

Evaluation of the antioxidant and anti-inflammatory activity of *Pycnanthus angolensis* and *Cnestis ferruginea*, two plants traditionally used in the treatment of breast cancer in Côte d'Ivoire

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Abstract

This study aims to evaluate the antioxidant and anti-inflammatory activities of plant extracts from *Cnestis ferruginea* and *Pycnanthus angolensis*. The antioxidant activity was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS, and FRAP assays, while the anti-inflammatory activity was assessed by the extracts' ability to inhibit bovine serum albumin denaturation.

The results show that the *Cnestis ferruginea* extract exhibits higher antioxidant activity, with IC₅₀ values of 97.46 µg/mL (DPPH), while the *Pycnanthus angolensis* extract displays a more moderate activity (91.37 µg/mL), compared to vitamin C (6.09 µg/mL). In contrast, the anti-inflammatory activity is higher for *Pycnanthus angolensis*, with an inhibition percentage of 68.42%, significantly greater than that of *Cnestis ferruginea* (47.36%) ($p < 0.05$) and comparable to sodium diclofenac (73.68%) ($p > 0.05$).

These results indicate that both extracts possess complementary biological activities. The *Pycnanthus angolensis* extract may contain compounds capable of directly acting on inflammatory mechanisms, independent of their antioxidant activity.

In conclusion, these plants are promising sources of bioactive compounds, potentially valuable in the prevention and management of diseases associated with oxidative stress and inflammation, pending further studies.

Keywords: Antioxidant activity; Anti-inflammatory; DPPH; ABTS; FRAP; Plant extracts

1. Introduction

Breast cancer is one of the most common and deadliest cancers among women worldwide, with a steadily increasing incidence, particularly in low- and middle-income countries, including those in sub-Saharan Africa [1]. This disease is characterized by uncontrolled cell proliferation, often associated with increased oxidative stress and chronic inflammatory processes that promote tumor progression and metastasis [2].

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Oxidative stress results from an imbalance between the production of free radicals and the body's antioxidant defense systems, leading to damage to biomolecules such as DNA, proteins, and lipids [3]. Furthermore, chronic inflammation plays a critical role in cancer initiation and progression through the production of pro-inflammatory cytokines and the activation of tumor-related signaling pathways [4]. Therefore, natural compounds with antioxidant and anti-inflammatory properties represent a promising approach for cancer prevention and management [2].

In Africa, and particularly in Côte d'Ivoire, traditional medicine plays a central role in healthcare systems. According to the World Health Organization, nearly 80% of African populations rely on medicinal plants for primary healthcare [1]. Among these plants, *Pycnanthus angolensis* and *Cnestis ferruginea* are widely used for the treatment of various ailments, including pain, inflammation, and certain chronic diseases.

Studies have shown that *Pycnanthus angolensis* contains various secondary metabolites such as flavonoids, tannins, saponins, and terpenoids, which are associated with significant antioxidant activities [5]. In addition, some extracts of this plant exhibit notable anti-inflammatory properties, contributing to the reduction of oxidative stress [6]. Similarly, *Cnestis ferruginea* is rich in bioactive compounds such as flavonoids, alkaloids, tannins, and saponins, which are responsible for several biological activities, including antioxidant and anti-inflammatory effects [7]. Previous research has also demonstrated its analgesic and anti-inflammatory properties, supporting its use in traditional medicine [6].

However, despite these findings, the specific biological activities of these plants in the context of breast cancer remain insufficiently documented. It is therefore essential to conduct experimental studies to validate their traditional uses and to better understand their mechanisms of action.

Thus, the present study aims to evaluate the antioxidant and anti-inflammatory activities of extracts from *Pycnanthus angolensis* and *Cnestis ferruginea*, two plants traditionally used in the treatment of breast cancer in Côte d'Ivoire, in order to contribute to their therapeutic valorization and to the search for new natural alternatives in cancer management.

Specifically, this work will aim to:

- Evaluate the in vitro antioxidant activity of total aqueous and hydroethanolic extracts of *Pycnanthus angolensis* and *Cnestis ferruginea* using DPPH and ABTS radical scavenging assays, as well as the ferric reducing antioxidant power (FRAP) assay;
- Assess the in vitro anti-inflammatory activity of these plant extracts using the protein denaturation inhibition assay.

