

Research Article

Anti-Inflammatory and Antioxidant Activities of *Pycnanthus angolensis* and *Daniellia oliveri* in Relation to Their Traditional Use in the Treatment of Dysmenorrhea

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Dysmenorrhea is a painful condition that precedes or accompanies menstruation. It is a real problem for women of reproductive age and impacts their quality of life. The present study aims to assess the anti-inflammatory and antioxidant properties of *Pycnanthus angolensis* and *Daniellia oliveri*, two plants traditionally used to treat dysmenorrhea. The anti-inflammatory activity of aqueous extracts of stem barks of *P. angolensis* and root barks of *D. oliveri* was carried out on Wistar rats using the carrageenan-induced paw edema method. The antioxidant activity was assessed in vitro using the 1,1-Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay. The results showed that aqueous extracts of both plants (at doses of 100 and 200 mg/kg) significantly reduced rat paw edema at 1, 2, 3, 4, 5 and 6-h postcarrageenan injection. The maximum edema-inhibiting activity was reached at the second hour with inhibition of 63.18% for the *P. angolensis* extract at 200 mg/kg and 58.82% for the *D. oliveri* extract at 200 mg/kg bw. For the antioxidant activity, the aqueous stem bark extract of *P. angolensis*, with an antiradical power (AP) of 2.15 μmol DPPH scavenged/mg extract, exhibited better anti-free radical activity compared with that of the aqueous root bark extract of *D. oliveri* (AP = 0.16 μmol DPPH scavenged/mg extract). These results show that aqueous extracts of *P. angolensis* and *D. oliveri* have anti-inflammatory and antioxidant properties.

Keywords: anti-inflammatory activity; antioxidant activity; *Daniellia oliveri*; dysmenorrhea; *Pycnanthus angolensis*

1. Introduction

Dysmenorrhea is a condition characterized by abdominal pain that occurs just before and/or during menstruation. It is one of the most common gynecological disorders among women of reproductive age [1], with a prevalence ranging from 16% to 91% in this population [2]. Dysmenorrhea is categorized as either primary or secondary. Primary dysmenorrhea occurs in the absence of any underlying

pathological condition, whereas secondary dysmenorrhea is associated with conditions such as endometriosis, uterine fibroids, or pelvic inflammatory disease [3, 4].

This condition significantly impacts the quality of life for women of reproductive age, leading to restrictions in daily activities [5], decreased academic performance among students [6], and poor sleep quality [7]. Additionally, it is often accompanied by other symptoms, including fatigue, nausea, vomiting, diarrhea, headaches, and lower back pain [8].

The main mechanism of dysmenorrhea, whether primary or secondary, involves hypercontractility of the uterine myometrium and vasoconstriction caused by elevated levels of prostaglandins, particularly Prostaglandin F2 α (PGF2 α), released by degenerating endometrial cells during menstruation [1, 9]. This process leads to the hypersensitization of pain fibers and ultimately results in pelvic pain [10, 11]. Oxidative stress is another potential mechanism of dysmenorrhea, contributing to uterine endothelial dysfunction and dysmenorrhea severity [12]. Myometrial contraction and vasoconstriction caused by prostaglandins also lead to uterine ischemia, resulting in pain and other symptoms such as vomiting or headache [1]. Oxidative stress has been reported to occur during ischemia [13] and may play an important role in the etiopathogenesis of dysmenorrhea [14].

The treatment of dysmenorrhea focuses on alleviating pain and symptoms either by targeting the physiological mechanisms underlying menstrual pain (such as the production of prostaglandins that promote inflammation) or by providing symptom relief. Therapeutic approaches include regular physical exercise and pharmacological treatments, particularly the use of nonsteroidal anti-inflammatory drugs or combined oral estrogen-progestogen hormonal therapy [15]. While effective treatments at low cost are widely available, they often have poor uptake or side effects. The exploration of herbal therapies for managing dysmenorrhea may also be considered, as plants are valuable sources of bioactive compounds used to treat various diseases.

