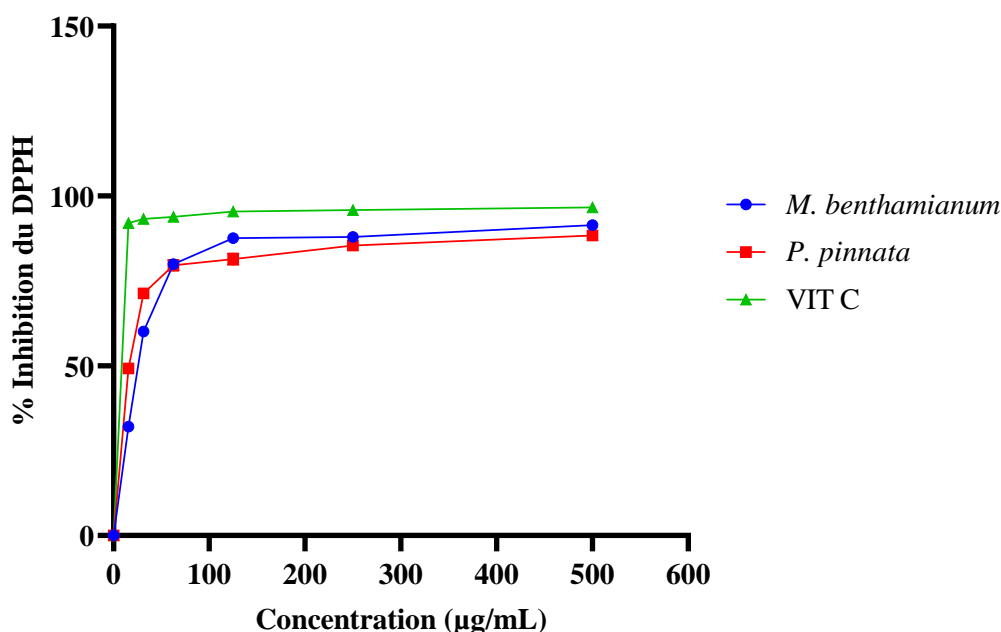


Figure 3: DPPH Radical Scavenging Activity of *M. benthamianum*, *P. pinnata* Extracts and Vitamin C.

These results indicate that both extracts possess significant DPPH radical scavenging capacities, with *P. pinnata* exhibiting the highest activity in this assay. Indeed, a lower IC₅₀ value reflects a greater ability to neutralize free radicals. This activity could be attributed to the presence of phenolic compounds, particularly flavonoids and tannins, which are known for their ability to donate electrons or hydrogen atoms in order to stabilize free radicals (Olugbami et al., 2014). The higher activity observed with *P. pinnata* may suggest a greater concentration of active phenolic compounds or the presence of molecules with stronger hydrogen-donating capacity.

These findings are consistent with the observations of Adu-Amankwaah et al. (2023), who reported that

polyphenol-rich extracts generally exhibit strong antiradical activity due to their abundance of hydroxyl groups capable of neutralizing free radicals. However, vitamin C remained significantly more effective, which can be explained by its nature as a pure compound possessing high antioxidant potential.

1.3.2 ABTS Radical Scavenging Activity

The ABTS assay results also demonstrated a dose-dependent inhibition of free radicals by the investigated extracts. The obtained IC₅₀ values were 114.17 ± X µg/mL for TROLOX, 135.00 ± X µg/mL for *M. benthamianum*, and 155.83 ± X µg/mL for *P. pinnata* (Figure 4).

