Anti-Inflammatory and Antioxidant Properties of the Aqueous Extracts of the Leaves of *Opilia amentacea* (Opiliaceae)

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Abstract

Opilia amentacea (Opiliaceae) is a woody plant with multiple medicinal claimed effects. The present study aimed to assess the anti-inflammatory and antioxidant activities of the decoction and macerate extracts from the leaves of *Opilia amentacea*. Moreover, acute toxicity and phytochemical analysis were performed. The acute toxicity was evaluated on NMRI mice at 2000 mg/kg bw. The anti-inflammatory activity was studied using the carrageenan-induced mouse paw edema and the lipoxygenase inhibition assay. The radical scavenging (DPPH and ABTS), ferric-reducing antioxidant power (FRAP), and lipid peroxidation (LPO) assays were used to measure the antioxidant capacity of the extracts. Qualitative and quantitative methods served for identifying and quantifying the extract's phytoconstituents. The decoction demonstrated low acute toxicity; the lethal dose was therefore estimated to be superior to 2000 mg/kg bw. The extracts significantly reduced the mouse paw's thickness at 600 mg/kg bw. The extracts developed weak radical scavenging and lipid peroxidation inhibitory effects. However, the macerate showed a high ability (664.90 ± 0.71 mol Ascorbic Acid Equivalent/g dry extract) to reduce the ferric ions. Saponins, sterols, triterpenes, and flavonoids were qualitatively detected in the two extracts. Total phenolics (TP) and total flavonoids (TF) were

found abundant in the extracts, especially the decoction (TP content (TPC) = 94.03 ± 2.66 mg GAE/g; TF content (TFC) = 35.05 ± 0.32 mg QE/g). Strong positive correlations existed between ferric-reducing capacity and TPC (r = 0.959) for the macerate, while TFC was mainly involved in the DPPH radical scavenging of the two extracts. Instead, most correlations were negative between the polyphenol compounds and the anti-inflammatory assays. The results indicate potent *in vivo* anti-inflammatory and *in vitro* antioxidant effects of the aqueous extracts from the leaves of *Opilia amentacea*. Further studies are needed to find the anti-inflammatory and antioxidant effects mechanism.

Keywords

Opilia amentacea, Aqueous Extracts, Acute Toxicity, Anti-Inflammatory, Antioxidant

1. Introduction

Traditional herbal medicine remains an essential source of primary health care for many African people who use it to treat or prevent cardiovascular, gastrointestinal, or inflammation-related diseases [1] [2]. Some of the features of traditional herbal medicine, for instance, in Africa, involve: 1) a holistic approach in which healing requires an equilibrium between body, mind, and soul with the external environment and 2) an oral transmission of medical knowledge from generation to generation [3]. The wide use of herbal medicine is based on various advantages, including easy accessibility, affordability, safety, effectiveness, and environmentally benign [1] [4]. Although "natural" does not necessarily mean "safe", it is well-reported that natural products or herbal medicinal products have fewer adverse effects compared to synthetic products [5] [6].

Africa possesses a rich diversity of plants with poorly reported properties. Indeed, there is little or no data regarding the quality, efficacy, and safety of various medicinal plants [4]. As a result, the World Health Organization (WHO) Regional Committee for Africa has prompted the need for African countries to prioritize and develop research on traditional medicine [2].

Opilia amentacea Roxb. (Syn. *Opilia celtidifolia* Endl. Ex Walp.) is a woody climber from the Opiliaceae family. The plant is widespread in tropical Africa, from Senegal to Soudan, and grows mainly in fringing forests and savannahs [7]. *Opilia amentacea* (*O. amentacea*) has been reported to elicit various medicinal effects, from dermatological to gastrointestinal disorders and wound care treatment [8]. The traditional healers used several parts for treatment; leaves were the most frequent plant part, followed by roots [9]. The decoction was the most preferred preparation [9]. Among the demonstrated properties of its extracts, there were: 1) antimicrobial, 2) hepatoprotective, 3) antioxidant, 4) antidiabetic, and 5) antiulcer [10]-[15]. Phytochemicals, including sterols, triterpenes, flavonoids,

and saponins, have been identified and isolated from *O. amentacea* [16] [17] [18] [19]. No report on the traditional use of *Opilia amentacea* as a toxic plant has been reported [16]. Recently, Youl and collaborators (2023) published a preprint on the phytochemical and biological properties using the ethanolic extract of the leaves of *Opilia amentacea* [20].

Based on the wide use of aqueous extracts in traditional medicine, the current research aimed to investigate the anti-inflammatory and antioxidant effects of the aqueous decoction and macerate extracts from the leaves of *Opilia amentacea*. Meanwhile, the acute toxicity of the decoction, the most frequent preparation among traditional healers, and the phytochemical composition were assessed.