In this context, we are interested in *Pycnanthus angolensis* (Myristicaceae) and *Daniellia oliveri* (Fabaceae), two plants of the Ivorian medicinal flora. *Pycnanthus angolensis* is a medium to large evergreen tree whose bark is used to treat dermatological infections, cough, chest pains, anemia, and malaria [16]. The bark is also used to treat a range of gynecological problems, from sterility to gonorrhea [17]. *Daniellia oliveri* is a deciduous tree that can reach 30 m in height. The bark is used to treat severe enteralgia, colic, intestinal obstruction, anorexia, anemia, bronchitis, pneumonia, hemorrhoids, bleeding, metrorrhagia, kidney problems, dysentery, gonorrhea, and dysmenorrhea. The roots are used for dysmenorrhea, stomach ache, metrorrhagia, syphilis, persistent constipation, dystocia, and helminthiasis [18].

Since inflammation and oxidative stress are involved in the mechanisms and the pathogenesis of dysmenorrhea, this study aims to assess the anti-inflammatory and antioxidant properties of *Pycnanthus angolensis* and *Daniellia oliveri*.

2. Materials and Methods

2.1. Plant Material. The plant material consists of *Pycnanthus angolensis* stem barks and *Daniellia oliveri* root barks. These organs were collected in the regions of N'douci (Southern Côte d'Ivoire) for *P. angolensis* and Bouaké (Central-Northern Côte d'Ivoire) for *Daniellia oliveri*. Samples of each species were sent to the National Floristic Center, Université Félix Houphouët-Boigny of Abidjan, where they were authenticated by comparison with specimens registered under the numbers UCJ012895 for *P. angolensis* and UCJ009287 for *Daniellia oliveri*.

2.2. Animals. Study was carried out using Wistar albino rats aged 12–14 weeks and weighing between 160 and 185 g. These animals were bred at the animal house of the Normal Superior School of Abidjan. They were maintained and fed pellets every other day and had free access to water. Rats were also kept at room temperature with 12 h of light during the day and 12 h of darkness in the night. All experimental procedures have been examined and approved by the Ethical Committee of Health Sciences, Université Félix Houphouët-Boigny of Abidjan.

2.3. Chemicals. Ascorbic acid, 1,1-Diphenyl-2-picrylhydrazyl (DPPH), and carrageenan were purchased from Sigma Chemical Co. (St. Louis, USA). Folin-Ciocalteu reagent, sodium carbonate (Na₂CO₃), and gallic acid were supplied by Merck Co (Germany). Diclofenac sodium tablets (25 mg, AdvaCare Pharma, USA) was used as a reference anti-inflammatory. All the other chemicals used in this study including solvents were of analytical grade.

2.4. Extract Preparation. Previously harvested *Pycnanthus angolensis* stem barks and *Daniellia oliveri* root barks were dried at room temperature for 3 weeks and later grounded to a powder using a grinder. One hundred (100) g of each plant powder was dissolved separately in 1 L of distilled water. The mixture was then homogenized for 5 min using an electronic mixer, and the resulting homogenate was successively filtered twice on cotton and once on Whatman filter paper (3 mm) [19]. After several homogenization and filtration cycles, the filtrates obtained were concentrated to dryness under reduced pressure at 30°C using a rotary evaporator (Büchi Rotavapor R-100, Schweiz). The resultant extracts constitute the aqueous extracts of *P. angolensis* stem barks and *D. oliveri* root barks.

2.5. Acute Toxicity. The acute toxicity of aqueous extracts of *P. angolensis* stem barks and *D. oliveri* root barks was performed according to Organization for Economic Cooperation and Development (OECD) guideline no 423 [20]. One group of three rats, fasted overnight, received a single oral dose of 2000 mg/kg bw of the aqueous extract of *P. angolensis*. Another group of three rats was treated under the same conditions with the aqueous extract of *D. oliveri*, while a final group of three rats received distilled water. After the treatment, food was withheld for further 3–4 h. Animals were observed individually at least once during the first 30 min after treatment, regularly during the first 24 h and daily for 14 days.