2. Materials and Methods

2.1. Plant Material

The plant material consisted of stem bark of *Pycnanthus angolensis* and leaves of *Cnestis ferruginea*, collected in the Lôh-Djiboua region, specifically in Lakota, in the southwest of Côte d'Ivoire. Samples of both plants were transferred to the National Floristic Center (CNF) of Félix Houphouët-Boigny University, where they were identified and registered under the voucher numbers UCJ012895 for *Pycnanthus angolensis* and UCJ004063 for *Cnestis ferruginea*. The plant organs were air-dried at room temperature for two weeks and then ground into powder using an electric grinder.

2.2. Preparation of Plant Extracts

The preparation of extracts from *Pycnanthus angolensis* and *Cnestis ferruginea* was carried out according to the method described by Zihiri et al. [8]. After collection, the stem bark of *Pycnanthus angolensis* and the leaves of *Cnestis ferruginea* were washed with water, shade-dried, and then pulverized using a blender.

Two types of extraction were performed: an aqueous extraction for *Pycnanthus angolensis* and a hydroethanolic extraction for *Cnestis ferruginea*.

To prepare the total aqueous extract of *Pycnanthus angolensis*, 100 g of powdered stem bark were homogenized in 1 L of distilled water using a blender at room temperature. The resulting homogenate was successively filtered through hydrophilic cotton and Whatman filter paper (3 mm). The extraction solvent was then evaporated in an oven set at 50°C. The dried residue was collected as a powder, constituting the total aqueous extract (E24).

For the hydroethanolic extract of *Cnestis ferruginea*, 100 g of powdered leaves were dissolved in 1 L of 70% ethanol. The mixture was homogenized for 5 minutes using a blender, and the resulting homogenate was filtered successively through cloth, three times through hydrophilic cotton, and once through Whatman filter paper (3 mm). This procedure was repeated three times, and the filtrates were pooled and dried in an oven at 45°C for 48 hours. The resulting powder constituted the hydroethanolic extract (E37). Both dry extracts were stored at 4°C until further use. The extraction yield was calculated using the following formula:

$$R \% = (m/m_0) \times 100$$

Where:

R = extraction yield (%)

m = mass (g) of the dry extract obtained

m₀ = mass (g) of the initial plant material

2.3. Evaluation of the Antioxidant Activity of *Pycnanthus angolensis* and *Cnestis ferruginea* Extracts

The antioxidant activity of E24 and E37 extracts was evaluated using DPPH and ABTS radical scavenging assays, as well as the ferric reducing antioxidant power (FRAP) assay.

2.3.1. Determination of Free Radical Scavenging Activity Using the DPPH Assay

The DPPH radical is widely used to evaluate the free radical scavenging activity of plant extracts. It is a stable free radical with a deep violet color that absorbs at 517 nm. In the presence of antioxidant compounds, DPPH is reduced to diphenylpicrylhydrazine, resulting in a color change from violet to yellow. The decrease in absorbance is inversely proportional to the antioxidant capacity of the tested sample.

The antiradical activity of *Pycnanthus angolensis* and *Cnestis ferruginea* extracts was determined according to the method described by Bammou et al. [9], with slight modifications. Briefly, 2 mL of a methanolic DPPH solution were mixed with 1.5 mL of extract solutions at different concentrations (500, 250, 125, 62.5, 31.25, and 15.625 µg/mL). The mixture was incubated in the dark at room temperature for 30 minutes.

Absorbance was measured at 517 nm using a UV-Visible spectrophotometer against a methanol blank. Vitamin C (ascorbic acid), at concentrations ranging from 0 to 500 µg/mL, was used as a reference standard.

The percentage inhibition (%I) was calculated using the following formula:

$$\%I = (A_0 - A_1/A_0) \times 100$$

Where:

%I = percentage inhibition

A₀ = absorbance of the DPPH solution without extract (control)

A₁ = absorbance of the DPPH solution in the presence of extract (sample)

The inhibitory concentration at 50% (IC₅₀), expressed in µg/mL, corresponds to the concentration of extract or vitamin C required to inhibit 50% of DPPH radicals. It was determined from the plot of percentage inhibition versus extract concentration.