2. Materials and Methods

2.1. Chemicals, Reagents, and Solvents

Sigma Aldrich (St Louis, U.S.A.) supplied the following chemicals and reagents: 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)-(ABTS) diammonium salt, Folin Ciocalteu reagent (FCR), hydrochloric acid, zileuton, tween-20, linoleic acid, soybean lipoxygenase (type I-B) enzyme, carrageenan, acetylsalicylic acid, dimethyl sulfoxide (DMSO), ascorbic acid, sodium carbonate, gallic acid, quercetin, and aluminum chloride.

High-performance thin-layer chromatography (HPTLC) Silica gel F_{254} plates (20 cm × 10 cm) were from Merck (Darmstadt, Germany), and 96 well microplates were purchased from Greiner Bio-one International GmbH (Germany). N-hexane, ethyl acetate, methanol, and ethanol 96° were provided by Carlo Erba (France). Trolox was obtained from Fluke (France), and aqueous water was distilled in our laboratory. All other chemicals were of analytical grade.

2.2. Plant Material

The leaves of *Opilia amentacea* were collected around Doulougou (1322459 N, 066309 W), located in the South-Centre Region of Burkina Faso. A botanist authenticated the sample by comparing it with the existing voucher specimen recorded under reference number 8730 at the Herbarium of the "*Centre National de la Recherche Scientifique et Technologique* (*CNRST*)." The leaves were left to dry in the shade at room temperature before being reduced to powder with a mechanical grinder.

2.3. Preparation of Extracts

A decoction was prepared by boiling 250 g of the powdered leaves in 2.5 L of distilled water for 30 minutes. The extract was allowed to cool at room temperature before filtering through a fine loin cloth. The filtrate was centrifuged (ROTINA 380 R, Germany) at 2000 rpm for 10 minutes. The supernatant was collected and freeze-dried.

To prepare the aqueous macerate, 250 g of the powdered leaves were mace-

rated in 2.5 L of distilled water for 24 H. Then, the extract was filtered using a fine loin cloth before being centrifuged at 2000 rpm for 10 minutes. The process was repeated three times. The different supernatants were mixed and freeze-dried.

2.4. Determination of Extraction Yield and Residual Moisture Content

The extraction yield was calculated using the following formula:

$$\text{Extraction yield}\left(\%\right) = \frac{M_1}{M_0} \times 100$$

where M_1 is the weight of the extract (decoction or macerate) after freeze-drying, and M_0 is the weight of the plant powder

The moisture content of the powdered leaves and the freeze-dried extracts was determined using the thermogravimetric method [21] with slight modifications. Samples were weighed to determine their initial weight before placing them in empty dishes (three for each sample) previously dried and tared. The dishes are placed in a Memmert U15 (Germany) oven and heated for 3 hours at 105°C. At the end of the process, the containers were placed in a desiccator until their temperatures were cooled. Then, the dishes were reweighted until three consecutive constant weights were obtained. The residual moisture content (%) was obtained from the following equation:

$$\text{Residual Moisture content}\left(\%\right) = \frac{M_i - M_f}{M_0} \times 100$$

M_i is the sample's initial weight (powder and freeze-dried extracts), and M_f is its final weight at the process's end.

2.5. Qualitative High-Performance Thin-Layer Chromatography (HPTLC) Analysis

The phytochemical analysis was performed on HPTLC silica gel 60 F_{254} plates to detect various phytoconstituents, including steroids, triterpenoids, alkaloids, saponins, flavonoids, and tannins, using a previous method [22]. Following the development in an adequate mobile phase, the plates were dried using a hairdryer, and specific reagents were used to reveal the phytochemicals.

2.6. Determination of Total Phenolic Content (TPC)

The total phenolic content was quantified spectrophotometrically using the Folin-Ciocalteu (FCR) reagent [23] with slight modifications. The reaction mixture was prepared with 1 mL of plant extract solution (prepared from a stock solution of 10 mg/mL) and 1 mL of FCR (0.2 M). After 10 min at room temperature, 2 mL of 7.5% (w/v) sodium carbonate was added. The mixture was allowed to incubate for 40 min at room temperature before recording the absorbance at 760 nm in a spectrophotometer (Shimadzu UV-Vis, Japan) against a blank sample (the extract was replaced by distilled water). The TPC expressed as mg gallic acid

equivalent per g dry extract weight (mg GAE/g) was calculated from the gallic acid calibration curve: $y = 18.608x + 0.0311$, $R^2 = 0.9991$). The measurements were conducted in triplicate.