2.6. Anti-Inflammatory Activity. The anti-inflammatory activity of aqueous extracts of *P. angolensis* stem barks and *D. oliveri* root barks was carried out on Wistar rats using the carrageenan-induced paw edema method [21]. Thirty (30) rats were divided into six groups of five each and fasted for 18 h prior to experimentation. The thickness of each animal's right hind paw was first measured before injecting

the carrageenan using a digital caliper. Then, the rats were treated as follows:

- Group 1 (carrageenan control) received distilled water orally.
- Group 2 (reference) received diclofenac sodium at 10 mg/kg bw orally.
- Groups 3 and 4 were orally administered with the aqueous extract of *P. angolensis* at doses of 100 and 200 mg/kg bw, respectively.
- Groups 5 and 6 were orally treated with the aqueous extract of *D. oliveri* at doses of 100 and 200 mg/kg bw, respectively.

One hour after the different treatments, 1% carrageenan (0.1 mL) was administered subcutaneously to the plantar fascia of the right hind paw of each rat. The thickness of the paw that received carrageenan was measured after 1, 2, 3, 4, 5, and 6 h using a digital caliper. Increase in thickness of the right hind paw was taken as an indicator of paw edema. Percentage increase in edema (% IE) was determined as the difference between thickness of the right hind paw at time zero (T_0) and thickness of this paw at time t (T_t):

$$\% \text{ IE} = \frac{T_t - T_0}{T_0} \times 100. \quad (1)$$

The anti-inflammatory activity was expressed as percentage inhibition of edema in the animals treated with extracts or sodium diclofenac in comparison to the carrageenan control group. The percentage inhibition of edema was calculated using the following formula:

$$\% \text{ Inhibition} = \frac{\% \text{ IE}_c - \% \text{ IE}_t}{\% \text{ IE}_c} \times 100, \quad (2)$$

where IE_c and IE_t represent the mean percentage increase in paw thickness in carrageenan control and treated groups, respectively.

2.7. Determination of Total Phenol Content. The total phenol content of aqueous extracts of *P. angolensis* stem barks and *D. oliveri* root barks was determined using Folin–Ciocalteu reagent [22]. To this end, 5 mL of Folin–Ciocalteu reagent (1:10 with distilled water) and 4 mL of sodium carbonate (1 M) were added to 0.5 mL of each plant extract (0.1 g/mL). After shaking, the reaction mixture was incubated at room temperature for 15 min, and absorbance was measured with a spectrophotometer at 765 nm. Gallic acid prepared in a methanol/water solvent mixture (50:50, v/v) was used as a standard at concentrations ranging from 0 to 250 mg/L. The total phenol content was expressed as mg of gallic acid equivalents (GAE) per gram of extract, using a standard curve of gallic acid. All measurements were carried out in three replicates.

2.8. Determination of Antioxidant Activity. The antioxidant activity of aqueous extracts of *P. angolensis* stem barks and *D. oliveri* root barks was determined using the DPPH radical

scavenging assay [23]. This assay is based on measuring the absorbance at 517 nm of a violet coloration characteristic of DPPH radicals, which are reduced to the yellow or purple hydrazine form in the presence of free radical scavengers or antioxidants. Thus, ethanol solution of DPPH (100 μM) was added to an equal volume of different concentrations of each extract (10–100 $\mu\text{g/mL}$ for *P. angolensis* and 100–800 $\mu\text{g/mL}$ for *D. oliveri*). The reaction mixture was shaken and incubated at room temperature in the dark for 15 min. The absorbance was then measured with a spectrophotometer at 517 nm. The experiment was repeated for three times. Ascorbic acid (3.125–50 $\mu\text{g/mL}$) was used as standard control. The percentage inhibition of DPPH radicals was calculated by the following equation:

$$\text{DPPH inhibition (\%)} = \frac{(A_0 - A_1)}{A_0} \times 100, \quad (3)$$

where A_0 is the absorbance of DPPH radicals without the sample extract and A_1 is the absorbance of DPPH radical with the sample extract.