2.3.2. Determination of Antioxidant Activity Using the ABTS Radical Scavenging Assay

The ABTS radical solution was prepared by mixing equal volumes of potassium persulfate (2.6 mM) and ABTS (7 mM). The mixture was incubated in the dark (covered with aluminum foil) at room temperature for 16 hours to allow radical formation.

Before use, the solution was diluted with 60 mL of methanol to obtain an absorbance between 1.0 and 1.5 at 734 nm. Then, 100 µL of extract at different concentrations (31.25, 62.5, 125, 250, 500, and 1000 µg/mL) were added to 2500 µL of freshly prepared ABTS solution. The reaction mixtures were incubated in the dark at room temperature for 30 minutes.

Absorbance was measured at 734 nm using a UV-Visible spectrophotometer. Trolox, at concentrations ranging from 12.5 to 500 µg/mL, was used as a reference standard. The percentage inhibition was calculated using the following formula:

$$\% I = (\text{Abs blank} - \text{Abs sample} / \text{Abs blank}) \times 100$$

Where:

%I = percentage inhibition

Abs_blank = absorbance of ABTS solution (control)

Abs_sample = absorbance of ABTS solution in the presence of extract or standard

2.3.3. Determination of Total Antioxidant Activity Using the FRAP Assay

The ferric reducing antioxidant power (FRAP) assay of *Pycnanthus angolensis* and *Cnestis ferruginea* extracts was performed according to the protocol described by Bammou et al. (2022) [9]. This method is based on the reduction of the ferric tripyridyltriazine complex (Fe^{3+} -TPTZ) to the ferrous form (Fe^{2+} -TPTZ) in the presence of antioxidants under acidic conditions. This reduction leads to the formation of an intense blue color with maximum absorbance at 593 nm.

The FRAP reagent was prepared by mixing 2.5 mL of TPTZ solution, 2.5 mL of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, and 25 mL of acetate buffer (pH 3.6). Then, 3500 µL of this reagent were added to 140 µL of methanolic extract solution. The mixture was incubated for 30 minutes at room temperature in the dark, and absorbance was measured at 593 nm using a spectrophotometer. Trolox was used as the reference standard.

2.4. Evaluation of the Anti-Inflammatory Activity of *Pycnanthus angolensis* and *Cnestis ferruginea* Extracts

The in vitro anti-inflammatory activity of *Pycnanthus angolensis* and *Cnestis ferruginea* extracts was evaluated using the protein denaturation inhibition method.

The procedure involved the preparation of three solutions:

- Test solution (0.5 mL): consisting of 0.45 mL of 5% bovine serum albumin (BSA) in aqueous solution and 0.05 mL of plant extract at a concentration of 250 µg/mL;
- Control solution (0.5 mL): consisting of 0.45 mL of 5% BSA solution and 0.05 mL of distilled water;
- Standard solution (0.5 mL): consisting of 0.45 mL of 5% BSA and 0.05 mL of sodium diclofenac at a concentration of 250 µg/mL.

All samples were first incubated at 37°C for 20 minutes, followed by heating at 57°C for 3 minutes. After cooling, 2.5 mL of phosphate-buffered saline (PBS) were added to each tube.

Absorbance was measured at 416 nm using a UV-Visible spectrophotometer. The percentage inhibition of protein denaturation was calculated using the following formula:

$$\%I = [(\text{Abs control} - \text{Abs sample}) / \text{Abs control}] \times 100$$

Where:

%I = percentage inhibition

Abs_control = absorbance of BSA + distilled water

Abs_sample = absorbance of BSA + plant extract

The control was considered to represent 100% protein denaturation. The results obtained for the extracts were compared with those of sodium diclofenac (250 µg/mL), used as a reference anti-inflammatory drug.

2.5. Statistical Analysis

Statistical analysis of the experimental data was performed using GraphPad Prism 8 (Microsoft, USA) and Excel 2010. Results were expressed as mean values.