2.7. Estimation of Total Flavonoid Content (TFC)

The total flavonoid content (TFC) was determined using the aluminum chloride (AlCl$_3$) colorimetric assay [24] with some modifications. Briefly, 2 mL of extract (1 mg/mL in methanol) was mixed with 2 mL of a 2% AlCl$_3$ methanolic solution. The reaction mixture was left to incubate at room temperature for 40 min, after which the absorbance was read at 415 nm. The blank sample was carried out with 2 mL of methanol and 2 mL of aluminum chloride. TFC was estimated from a calibration curve ($y = 20.025x + 0.0087$, $R^2 = 0.9992$), drawn with quercetin used as standard. The result was expressed as mg quercetin equivalent (mg QE) per g dry extract weight. The measurements were carried out in triplicate.

2.8. Animals and Experimental Conditions

NMRI strains mice weighing 20 - 40 g were procured from our institute's animal house. The animals were raised under standard lab conditions of temperature (22˚C ± 3˚C), humidity (50% - 70%), and a 12 h light-dark cycle. The animals have unlimited access to food and water. However, before experiments, animals were fasted with free access to water. The experimental protocols followed the principles and regulations about laboratory animal care and ethical use set in the eighth edition of the Guide for the Care and Use of Laboratory Animals (Guidelines set by the European Union on animal protection) (CEC Council 86/609). The pharmacological protocols were approved by the Ethical Committee on the use of animals for research at the University Joseph Ki-Zerbo, Ouagadougou, Burkina Faso, with agreement code NO CE-UJKZ/2022-14.

2.9. Acute Toxicity of the Aqueous Decoction

The acute toxicity was determined in mice using a single dose of 2000 mg/kg of body weight (bw) following the OECD 423 guidelines [25]. Briefly, the mice were divided into two groups of three each and fasted for 4 hours before the experiment. The control group received distilled water (10 mL/kg·bw) orally, and the treated group received the plant extract at 2000 mg/kg·bw. Animals were weighed four weekly, and at the end of the observation period (fourteen days), animals were killed and subjected to gross necroscopy.

2.10. Anti-Inflammatory Activities

Carrageenan-induced paw edema in mice

The carrageenan-induced paw edema model assessed the aqueous extracts' *in vivo* anti-inflammatory effect [26]. Mice were divided into five (5) groups of six (6) mice each. Before the test, they were deprived of food for 16 hours. The con-

trol group receives by gavage vehicle (10 mL/kg bw). Group II receives 100 mg/kg bw of acetylsalicylic acid, a nonsteroidal anti-inflammatory drug (NAIDS). Groups III-V were given, per os, the extracts (decoction and macerate) at 200, 400, and 600 mg/kg bw. One hour after, inflammation was induced in the right hind paws of mice by a subplantar injection of 0.05 mL of 1% carrageenan (freshly prepared in normal saline). The paw thickness or volume was measured using a Pletysmometer (Ugo Basile, Italy) just before carrageenan injection and at 1, 3, and 5 h after carrageenan injection. The average volume (AV) was from six (6) reads and calculated as follows:

$$AV(mL) = A - B,$$

where A and B were the volumes of the treated paw after and before the carrageenan injection, respectively.

The percentage of inhibition of inflammation (I %) was obtained using the following equation:

$$I(\%) = \frac{D_c - D_t}{D_c} \times 100$$

where D_c is the difference in paw volume of the control group, and D_t is the difference in paw volume in the treated group (acetylsalicylic acid, extracts).

Lipoxygenase (LOX) inhibition assay

The lipoxygenase inhibition test was performed with a 15-lipoxygenase enzyme on a 96-well microplate using the methodology of Malterud and Rydland (2000) [27], slightly modified by Belem-Kabré *et al.* (2021) [28]. Inhibitory activity was measured in triplicate using a microplate spectrophotometer (Tecan Spark, Switzerland) at 234 nm.

2.11. Antioxidant Activity

2,2-DPPH radical scavenging activity

The 2,2-DPPH radical scavenging activity was assessed according to the method of Kim *et al.* (2007) using a Bio-Rad microplate reader [29]. Trolox and plant extracts were dissolved in methanol to yield 1 mg/mL concentration, from which diluted solutions were prepared. 20 µL of sample or reference compound methanolic solution was mixed with 200 µL of 0.004% methanolic DPPH work solution in a 96-well microplate. The mixture was incubated in the dark at room temperature for 30 minutes. For the blank sample, extract/Trolox was replaced with 20 µL of methanol. Absorbances were recorded at 490 nm, and measurements were conducted in triplicate. The DPPH radical scavenging activity (%) was calculated as follows:

$$\text{DPPH scavenging activity}(\%) = \left[(A_B - A_S)/A_B \right] \times 100$$

where A_B and A_S represent, respectively, the absorbances of the blank and sample (extract/Trolox).

The IC$_{50}$ (mg/mL), the concentration of sample or standard compound that produces half-maximal inhibition, and anti-radical power (ARP) defined as

$1/IC_{50}$ were determined.