The concentration of the extract required to scavenge 50% of DPPH radicals (IC_{50}) (μg extract/mL of reaction solution) was determined. The value obtained was used to calculate the efficient concentration 50 (EC_{50}) (mg extract/ μmol of DPPH) using the following formula:

$$\text{EC}_{50} = \frac{\text{IC}_{50}}{\text{concentration of DPPH solution}}. \quad (4)$$

The inverse of this last parameter corresponds to the antiradical power (AP) = $1/\text{EC}_{50}$ expressed as μmol DPPH scavenged/mg extract.

2.9. Statistical Analysis. Statistical analysis of the data was performed using Graph Pad Prism 9.0 (Microsoft, USA). Results were expressed as mean with standard error (mean $\pm$ SE). The independent t -test was used to compare the mean of the total phenol content of the two plant extracts. The differences between the means of antifree radical parameters and between the means of percentage inhibition of inflammation were determined using one-way analysis of variance followed by Turkey's multiple comparison test. The number of groups used in these last two analyses was at least three. The difference was significant for a p value of less than 5% ($p < 0.05$).

3. Results

3.1. Acute Toxicity. Administration of aqueous extracts of *P. angolensis* stem barks and *D. oliveri* root barks at a single dose of 2000 mg/kg bw did not result in any change in rat behavior during the observation period. No signs of agitation, convulsion, torsion, or diarrhea were observed. Similarly, no mortality was recorded in animals after 24 h and then after 2 weeks of observation. These results indicate that the lethal dose 50 (LD_{50}) of the two plant extracts is greater than 2000 mg/kg bw.

3.2. Effect of *Picnanthus angolensis* and *Daniellia oliveri* Extracts on Carrageenan-Induced Edema. The results show that aqueous extracts of *D. oliveri* at 100 mg/kg bw and *P. angolensis* at 100 and 200 mg/kg bw significantly ($p < 0.05$) inhibited carrageenan-induced rat paw edema at the first hour compared with diclofenac sodium (10 mg/kg bw) (Figure 1). The percentages of inhibition recorded were 46.03%, 49.66%, 52.40%, and 12.83% in the groups of animals pretreated, respectively, with the aqueous extract of *D. oliveri* at 100 mg/kg bw, the aqueous extract of *P. angolensis* at 100 and 200 mg/kg bw, and diclofenac sodium (10 mg/kg bw). At the second hour, edema inhibition was statistically identical and greatest in all groups of pretreated rats, but at the third hour, a decrease in inhibition was recorded in these groups. From the fourth hour, edema inhibition percentages increased again in rats pretreated with *D. oliveri* and *P. angolensis* extracts, with the exception of those given diclofenac sodium. This increase continued until the sixth hour, where these plant extracts significantly inhibited edema compared with diclofenac sodium. The aqueous extract of *P. angolensis* at 200 mg/kg, with an inhibition of 65.66%, exhibited a better anti-inflammatory effect than the 100 mg/kg dose (56.42%) and the aqueous extract of *D. oliveri* (44.09% and 44.18%, respectively, for the 100 and 200 mg/kg bw doses).

3.3. Total Phenol Content. The total phenol content of aqueous extracts of *Picnanthus angolensis* stem barks and *Daniellia oliveri* root barks is shown in Table 1. The values, expressed as mg gallic acid equivalent per g of extract (mg GAE/g extract), were determined from the standard curve for gallic acid, whose equation is $y = 0.005903x$ and $R^2 = 0.9780$. The results show that the aqueous stem bark extract of *P. angolensis* (18.42 ± 0.36 mg GAE/g extract) has a significantly higher total phenol content ($p < 0.05$) than the aqueous root bark extract of *D. oliveri* (2.42 ± 0.06 mg GAE/g extract).