3. Results and Discussion

3.1. Extraction Yield

The extraction of bioactive compounds from the bark of *Pycnanthus angolensis* and the leaves of *Cnestis ferruginea* yielded 2.8% and 12.55% dry extracts, respectively. Statistical analysis revealed that the extraction yield of *C. ferruginea* was significantly higher than that of *P. angolensis* ($p < 0.05$), indicating a marked difference in the extractability of secondary metabolites between the two species. Notably, the hydroethanolic extract of *C. ferruginea* (E37; 12.55%) was more than four times higher than that of *P. angolensis* (E24; 2.8%).

This variation can be explained by several factors. First, the use of a hydroethanolic solvent enhances extraction efficiency due to its dual polarity, allowing the solubilization of both polar and semi-polar compounds [10]. Second, the plant organ plays a critical role: leaves of *C. ferruginea* are generally richer in readily extractable secondary metabolites such as polyphenols, flavonoids, and tannins, whereas the bark of *P. angolensis* is more lignified and contains less soluble structural components.

Furthermore, both intrinsic and extrinsic factors may contribute to these differences. Intrinsically, genetic characteristics influence the biosynthesis and accumulation of secondary metabolites. Extrinsically, environmental conditions such as soil type, sunlight exposure, rainfall, and seasonal variation significantly affect plant secondary metabolism [11]. Additionally, the developmental stage at harvest is a determining factor, as the concentration of bioactive compounds can vary throughout the phenological cycle. These findings are consistent with previous studies demonstrating that ecological and physiological factors strongly influence both the quality and quantity of secondary metabolites in medicinal plants [12]. Thus, the observed yield differences likely result from a complex interaction between plant material, extraction method, and environmental conditions, conferring *C. ferruginea* a higher extractive and potentially bioactive capacity under the studied conditions.

3.2. Antioxidant Activity of *P. angolensis* and *C. ferruginea* Extracts

The antioxidant activity of aqueous extract of *P. angolensis* (E24) and hydroethanolic extract of *C. ferruginea* (E37) was evaluated using DPPH, ABTS, and FRAP assays, which explore different antioxidant mechanisms.

3.2.1. DPPH Radical Scavenging Assay

The IC_{50} values obtained were 91.37 $\mu\text{g/mL}$ for E24, 97.46 $\mu\text{g/mL}$ for E37, and 6.09 $\mu\text{g/mL}$ for vitamin C. Statistical analysis indicated that E24 exhibited significantly higher radical scavenging activity than E37 ($p < 0.05$), although both extracts were markedly less active than vitamin C ($p < 0.05$).

These results indicate a moderate antioxidant activity for both extracts. Previous studies on *P. angolensis* reported DPPH IC_{50} values ranging from 50 to 150 $\mu\text{g/mL}$ depending on extraction methods, consistent with the present findings [13]. This activity is likely attributed to lignans and phenolic compounds capable of donating hydrogen atoms to neutralize free radicals. In contrast, studies on *C. ferruginea* have reported variable antioxidant activities depending on the solvent used, with hydroethanolic extracts often showing higher activity than aqueous extracts [14]. The slightly lower activity of E37 in this study may therefore be due to differences in phytochemical composition or lower concentrations of strong hydrogen-donating compounds.

3.2.2. ABTS Radical Cation Scavenging Assay

The IC_{50} values were 777.33 $\mu\text{g/mL}$ for E24, 703.33 $\mu\text{g/mL}$ for E37, and 113.33 $\mu\text{g/mL}$ for Trolox. E37 exhibited significantly higher activity than E24 ($p < 0.05$), although both extracts were significantly less active than Trolox ($p < 0.05$).

The relatively high IC_{50} values indicate a weak capacity to neutralize the $\text{ABTS}^{\bullet+}$ radical compared to the standard. However, the superior activity of E37 aligns with reports indicating that hydroethanolic extracts are generally more effective in ABTS assays due to their ability to extract a broader range of phenolic compounds, including both hydrophilic and lipophilic molecules [15]. Moreover, previous studies have demonstrated a positive correlation between total polyphenol content and ABTS activity, particularly in flavonoid-rich species [16], suggesting that the observed activity of E37 may be related to a higher concentration or diversity of such compounds.