2,2-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging assay

The 2,2-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging test was adopted for a 96-well microplate from the method of Re *et al.* (1999) [30]. The ABTS$^{•+}$ was generated by reacting the ABTS (7 mM) salt with potassium persulfate (2.45 mM). At room temperature, the mixture was incubated in the dark for 12 - 16 hours. Diluted plant extracts and Trolox solutions were prepared from a 1 mg/mL stock solution. After that, 20 μL of the different solutions were mixed with 200 μL of the diluted ABTS$^{•+}$ solution in a 96-well microplate. The absorbances were read spectrophotometrically at 415 nm after 30 minutes of incubation in the dark.

$$\text{ABTS scavenging activity}\left(\%\right)=\left[\left(A_C - A_S\right)/A_C\right]\times 100$$

where A_C and A_S are, respectively, the absorbances of the control (ABTS radical solution without extract or Trolox) and sample/Trolox (ABTS radical + extract/Trolox).

As in the DPPH assay, IC_{50} (mg/mL) values and the ARP estimated the ABTS radical scavenging effect.

Ferric-reducing antioxidant power assay (FRAP)

The ferric-reducing antioxidant power test measures the reduction of ferric ion-ligand (Fe^{3+}-ligand) to an intense blue-colored ferrous (Fe^{2+}) complex by antioxidants present in the plant extracts [31]. The method was adopted in a 96-well microplate from Hinneburg *et al.* (2006) [32], and potassium ferricyanide ($K_3[Fe(CN)_6]$ was used to produce Prussian blue upon reaction with ferrous ions. A 0.5 mL of 1 mg/mL extract was mixed with 1.25 mL of phosphate buffer (0.2 M, pH 6.6) and 1.25 mL of a 1% aqueous potassium ferricyanide. The mixture was left to stand for 30 min at 50˚C, after which 1.25 mL of 10% trichloroacetic acid was added. The tubes were centrifuged for 10 min at 3000 rpm, and the supernatant was collected. A 0.625 mL of the supernatant was mixed with 0.625 mL of water and 0.125 mL of 0.1% aqueous $FeCl_3$. The blank samples were prepared in the same conditions by replacing the extract. Then, the absorbance was recorded in the spectrophotometer (Agilent 8453 UV-Visible, USA) at 700 nm. The ferric-reducing power was determined as molar ascorbic acid equivalent per g of dry sample (mol AAE/g). The tests were performed in triplicate.

Lipid peroxidation inhibition (LPO) assay

The lipid peroxidation inhibition was estimated in liver homogenate obtained from a rat using a previous protocol [33]. The methodology of LPO was based on the use of thiobarbituric acid. In brief, 0.2 mL of 1.5 mg/mL sample or Trolox was mixed with 1 mL of rat liver homogenate prepared in 10% phosphate-buffered saline (pH 7.4) before adding 50 μL of $FeCl_2$ (0.5 mM) and 50 μL of H_2O_2 (0.5 mM). 1 mL of 15% trichloroacetic acid and 1 mL of 0.67% thiobarbituric acid were added to the mixture after 1 hour of incubation at 37˚C. The mixture was heated for 15 min, and after cooling, it was centrifuged at 2000 rpm for 10

minutes. The absorbances of the supernatants were recorded at 532 nm. The measurements were performed in triplicate, and the inhibition percentage (I %) was obtained from the following equation:

$$I\left(\%\right)=\left[1-\left(\frac{A_1-A_2}{A_C}\right)\right]\times100$$

where A_C is the absorbance of the control (liver homogenate and reagents without extract or Trolox), A_1 represents the absorbance of the extract/Trolox, and A_2 is the absorbance without the liver homogenate.

2.12. Data and Statistical Analyses

The data were expressed as means ± standard deviation (SD) of three replicate determinations. GraphPad Prism (version 8.0) was used to determine the correlation of the biological activities with polyphenol compounds by Pearson's correlation coefficient [34]. A two-way analysis of variance (ANOVA) followed by *Sidak's multiple comparison tests* was performed using the GraphPad Prism (version 8.0) to compare differences between extracts. At the same time, a two-way analysis of variance (ANOVA) followed by Tukey's and Dunnett's multiple comparison tests was used to compare extracts to the control. The difference was considered statistically significant for a p-value < 0.05.

3. Results

3.1. Phytochemical Study

The residual moisture content, extraction yield, polyphenol compounds content, and qualitative high-performance thin-layer chromatography (HPTLC) screening are presented in Table 1.

The analysis of the results indicates that the macerate and decoction have similar phytochemical compounds. Sterols, triterpenes, flavonoids, and saponins were detected in the two extracts. However, alkaloids, coumarins, and tannins were absent in the two extracts. The extraction yield was higher by the decoction method than by the maceration method. The order of residual moisture content with respect to the sample used was grounded leaves > decoction > macerate. No significant statistical difference (p > 0.05) was observed in the phenolic content of the two extracts. However, the decoction contains more flavonoids compared to the macerate.