3.4. DPPH Radicals Scavenging Activity. The inhibition of DPPH radicals by *Picnanthus angolensis* and *Daniellia oliveri* extracts and ascorbic acid is shown in Figure 2. Scavenging DPPH radicals increased with increasing concentration of extracts. IC_{50} , EC_{50} , and antiradical power (AP) values for plant extracts and ascorbic acid are shown in Table 2. Lower IC_{50} values and higher AP values correspond to higher scavenging activity. The results show that the aqueous stem bark extract of *P. angolensis*, with an AP of $2.15 \mu\text{mol}$ DPPH scavenged/mg extract, exhibited better anti-free radical activity compared with the aqueous root bark extract of *D. oliveri* (AP = $0.16 \mu\text{mol}$ DPPH scavenged/mg extract). However, the antifree radical activity of ascorbic acid (AP = $20.2 \mu\text{mol}$ DPPH scavenged/mg extract) is far superior to that of the two plant extracts.

4. Discussion

Dysmenorrhea is a painful condition caused by the release of high levels of prostaglandins in the menstrual fluid, resulting

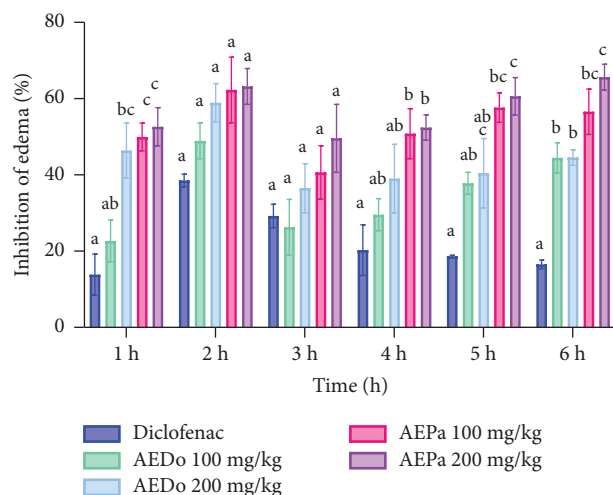


FIGURE 1: Time course inhibition of paw edema in rats treated with diclofenac and plant extracts. AEPa: the aqueous extract of *P. angolensis*; AEGs: the aqueous extract of *D. oliveri*. Values are expressed as mean $\pm$ standard error (SE) ($n = 5$). The letters a, b, and c denote statistical significance. Means with different letters are significantly different ($p < 0.05$).

TABLE 1: Total phenol content of aqueous extracts of *Picnanthus angolensis* and *Daniellia oliveri*.

Extracts	Total phenol content (mg GAE/g extract)
Aqueous extract of <i>Picnanthus angolensis</i>	18.42 ± 0.36^a
Aqueous extract of <i>Daniellia oliveri</i>	2.42 ± 0.06^b

Note: Values are expressed as mean $\pm$ standard error (SE) ($n = 3$). The letters a and b denote statistical significance. Means with different letters are significantly different ($p < 0.05$).

in uterine contraction and vasoconstriction [1, 9]. Myometrial contraction and vasoconstriction caused by prostaglandins lead to uterine ischemia, causing oxidative stress [13], which may play an important role in the etiopathogenesis of dysmenorrhea [14]. This study was carried out to investigate the anti-inflammatory and antioxidant properties of *Picnanthus angolensis* and *Daniellia oliveri* in relation to their traditional use in the treatment of dysmenorrhea. The acute oral toxicity study of aqueous extracts of *P. angolensis* stem barks and *D. oliveri* root barks showed that the lethal dose 50 (LD_{50}) of these extracts is greater than 2000 mg/kg b.w. Thus, both plant extracts are classified in category five of the globally harmonized system of classification of chemicals, which characterizes substances of low toxicity [20]. The anti-inflammatory activity of these extracts was determined using the carrageenan-induced paw edema model in rats. The inflammatory process is complex, involving several cellular signaling pathways in addition to the production of free radicals responsible for tissue degeneration. Carrageenan-induced inflammation involves many mediators which induce the inflammatory reaction in two different phases [24]. The first phase occurs within an hour of administration, resulting from the release of histamine, serotonin, and bradykinin from mast cells. These