3.2.3. Ferric Reducing Antioxidant Power (FRAP)

The concentrations of Fe^{2+} ions generated were 106.08 $\mu\text{g/mL}$ for E24 and 162.22 $\mu\text{g/mL}$ for E37. E37 exhibited a significantly higher reducing power than E24 ($p < 0.05$), indicating a greater ability to reduce Fe^{3+} to Fe^{2+} .

This finding confirms the superiority of E37 in electron transfer mechanisms. Similar observations have been reported in the literature, where polyphenol-rich extracts particularly those containing flavonoids and tannins exhibit strong reducing power [15]. In the case of *C. ferruginea*, several studies have highlighted the presence of phenolic compounds acting as potent reducing agents [14]. Conversely, the lower reducing power of E24 may reflect either a lower concentration of electron-donating compounds or qualitative differences in its phytochemical composition.

3.2.4. Overall Antioxidant Profile

The results reveal a test-dependent antioxidant profile, consistent with the literature emphasizing the complementarity of antioxidant assays [17]. Specifically, *P. angolensis* (E24) showed better performance in the DPPH assay, whereas *C. ferruginea* (E37) exhibited significantly higher activity in ABTS and FRAP assays ($p < 0.05$).

This divergence suggests qualitative differences in phytochemical composition: E24 may be richer in hydrogen atom donors, while E37 likely contains more compounds involved in electron transfer mechanisms. Similar trends have been reported in comparative studies of plant extracts, where antioxidant activity varies according to the dominant reaction mechanism [18].

Overall, although both extracts exhibit noteworthy antioxidant potential, their activities remain lower than those of standard compounds, likely due to the higher purity and concentration of the latter. Nevertheless, these extracts represent promising natural sources of bioactive compounds with potential applications in pharmaceutical and nutraceutical fields.

3.3. Anti-inflammatory Activity of the Extracts

The anti-inflammatory activity of the extracts was evaluated based on their ability to inhibit the denaturation of bovine serum albumin (BSA), a key mechanism involved in inflammatory processes.

The results showed that E24 (*P. angolensis*) exhibited an inhibition percentage of 68.42%, significantly higher than that of E37 (*C. ferruginea*) (47.36%) ($p < 0.05$). Moreover, no statistically significant difference was observed between E24 and diclofenac sodium (73.68%) ($p > 0.05$), indicating comparable efficacy to the standard anti-inflammatory drug.

The high activity of E24 may be attributed to the presence of bioactive compounds capable of inhibiting protein denaturation, a process closely associated with inflammation. These compounds may act through multiple mechanisms, including inhibition of inflammatory mediators such as prostaglandins and cytokines, or stabilization of protein structures [19].

Interestingly, the higher anti-inflammatory activity of E24 despite its comparatively lower antioxidant activity (ABTS and FRAP) suggests that these two properties are not strictly correlated. Some compounds may exert anti-inflammatory effects independently of their antioxidant capacity by specifically targeting biochemical pathways involved in inflammatory responses [20].

4. Conclusion

This study highlights the antioxidant and anti-inflammatory potential of hydroethanolic leaf extracts of *Cnestis ferruginea* and aqueous bark extracts of *Pycnanthus angolensis*, evaluated using DPPH, ABTS, FRAP assays, and protein denaturation inhibition. The extract of *C. ferruginea* exhibited superior antioxidant activity, whereas *P. angolensis* demonstrated a pronounced anti-inflammatory effect, comparable to that of diclofenac sodium.

These findings suggest that antioxidant and anti-inflammatory properties are not necessarily correlated, as certain compounds may specifically target inflammatory pathways independently of their antioxidant capacity. Overall, the results underscore the pharmacological relevance of these plant species as promising sources of bioactive compounds for the management of conditions associated with oxidative stress and inflammation.

However, further investigations, including in vivo studies, toxicity assessments, and clinical trials, are required to confirm their efficacy and safety, and to support their potential therapeutic application.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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