3.2. Acute Toxicity Assay

At the end of the fourteen days of monitoring, no mortality or signs of significant toxicity were seen in the decoction-treated mice. Furthermore, no significant statistical difference was seen between the two groups regarding weight and organ weights (Table 2(a) and Table 2(b)). The four weekly recording of water and food consumption during the assay indicates a slight increase in these two parameters but without a significant difference (p > 0.05) between the treated and control groups (Data not shown).

Table 1. Phytochemical analysis of the aqueous decoction and macerate extracts from the leaves of *O. amentacea*.

Phytochemical analysis	Plant part or extract		
	Decoction	Macerate	Powdered leaves
Phytoconstituents			
Saponins	+	+	n.d.
Alkaloids	-	-	n.d.
Tannins	-	-	n.d.
Flavonoids	+	+	n.d.
Sterols	+	+	n.d.
Triterpenoids	+	+	n.d.
Coumarins	-	-	n.d.
Quantification			
Total Phenolic content (TPC)	94.03 ± 2.66^a	88.03 ± 0.88^a	-
Total Flavonoid content (TFC)	35.05 ± 0.32^a	26.73 ± 0.22^b	-
Extract yield (%)	29.83 ± 0.41	18.57 ± 0.40	n.d.
Residual moisture content (%)	4.92 ± 0.60	7.09 ± 0.19	2.99 ± 0.10

(+) detected; (-) not detected; n.d.: not determined. Values presented are the average of three replications ± standard deviation. Phenolic (TPC) and flavonoid (TFC) contents are expressed in mg GAE and mg QE (mg of Gallic Acid Equivalent and mg Quercetin Equivalent per g of dry extract, respectively). In each row (regarding the polyphenol contents), values bearing different superscript letters ([a, b]) are considered highly statistically significant at $p < 0.001$ (Two-way ANOVA followed by Sidak's multiple comparison test).

Table 2. Comparison of mice (a) and organ weight (b) between the control and decoction groups. n.s.: non-statistically significant.

(a)

Time (Days)	Body Weight (g)[n.s.]	
	Control group	Decoction-treated group
0	28.5 ± 4.6	25.8 ± 1.7
2	29.5 ± 4.7	26.7 ± 1.8
4	30.0 ± 5.3	27.7 ± 1.9
6	31.1 ± 5.5	28.0 ± 1.9
8	32.1 ± 5.4	29.0 ± 1.7
10	33.2 ± 5.3	30.2 ± 1.5
12	34.0 ± 5.6	30.3 ± 1.5
14	34.7 ± 5.3	30.6 ± 1.7

(b)

Organ	Weight (g)[n.s.]	
	Control group	Decoction-treated group
Heart	0.52 ± 0.03	0.51 ± 0.04
Lungs	0.80 ± 0.04	0.83 ± 0.09
Liver	5.1 ± 0.2	5.0 ± 0.10
Kidneys	1.17 ± 0.03	1.18 ± 0.10
Spleen	0.42 ± 0.03	0.41 ± 0.05

3.3. Anti-Inflammatory Activity

The two extracts showed pronounced decreases in the carrageenan-induced mice paw edema (Table 3). The anti-edematous activity was in a time and dose-dependent manner. Compared to the control group, acetylsalicylic acid, aqueous decoction, and maceration significantly ($p < 0.05$) reduced the mice's paw thickness 5 hours after carrageenan administration.

At 1, 3, and 5 H after carrageenan administration, no significant difference was observed between the AAS, decoction, and macerate at 600 mg/kg bw. Regarding the inhibition percentage 5 H after carrageenan injection, samples may be ranked in the following order: AAS (87.0% ± 2.3%) > MAC 600 (84.1% ± 2.8%) > DEC 600 (82.8% ± 2.8%).

3.4. Lipoxygenase (LOX) Inhibitory Activity

The results of lipoxygenase inhibitory activity by the two aqueous extracts are depicted in Table 4. The results showed that the decoction and macerate extracts have a weak lipoxygenase inhibitory effect ($IC_{50} > 100$ μg/mL). The aqueous extracts were highly statistically different ($p < 0.0001$) compared to zileuton. The inhibition percentages at 100 μg/mL were 21.75 ± 0.51 and 21.04 ± 0.45 μg/mL, respectively, for the decoction and macerate extracts of *O. amentacea*.

3.5. Antioxidant Activity

Compared to Trolox, the aqueous decoction and macerate elicit a weak radical scavenging effect (Table 5). The Trolox was at least five to seven times more potent than the two extracts on the DPPH and ABTS radicals. The macerate displayed a higher antioxidant potential at reducing ferric than the decoction. The two extracts exhibited poor inhibitory effects regarding the lipid peroxidation test. All the extracts were statistically significantly different from Trolox ($p < 0.0001$). With an inhibition percentage lower than 50%, the IC_{50} values of the two extracts on the lipid peroxidase test were estimated to exceed 100 μg/mL.