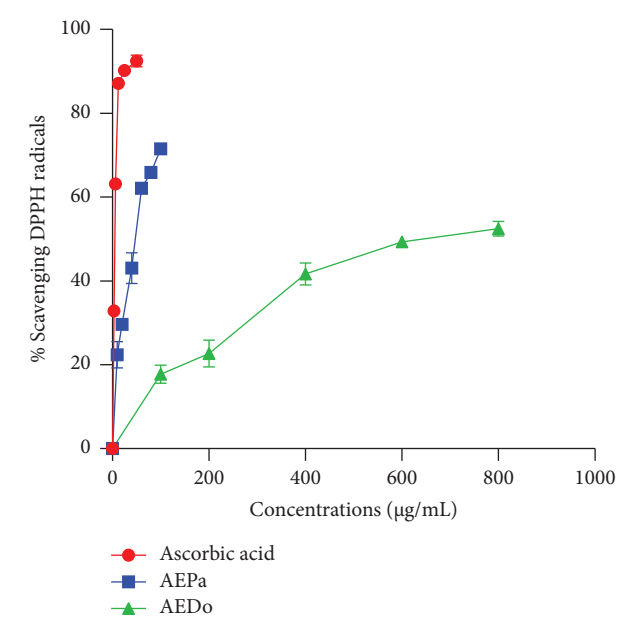


FIGURE 2: DPPH radicals scavenging curve by aqueous extracts of *Pycnanthus angolensis* and *Daniellia oliveri* and ascorbic acid. AEPa: the aqueous extract of *P. angolensis*; AEDo: the aqueous extract of *D. oliveri*. Values are expressed as mean of percentage scavenging of DPPH radicals $\pm$ standard error (SE) ($n = 3$).

TABLE 2: DPPH scavenging activity of aqueous extracts of *Pycnanthus angolensis* and *Daniellia oliveri*.

Extracts	IC ₅₀ (µg/mL)	EC ₅₀ (mg/µmol)	AP (µmol/mg)
AEPa	46.5 $\pm$ 0.75 ^a	0.465 $\pm$ 0.02 ^a	2.15 $\pm$ 0.05 ^a
AEDo	630.48 $\pm$ 2.26 ^b	6.31 $\pm$ 0.45 ^b	0.16 $\pm$ 0.07 ^b
Ascorbic acid	4.95 $\pm$ 0.08 ^c	0.05 $\pm$ 0.01 ^c	20.2 $\pm$ 0.24 ^c

Note: Values are expressed as mean $\pm$ standard error (SE) ($n = 3$). The letters a, b, and c denote statistical significance. Means in the same column with different letters are significantly different ($p < 0.05$). Abbreviations: AEDo, aqueous extract of *Daniellia oliveri*; AEPa, aqueous extract of *Pycnanthus angolensis*; AP, antiradical power; EC₅₀, efficient concentration 50; IC₅₀, inhibitor concentration 50.

factors trigger vascular changes leading to plasma exudation [25]. The second phase is characterized by swelling caused by the increased release of arachidonic acid metabolites such as prostaglandins and leukotrienes in the inflammatory zone [26]. Carrageenan-induced edema peaks at 3 h postinjection. The increase in paw thickness in edematous control rats from the first to the sixth hour indicates that any reduction in edema during this period could be due to the effects of the administered products.