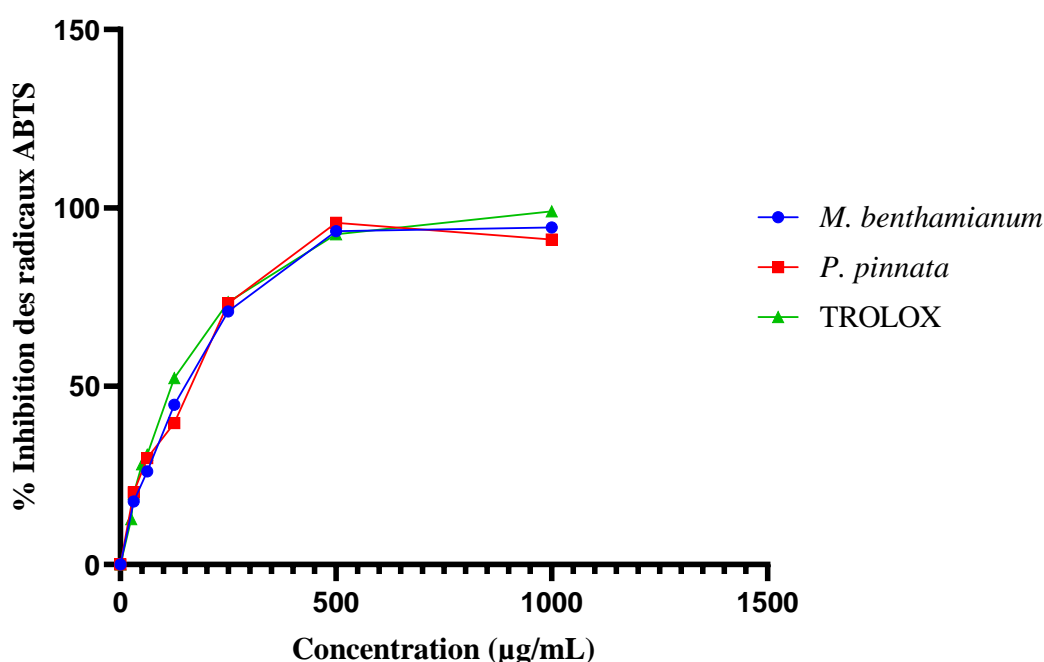


Figure 4: ABTS Radical Scavenging Activity of *M. benthamianum*, *P. pinnata* Extracts and TROLOX.

Statistical analysis indicated that *M. benthamianum* exhibited a significantly lower IC₅₀ value than *P. pinnata* ($p < 0.05$), reflecting greater antiradical activity in this assay. However, both extracts remained significantly less active than TROLOX ($p < 0.05$), used as the antioxidant reference standard.

Contrary to the results observed with the DPPH assay, *M. benthamianum* appeared to be the most active extract in the ABTS assay. This divergence may be explained by the structural differences between DPPH and ABTS radicals, as well as by the chemical nature of the compounds present in the extracts. Since the ABTS radical is soluble in both aqueous and organic media, it can interact with a broader range of antioxidant compounds, particularly certain hydrophilic compounds present in *M. benthamianum* (Costa et al., 2023). These results therefore suggest that the extracts possess distinct

antioxidant profiles depending on the nature of the radical tested.

1.3.3 Ferric Reducing Antioxidant Power (FRAP)

The reducing power of the extracts, expressed as the concentration of ferrous ions (Fe²⁺) generated after reduction of ferric ions (Fe³⁺), was determined from the calibration curve equation ($y = 0.0056x$).

The obtained results showed values of 1301.43 ± X µg/mL Fe²⁺ for *M. benthamianum* and 672.14 ± X µg/mL Fe²⁺ for *P. pinnata*. Statistical analysis revealed that the reducing power of *M. benthamianum* was significantly higher than that of *P. pinnata* ($p < 0.05$), indicating a greater capacity for electron transfer and ferric ion reduction.

The FRAP assay is based on the electron transfer mechanism, which is considered a major indicator of antioxidant potential. A higher value reflects stronger reducing capacity and therefore greater antioxidant potential (Olugbami *et al.*, 2014). The results suggest that *M. benthamianum* probably contains a higher concentration of reducing compounds or molecules with greater electron-donating ability. Several studies have demonstrated that reducing power is strongly correlated with the presence of polyphenols and constitutes a reliable marker of antioxidant activity (Gouvinhas *et al.*, 2018; Bibi *et al.*, 2020).

Comparative analysis of the three assays showed that both extracts possess significant antioxidant activity, although their effectiveness varies depending on the mechanism evaluated. *Paullinia pinnata* exhibited greater activity in the DPPH assay, whereas *Mezoneuron benthamianum* was more active in the ABTS and FRAP assays. These differences suggest that the extracts do not share the same phytochemical profile and that their bioactive compounds may act through distinct antioxidant mechanisms.

These findings are consistent with several studies reporting notable antioxidant activity of *P. pinnata* and *M. benthamianum*, although generally lower than that of reference antioxidant molecules (Dermame *et al.*, 2018). However, some plant extracts, such as those from *Vitis vinifera* (Katalinić *et al.*, 2006) and *Peumus boldus* (Amarowicz *et al.*, 2004), have demonstrated antioxidant activities superior to certain reference standards, probably due to their higher content of polyphenols and other reducing compounds. Thus, the obtained results demonstrate that *Mezoneuron benthamianum* and *Paullinia pinnata* possess genuine antioxidant potential, although differing according to the mechanism of action investigated. This suggests the presence of distinct phytochemical profiles and confirms their relevance as potential sources of natural antioxidant compounds.

5. CONCLUSION

The present study aimed to evaluate the anti-inflammatory and antioxidant activities of hydroethanolic extracts of *Paullinia pinnata* and *Mezoneuron benthamianum*, two plants widely used in Ivorian traditional medicine. The results demonstrated a notable inhibition of protein denaturation by extracts from both plants, highlighting their anti-inflammatory potential. These plant extracts were also capable of neutralizing free radicals, thereby demonstrating antioxidant potential with varying levels of effectiveness.

Although the activities of these two plants were lower than those of the reference substances used, namely diclofenac, vitamin C, and TROLOX, their biological potentials against inflammation and oxidative stress remain significant. These two processes are involved in the pathogenesis of numerous diseases, particularly breast cancer.

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