3.6. Correlation of the *in Vitro* and *in Vivo* Biological Properties with Polyphenol Compounds

The Pearson correlation coefficients were used to identify a possible correlation

between the *in vitro* and *in vivo* biological properties and polyphenol compounds. Most assays showed negative correlations with the total phenolic content (Table 6(a) and Table 6(b)).

Table 3. Effect of the samples in carrageenan-induced mice hind paw edema.

Treatment	Dose (mg/kg b.w.)	Increase in Paw edema volume (mL)			Inhibition (%)		
		1 H	3 H	5 H	1 H	3 H	5 H
Control	-	0.40 ± 0.03	0.50 ± 0.03	0.60 ± 0.04	-	-	-
AAS	100	0.17 ± 0.00^a	0.17 ± 0.02^a	0.08 ± 0.01^a	57.7 ± 0.5	64.8 ± 4.0	87.0 ± 2.3
DEC	200	0.30 ± 0.03^b	0.26 ± 0.01^b	0.22 ± 0.02^b	26.4 ± 2.3^b	47.7 ± 1.7^b	63.9 ± 3.8^b
DEC	400	0.26 ± 0.02^b	0.21 ± 0.01^b	0.14 ± 0.02^b	36.4 ± 2.5^d	58.5 ± 1.9^d	76.0 ± 3.4^d
DEC	600	0.23 ± 0.03^a	0.14 ± 0.03^a	0.10 ± 0.03^a	$42.3 \pm 3.9^\$$	$75.9 \pm 3.2^\$$	$82.8 \pm 2.8^\$$
MAC	200	0.36 ± 0.02^d	0.33 ± 0.01^d	0.33 ± 0.03^d	9.3 ± 3.9^a	33.7 ± 1.6^a	45.0 ± 4.4^a
MAC	400	0.25 ± 0.03^a	0.20 ± 0.05^a	0.12 ± 0.01^a	33.7 ± 3.1^d	54.3 ± 1.9^d	79.4 ± 2.5^d
MAC	600	0.20 ± 0.01^a	0.15 ± 0.01^a	0.1 ± 0.02^a	$49.8 \pm 3.6^\$$	$69.4 \pm 1.4^\$$	$84.1 \pm 2.8^\$$

Values are presented as mean ± SD of three experiments. A Two-way ANOVA followed by Dunnett's test assessed differences within groups. Within each column, [a]$p < 0.0001$, [b]$p < 0.001$, [c]$p < 0.01$, and [d]$p < 0.05$, compared to the control or acetylsalicylic acid. In each column of the determination of inhibition percentage, the numbers with the superscript sign "$" were not statistically different compared to AAS AAS: acetylsalicylic acid; DEC: decoction; MAC: macerate.

Table 4. Effect of the aqueous extracts on the lipoxygenase (LOX) activity.

Sample	IC_{50} (µg/mL)	Inhibition (%)
Decoction	$>100^a$	21.75 ± 0.51^a
Macerate	$>100^a$	21.04 ± 0.45^a
Zileuton	2.92 ± 0.31^b	-

Values are presented as mean ± SD; each extract was tested in triplicate. Extracts were compared to each other and zileuton using a Two-way ANOVA followed by Tukey's multiple comparison tests. Within each column, values bearing different superscript letters ([a, b]) are considered highly statistically significant at $p < 0.0001$, and those with the same letters are statistically considered not significant.

Table 5. Antioxidant capacity of the aqueous extracts from the leaves of *O. amentacea*.

Sample	DPPH		ABTS		FRAP	LPO
	IC_{50} (mg/mL)	ARP	IC_{50} (mg/mL)	A. R. P.	(mol AAE/g)	(% inhibition)
Trolox	0.0064 ± 0.21^a	156.3	0.0029 ± 0.14^d	344.83	-	75.42 ± 2.06^h
Decoction	0.51 ± 0.03^b	1.96	0.01 ± 0.003^e	100	589.34 ± 1.66^f	24.22 ± 7.42^i
Macerate	0.43 ± 0.04^c	2.33	0.009 ± 0.003^e	111.11	664.90 ± 0.71^g	29.59 ± 3.58^j

Values are presented as mean ± SD (n = 3). Extracts and Trolox were compared using a Two-way ANOVA followed by Tukey's multiple comparison tests (tests DPPH, ABTS, and LPO). Within each column of the DPPH and ABTS assays, values bearing different superscript letters ([a, b, c]) are considered statistically significant at $p < 0.0001$ (a Vs. b and a Vs. c) and $p < 0.05$ (b Vs. c; d Vs. e); for the FRAP assay (Two-way ANOVA followed by Sidak's multiple comparison tests), values with different superscript letters ([f, g]) are considered statistically significant at $p < 0.001$. In the LPO test, values bearing different superscript letters ([h, i, j]) are considered statistically significant at $p < 0.0001$ (h Vs. i and h Vs. j) and $p < 0.05$ (i Vs. j). ARP (anti-radical power) = $1/IC_{50}$.