The results of the present study show that aqueous extracts of *D. oliveri* root barks and *P. angolensis* stem barks inhibited edema during the experimental period. The edema-inhibiting effect was already observed 1 h after administration of carrageenan. As with diclofenac sodium, the reference drug, the maximum edema-inhibiting effect of plant extracts was reached at the second hour. Moreover, the maximum effect obtained with this drug was statistically identical to that of plant extracts. These observations indicate that *D. oliveri* and *P. angolensis* extracts possess anti-

inflammatory activity and are capable of combating the early stages of inflammation. However, the anti-inflammatory effect of the aqueous extract of *P. angolensis* is better than that of the aqueous extract of *D. oliveri*. The rapid anti-inflammatory effect of these extracts may be explained by their good bioavailability like diclofenac sodium, whose pharmacokinetic properties are represented by a mean time to maximum plasma concentration of 1 h and a bio-availability of 65% when administered orally [27]. This rapid effect could also be explained by the inhibition of mediators such as histamine, serotonin, and bradykinin involved in the initial phase of inflammation which occurs between 0 and 2.5 h after injection of the phlogogenic agent [28]. The decrease in edema inhibition at the third hour was followed by an increase from the fourth to the sixth hour, and the effect of extracts was significantly greater than that of diclofenac sodium. This shows that extracts are capable of inhibiting the mediators involved in the late phase of inflammation. This late phase is the result of cyclo-oxygenase-mediated overproduction of prostaglandins in the tissues [29] and may continue beyond 5 h after carrageenan injection [30].

The DPPH radical scavenging test is a simple, rapid, and widely used method for assessing the ability of substances to scavenge free radicals. DPPH is a naturally stable free radical whose color changes from purple to yellow or mauve when reduced by hydrogen or electron donor compounds. Substances which are able to perform this reaction are considered as radical scavengers and therefore antioxidants [31]. The results of this study indicate that the aqueous extracts of *P. angolensis* and *D. oliveri* are capable of scavenging DPPH radicals, with an antiradical power of the *P. angolensis* extract (2.15 µmol DPPH scavenged/mg extract) better than that of *D. oliveri* (0.16 µmol DPPH scavenged/mg extract). The results also show that the aqueous extract of *P. angolensis* (18.42 $\pm$ 0.36 mg GAE/g extract) has a significantly higher total phenol content ($p < 0.05$) than the aqueous extract of *Daniellia olivera* (2.42 $\pm$ 0.06 mg GAE/g extract). The antiradical activity of these plant extracts is probably linked to their total phenol contents. In general, higher total phenol and flavonoid contents result in better DPPH scavenging activity [32, 33]. Previous studies showed that phenolic compounds, notably flavonoids and tannins, are powerful antioxidants capable of inhibiting the inflammatory reaction [34, 35]. The anti-inflammatory activity of aqueous extracts of *P. angolensis* stem barks and *D. oliveri* root barks could therefore be due to the phenolic compounds they contain. These pharmacological properties are thought to make these two plants effective remedies for dysmenorrhea.

5. Conclusion

The present study showed that the aqueous extracts of stem barks of *Pycnanthus angolensis* and root barks of *Daniellia oliveri* significantly reduce rat paw edema, with a peak inhibition of over 50% at 2 hours postcarrageenan injection. Both *Pycnanthus angolensis* and *Daniellia oliveri* extracts exhibit antioxidant activity by scavenging DPPH free

radicals, with *Pycnanthus angolensis* showing superior activity. These findings support their potential use in traditional medicine for treating dysmenorrhea, a condition associated with inflammation and oxidative stress.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics Statement

The authors declare that the use of animals in the present study was approved by the Ethical Committee of Health Sciences, Université Félix Houphouët-Boigny of Abidjan.

Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

K.K.A.M. conceived the study, participated in its execution, and drafted the manuscript. K.S.P. performed the statistical analysis and helped to draft the manuscript. A.A.T.I. carried out the experiment. T.J.N.D. coordinated the study. N.J.D. participated in the design of the study. All authors read and approved the final manuscript.

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