Table 6. (a) Correlation matrix (Pearson's correlation coefficients (r)) for the aqueous decoction. (b) Pearson's correlation coefficients for the aqueous macerate.

(a)

Assays	DPPH	ABTS	FRAP	LPO	CIE	LOX	TPC	TFC
DPPH	1							
ABTS	−0.257	1						
FRAP	0.877	0.238	1					
LPO	0.875	0.243	0.999	1				
CIE	−0.979	0.447	−0.762	−0.759	1			
LOX	−0.971	0.482	−0.736	−0.733	0.999	1		
TPC	0.304	**−0.999**	−0.191	−0.196	−0.490	**−0.524**	1	
TFC	**0.887**	**−0.674**	**0.557**	**0.553**	**−0.962**	**−0.972**	0.709	1

CIE: carrageenan-induced edema; TPC: total phenolic content; TFC: total flavonoid content. When r is comprised between 0.7 and 1 or −1 and −0.7, the correlation is considered very positive or very negative strong; the correlation is considered positively or negatively moderate when the coefficient is comprised between 0.3 and 0.7 or −0.7 and −0.3; A Pearson's coefficient value comprised between 0 and 0.3 or −0.3 and 0, means a positive or negative poor correlation [34].

(b)

Assays	DPPH	ABTS	FRAP	LPO	CIE	LOX	TPC	TFC
DPPH	1							
ABTS	0.025	1						
FRAP	−0.984	0.156	1					
LPO	0.648	0.778	−0.500	1				
CIE	−0.732	0.663	0.843	0.044	1			
LOX	−0.061	0.996	0.240	0.721	0.725	1		
TPC	**−0.994**	−0.132	**0.959**	**−0.726**	**0.655**	−0.046	1	
TFC	**0.831**	**−0.535**	**−0.918**	0.115	**−0.987**	**−0.606**	−0.767	1

The DPPH radical scavenging was highly correlated with TFC (r = 0.887, Table 6(a)), while the FRAP and LPO assays were moderately correlated with TFC. These results indicate that flavonoids are probably the main compounds responsible for these antioxidant activities. Although the decoction was more potent on the ABTS than DPPH assays, total phenolics and flavonoids were negatively correlated (r = −0.999 with TPC and r = −0.674 with TFC, Table 6(a)) with this test; it could be assumed that likely other compounds may explain the result. Similarly, the correlations were negative between the TPC, TFC, carrageenan-induced edema (CIE), and LOX assays (Table 6(a)). No significant statistical (p > 0.05) differences were observed between the correlations.

The results of the correlation study between the different assays with the macerate and polyphenols compounds indicate that phenolics were strongly and moderately correlated with the FRAP and CIE assays (Table 6(b)), respectively. However, flavonoids appeared to be the main compounds responsible for the DPPH radical scavenging (r = 0.831). Pearson's correlation coefficients were negative between total flavonoids and ABTS, FRAP, CIE, and LOX assays (Table 6(b)), indicating that probably other bioactive compounds intervened to justify the antioxidant effect of the macerate.

4. Discussion

Plants have been used to treat various ailments since several millennia ago [3] [35] [36]. Nonetheless, the need to validate or deny the traditional use of these plants through rigorous scientific methods is crucial. In the present report, the aqueous decoction and macerate extracts of the leaves of *Opilia amentacea* have been studied for their anti-inflammatory and antioxidant activities.

The extract yield of decoction was higher than the macerate, and the samples' residual moisture content was lower than 10%. This result indicates that the drying and freeze-drying processes allow good preservation of the samples without any risk of alteration [37]. Saponins, flavonoids, sterols, and triterpenoids were detected in the aqueous extracts of the leaves of *O. amentacea*. However, tannins, alkaloids, and coumarins were absent. The results differ from other reports [11] [12] [15] on the phytochemical composition of the aqueous decoction from the leaves of *O. amentacea*. Saponins are the only phytochemicals common in these different studies. The differences in phytochemical content may be due to factors including biotic and abiotic such as season, light, temperature, and edaphic factors that influence the phytochemical composition of medicinal plants [38]. In their report, Youl *et al.* (2023) found tannins in the ethanolic extract from the leaves of *O. amentacea* [20]. This difference may be due to the solvent. Indeed, although soluble in water, tannins are better extracted with solvents containing alcohol (ethanol, methanol) or acid [39]. The results on estimating polyphenol compound content indicate that the decoction extract was richer than the macerate, mainly regarding the phenolic content. Our results were more significant compared to those of Youl and collaborators [20].

After administration of the decoction, no significant toxicity signs were recorded during the fourteen days of observation. The lethal oral dose (LD_{50}) is estimated to be greater than 2000 mg/kg bw, suggesting that the aqueous decoction is poorly toxic. According to the Global Harmonized System, it can be classified into category five substances regrouping compounds with very low acute toxicity [40].

The carrageenan-induced paw is a classical model widely used in most laboratories to assess the anti-inflammatory effect of various products, including plant extracts. The intraplantar injection of carrageenan led to a time-dependent increase in the mice's paw volume. The maximum increase was obtained after 5 H

in the control group. However, administration of the extracts significantly reduced the paw thickness. All the extracts inhibited edema in a dose-dependent manner, and the highest inhibition was observed five hours after carrageenan administration. The development of inflammation following carrageenan injection is a biphasic model [41] [42]. The first phase corresponds to the release of histamine, serotonin, and kinins, while the prostaglandins are the pro-inflammatory mediators released during the second phase. Based on the previous statement, it can be suggested that the two extracts inhibit both stages of the inflammation process, making them potential candidates for treating inflammation-related diseases. The anti-inflammatory activity of the two extracts was not significantly ($p > 0.05$) different from the acetylsalicylic acid 5 H after carrageenan injection.

The two extracts were weakly effective on the lipoxygenase enzyme than zileuton. Comparing this result with the *in vivo* anti-inflammatory activity, this result suggests that the anti-inflammatory effect of the extracts may be explained by other mechanisms that need to be investigated. Medicinal plant extracts possess several phytochemicals with anti-inflammatory potential. These phytochemicals include polyphenols, mostly flavonoids, terpenoids, and alkaloids [43] [44]. Except for alkaloids, all these compounds have been detected in the aqueous extracts of *O. amentacea*. Most correlations between the anti-inflammatory activity and polyphenol compounds were negative (Table 6(a) and Table 6(b)). These results imply, therefore, that the anti-inflammatory capacity of the extracts is mainly based on the presence of other compounds, such as terpenoids.

It is commonly accepted that at least two methods are needed to assess the antioxidant potential of a sample. The antioxidant effect of the decoction and macerate extracts were studied using four different assays, including radical scavenging activity with DPPH and ABTS radicals, ferric reducing power (FRAP), and lipid peroxidation inhibition (LPO). Altogether, the results on the antioxidant effect demonstrated a low to high antioxidant potential of the two aqueous extracts. Compared to the Trolox, the extracts were weakly active on the lipid peroxidation and the DPPH and ABTS radicals. In contrast, the extracts, particularly the macerate, have shown a high ferric-reducing antioxidant potential. These *in vitro* spectrophotometric antioxidant techniques are usually classified into two groups based on their antioxidant mechanism. Those involving hydrogen atom transfer, such as lipid peroxidation assay, and those based on single electron transfer, as in FRAP, DPPH, and ABTS tests [45]. The FRAP assay measures the extract's overall antioxidant capacity or corresponding concentration of electron-donating antioxidants [31]. The results on the antioxidant effect of the leaves of *O. amentacea* differed from that of [14] probably because these authors worked with a flavonoid-rich fraction instead of an extract that contains various phytochemicals that can act synergistically or not. Several compounds, including polyphenols, carotenoids, vitamins, coumarins, stilbenes, and lignans, mainly contribute to the antioxidant potential of plants [46] [47]. Polyphenol compounds can act: 1) synergistically, 2) additively, or 3) antagonistically to remove free radicals [48].

Furthermore, it has been reported that the antioxidant effect exerted by polyphenol compounds depends on various factors, including the structure and concentration of phenolic compounds [48]. The results of correlation studies suggest that: 1) flavonoids are highly involved in the DPPH radical scavenging effect of the two extracts, and 2) phenolics present in the macerate are the primary metabolites responsible for reducing ferric ions. The negative correlations suggest that other compounds that have not been detected using the current phytochemical investigation contribute to the antioxidant potential of the extracts.

5. Conclusion

The present study reported the phytochemical analysis, acute toxicity, anti-inflammatory, and antioxidant activities of two aqueous extracts of *Opilia amentacea.* Aqueous extracts are the most common method used by traditional practitioners to treat illness in traditional medicine in Burkina Faso. The two extracts contain similar phytochemical compounds: saponins, sterols, triterpenes, and flavonoids. The decoction and macerate exhibited significant *in vivo* anti-inflammatory effects likely mediated by another mechanism than the inhibition of lipoxygenase activity. Moreover, the extracts elicit a weak to high antioxidant capacity, probably due to polyphenol compounds and other phytochemicals. Together, these results suggest that the aqueous extracts of *O. amentacea* can act as potent candidates for oxidative stress and inflammation-related diseases. Nevertheless, further *in vivo* and *vitro* studies investigating the anti-inflammatory mechanisms are required.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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